



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 archesporium. The occurrence of cytologically 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 (Goška, 1997; Svirshchetskaya 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 *Boecheera 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, apogamy or adventitious embry-

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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), Zachariev (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, Maletskii and Maletskaya (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 embryogenesis 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 of apomixis, pollinators for the formation of heterosis hybrids, and also as pure-line apomictic varieties.

Shiryayeva (1983) and Perfilieva (2003) shared the view that apomixis occurs in sugar beet. Bogomolov (2005) obtained sugar beet apomictic gamma-lines by pollinating male-sterile sugar beet plants with gamma-irradiated pollen of the wild beets *B. corolliflora* and *B. trigyna*.

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_{III} 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 O996 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 *Pgi SS*, *Pgm FF* × male-fertile line genotype *Pgi FF*, *Pgm 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.

TABLE 1. Results of reproduction experiments

Male function of plant/ploidy	Experiment type			
	Isolation	Cross-pollination	Open-pollination	Emasculation
male-sterile/2x	autonomous apomixis	- reduced parthenogenesis - unreduced parthenogenesis - hybridization - B _{III} hybrids	hybridization	
hemimale-sterile/2x	- unreduced parthenogenesis - self-fertilization		- hybridization - self-fertilization	
male-fertile/2x, 4x	- reduced parthenogenesis - self-fertilization		- reduced parthenogenesis - hybridization - self-fertilization	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 Kuźdowicz (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: 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 phosphatase 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 χ^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).

SEED PROGENIES FORMED AFTER AMPHIMIXIS AND APOMIXIS

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.

TABLE 2. Seed progenies (sublines) formed as a result of amphimixis and apomixis; seed weight [g]

Origin-line	No. of tested mother plants	No. of seed progeny	Seed progeny resulting from facultative apomixis			
			No. of progenies (sublines) (%) from number of mother plants (%) from total progeny numbers	Apomixis no pollen	Apomixis and self-pollination	Apomixis and hybridization
MSMP46/94	104	55	1 (1.0) (1.8)	Ap0012 3.3g		
FC708CMS	173	111	1 (0.6) (0.9)		P0219 7.0g	
FC607CMS	230	142	6 (2.6) (4.2)	Ap037 0.3g	P029 105.0g Sp0393 4.8g	Sw01-3 44.4g Sw0431 207.0g Sw0440 212.4g
FC403CMS	17	10	1 (5.9) (10.0)		Ap0316 16.8g	
O996	29	25	4 (13.8) (16.0)		Sp012 7.8g P03104 22.9g P03109 75.0g SP0479 62.6g	
Total	553	343	13 (2.4) (3.8)	2	8	3

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).

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

Genotype of mother plant		Symbol	No. of tested plants	No. of homozygotic loci (pure after mother)	Segregation at heterozygotic loci in total	χ^2 tests and probability P for segregation ratio	
homozygotic	heterozygotic					1:2:1 1FF:2FS:1SS	3:8:3 3FF:8FS:3SS
3 MDH1 NN, IDH1 FF, GDH SS	5 PGI FS, PGM FS, MDH2 FS, LAP FS, ACP2 FS	Ap 0012	48	3	38FF+89FS+40SS	$\chi^2 = 0.7725$ 0.70>P>0.50	$\chi^2 = 1.0649$ 0.70>P>0.50
4 PGM FF, MDH2 SS, IDH1 FF, GDH SS	2 PGI FS, MDH1 NP	Ap 037	9	4	4FF(NN)+8FS(NP)+6 SS(PP)	$\chi^2 = 0.2222$ 0.70>P>0.50	$\chi^2 = 1.1791$ 0.30>P>0.20

TABLE 4. Autonomous apomixis in emasculated and isolated flowers

Line	Mother plant	% of apomictically formed seeds	Collected seed progeny
FC607CMS	a	5.8	P029
	b	22.2	not collected
FC708CMS	c	2.3	P0219
	d	14.0	P03104
O996	e	16.0	P03109
	f	4.6	SP0479

Apomictically formed embryos identified by flow cytometry showed gametic ploidy level. Apomictically formed embryos identified by isozyme profile were metamorphous, 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 χ^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 5. Identification of facultative agamospermy

Method	Mother plant	Seed progeny resulting from facultative agamospermy		
		Progeny	Apomictic embryos	Amphimictic embryos
Flow cytometry	diploid	Sp012	1% haploids	99% diploids
	diploid	Sw01-3	1% haploids	99% diploids
	tetraploid	Sp0393	20% diploids	80% tetraploids
Isozyme electrophoresis	homozygote at 2 marker loci: <i>Pgi</i> , <i>Pgm</i>	Sw0431	10% homozygotic maternal embryos	90% heterozygotic hybrid embryos
	homozygote at 2 marker loci: <i>Pgi</i> , <i>Pgm</i>	Sw0440	61% homozygotic maternal embryos	39% heterozygotic hybrid embryos

TABLE 6. Frequency of plants with normal meiosis and forming unreduced pollen grains (to various degrees)

Line ploidy	% of plants with normal meiosis (number of pores)	% of plants with disturbed meiosis	
		analyzed plants (number of pores)	plants with unreduced pollen (number of pores)
FC607CMS 2x	38 (5-8)	62 (7-13)	38 (10-13)
FC607CMS 4x	35 (10-15)	65 (5-22)	15 (20-22)
Total 5 lines	46	54	21

(Tab. 4). 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 metromorphous, 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-fer- tile) of various genotypes ($\text{♀ } Pgi\ SS, Pgm\ FF \times \text{♂ } Pgi\ FF, Pgm\ SS$). 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 χ^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 B_{III} embryos. The remaining ~75% were the expected triploid seeds. Haploids and B_{III} 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 (Shiryayeva, 1983; Maletskii and Maletskaya, 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).

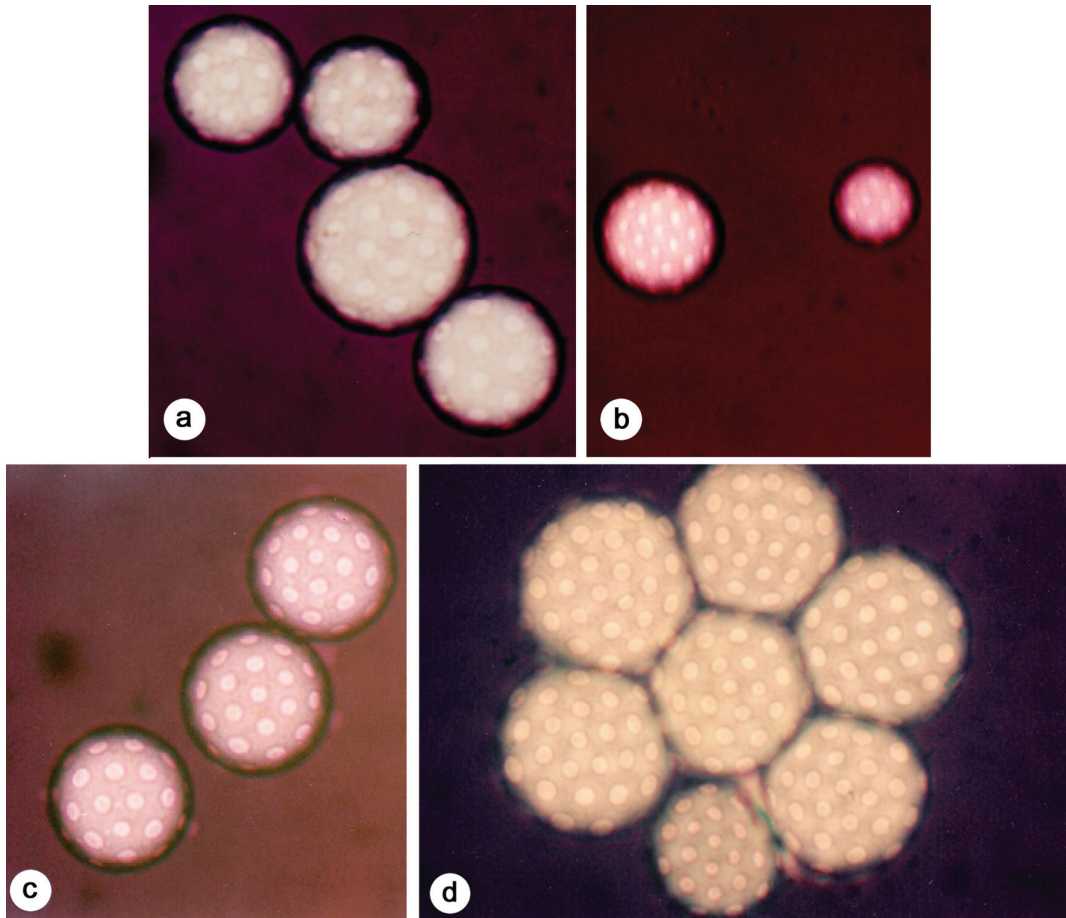


Fig. 1. Pollen grain morphology. **(a)** Pollen grains of diploid mother plant: three reduced grains with 7 pore regions, one unreduced grain with 12 pore regions. $\times 1000$, **(b-d)** From tetraploid mother plants: **(b)** Pollen grain with 15 pore regions – reduced in the tetraploid. Pollen grain with 7 pore regions – monohaploid. $\times 500$, **(c)** 10–12 entire pore regions are visible on the surface of pollen grains, indicating that the grains are cytologically reduced in the tetraploid. $\times 1000$, **(d)** One smaller grain shows 13 pore regions, indicating reduced number of chromosomes in tetraploid plant pollen. The remaining grains show 16–20 pore regions; they probably result from disturbed meiosis or lack of reduction. $\times 1000$.

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 archesporium much more frequently than in the male archesporium. 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*. Poddubnaya-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 automictic 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 Goška (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. 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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REFERENCES

- BAROCKA KH. 1966. Die Sektion *Corollinae* der Gattung *Beta* (Tournef.) L. *Zeitschrift für Pflanzenzüchtung* 56: 379–388.
- BAYER RJ, RITLAND K, and PURDY BG. 1990. Evidence of partial apomixis in *Antennaria media* (Asteraceae: Inuleae) detected by the segregation of genetic markers. *American Journal of Botany* 77(8): 1078–1083.
- BOGOMOLOV MA. 2005. Apomiksii i ego rol' v selektsii sakharnoy svekly. *Sakharnaya svekla* 8: 19–22.
- CLEIJ G, DE BOCK TSM, and LEKKERKERKER B. 1968. Crosses between *Beta intermedia* Bunge and *B. vulgaris* L. *Euphytica* 17: 11–20.
- CLEIJ G, DE BOCK TSM, and LEKKERKERKER B. 1976. Crosses between *Beta vulgaris* L. and *B. lomatozona* F. et M. *Euphytica* 25: 539–547.
- DALKE L. 1980. Samozgodność u jednonasiennego buraka cukrowego i możliwości jej wykorzystania w hodowli. *Hodowla Roślin Aklimatyzacja i Nasiennictwo* 24(3): 169–202.
- ELLERTON S, and HENDRIKSEN AJT. 1959. Note on the probable cause of occurrence of tetraploid plants in commercial triploid varieties of sugar beet. *Euphytica* 8(2): 99–103.
- FILUTOWICZ A, and DALKE L. 1970. Charakterystyka gatunków sekcji *Corollinae* rodzaju *Beta* i możliwości ich wykorzystania w hodowli buraka cukrowego. *Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin* 5–6: 15–23.
- FILUTOWICZ A, and DALKE L. 1972. Mieszanie buraka cukrowego (*Beta vulgaris* L.) z *Beta lomatozona* F. et M. *Hodowla Roślin Aklimatyzacja i Nasiennictwo* 16(2): 85–93.
- GALBRAITH DW, HARKINS KR, MADDOX JM, AYRES NM, SHARMA DP, and FIROOZABADY E. 1983. Rapid flow cytometric analysis of the cell cycle in intact plant tissues. *Science* 220: 1049–1051.
- GERLACH D. 1965. Befruchtung und Autogamie bei *Rubus caesius*. *Biologisches Zentralblatt* 84(5): 611–633.
- GOEL S, CHEN Z, AKIYAMA Y, CONNER JA, BASU M, GUALTIERI G, HANNA WW, and OZIAS-AKINS P. 2006. Comparative physical mapping of the apospory-specific genomic region in two apomictic grasses: *Pennisetum squamulatum* and *Cenchrus ciliaris*. *Genetics* 173(1): 389–400.
- GOŚKA M. 1997. *Monografie i Rozprawy Naukowe. Haploidy i Podwojone Haploidy Buraka Cukrowego (Beta vulgaris L.) oraz Możliwości Ich Wykorzystania w Hodowli*. Instytut Hodowli i Aklimatyzacji Roślin, Radzików.
- HUO H, CONNER JA, and OZIAS-AKINS P. 2009. Genetic mapping of the apospory-specific genomic region in *Pennisetum squamulatum* using retrotransposon-based molecular markers. *Theoretical and Applied Genetics* 119(2): 199–212.
- JASSEM B. 1976. Embryology and genetics of apomixis in the section *Corollinae* of the genus *Beta*. *Acta Biologica Cracoviensia Series Botanica* 19: 149–172.
- JASSEM B. 1990. Apomixis in the genus *Beta*. *Apomixis Newsletter* 2: 7–23.
- JASSEM B, and JASSEM M. 1970. Embryological investigations into the polyploid *Beta* species of the *Corollinae* section. *Genetica Polonica* 11(3–4): 367–377.
- JASSEM M. 1973. Endosperm development in diploid, triploid and tetraploid seed of sugar beet (*Beta vulgaris* L.). *Genetica Polonica* 14(3): 295–303.
- JOHRI BM, AMBEGAOKAR KB, and SRIVASTAVA PS. 1992. *Comparative Embryology of Angiosperms*, vol. 1. Springer-Verlag, Berlin, Heidelberg, New York, London, Paris, Tokyo, Hong Kong, Barcelona, Budapest.
- KOJIMA A, and NAGATO Y. 1992. Diplosporous embryo-sac formation and the degree of diplospory in *Allium tuberosum*. *Sexual Plant Reproduction* 5: 72–78.
- KOLLMANN J, STEINGER T, and ROY BA. 2000. Evidence of sexuality in European *Rubus* (Rosaceae) species based on AFLP and allozyme analysis. *American Journal of Botany* 87(11): 1592–1598.
- KUŹDOWICZ A. 1958. Identyfikacja stopnia poliploidalności u buraka (*Beta vulgaris* L.) na podstawie liczby ujęć łagiewkowych pyłku. *Acta Societatis Botanicorum Poloniae* 27(3): 491–500.
- LEVITES EV. 1986. *Genetika Izofermentov Rasteniy*. Nauka, Novosibirsk.
- LEVITES EV, DENISOVA FS, and FILATOV GP. 1994. Genetic control of isozymes in the sugar beet. In: Markert CL, Scandalios JG, and Serov OL [eds], *Isozymes: Organization and Roles in Evolution, Genetics and Physiology*, 171–178. World Scientific, Singapore, New Jersey, London, Hong Kong.
- ŁOMNICKI A. 1995. *Wprowadzenie do Statystyki dla Przyrodników*. Wydawnictwo Naukowe PWN, Warszawa.
- MALETSKII SI. 2000. *Binominal'nye raspredeleniya v genicheskikh issledovaniyakh na rasteniyakh*. Rossiyskaya Akademiya Nauk Sibirskoe Otdelenie Institut Tsitologii i Genetiki, Ministerstvo Sel'skogo Khozyaystva i Prodovol'stviya Rossii Novosibirskiy Gosudarstvennyy Agrarnyy Universitet, Novosibirsk.
- MALETSKII SI, and LEVITES EV. 2005. *Epigenetika Rasteniy, Sbornik Nauchnykh Trudov*. Rossiyskaya Akademiya Nauk Sibirskoe Otdelenie Institut Tsitologii i Genetiki, Novosibirsk.
- MALETSKII SI, and MALETSKAYA EI. 1996. Samofertil'nost' i agamospermiya u sakharnoy svekly (*Beta vulgaris* L.). *Genetika* 32(12): 1643–1650.

- MATZK F, MEISTER A, and SCHUBERT I. 2001. An efficient screen for reproductive pathways using mature seeds of monocots and dicots. *The Plant Journal* 21: 97–108.
- MURAWSKI DA and BAWA KS. 1994. Genetic structure and mating system of *Stemonoporus oblongifolius* (Dipterocarpaceae) in Sri Lanka. *American Journal of Botany* 81(2): 155–160.
- NOGLER GA. 1984. Gametophytic apomixis. In: Johri BM [ed.], *Embryology of Angiosperms*, 475–518. Springer-Verlag, Berlin, Heidelberg, New York, Tokyo.
- OWEN FV. 1945. Cytoplasmically inherited male-sterility in sugar beets. *Journal of Agricultural Research* 71(10): 423–440.
- PERFILIEVA LP. 2003. Apomiksyz u riznykh form tsukrovoho buriaka. *Zbirnyk naukovykh prats Umanskoho derzhavnogo ahrarnoho universytetu (spetsialnyi vypusk) Biolohichni Nauky i Problemy Roslynnystva*: 384–389.
- PODDUBNAYA-ARNOLDI VA. 1982. *Kharakteristika Semeystv Pokrytosemennykh Rasteniy po Tsitoembriologicheskim Priznakam*. Nauka, Moskva.
- RAVI M, MARIMUTHU MPA, and SIDDIQI I. 2008. Gamete formation without meiosis in *Arabidopsis*. *Nature* 451: 1121–1124.
- RODRIGUES JCM, and KOLTUNOW AMG. 2005. Epigenetic aspects of sexual and asexual seed development. *Acta Biologica Cracoviensia Series Botanica* 47(1): 37–49.
- SAVIDAN Y. 2001. Gametophytic apomixis. A successful mutation of the female gametogenesis. In: Bhojwani SS, and Soh WY [eds], *Current Trends in the Embryology of Angiosperms*, 419–433. Kluwer Academic Publishers, Dordrecht, Boston, London.
- SAVIDAN Y, CARMAN JG, and DRESSELHAUS T. 2001. *The Flowering of Apomixis: from Mechanisms to Genetic Engineering*. Mexico, DF: CIMMYT, IRD, European Commission DG VI (FAIR).
- SCANDALIOS JG. 1969. Genetic control of multiple molecular forms of enzymes in plants: a review. *Biochemical Genetics* 3: 37–79.
- SEDOVSKIY AI, NURZHANOVA AA, and SEILOVA LB. 2001. Sugar-beet (*Beta vulgaris* L.) breeding using apomictic forms. *Proceedings of the Asian Agriculture Congress*, 24–27 April 2001, 138. Manila, Philippines.
- SEILOVA LB. 1996. *Apomiksyz u sakharnoy svekly i ego ispol'zovanie v prakticheskoy selektsii*. Na pravakh rukopisi. Kazakhskaya Akademiya Sel'skokhozyaystvennykh Nauk, Almaty.
- SHARBEL TF, VOIGT ML, CORRAL JM, THIEL T, VARSHNEY A, KUMLEHN J, VOGEL H, and ROTTER B. 2009. Molecular signatures of apomictic and sexual ovules in the *Boechera holboellii* complex. *Plant Journal* 58(5): 870–882.
- SHIRYAEVA EI. 1983. Tsitoembriologicheskoe izuchenie apomiksisa u *Beta vulgaris* L. *Tezisy Dokladov VII Delegatskogo S'ezda Wsesoyuznogo Botanicheskogo Obshchestva*, Donetsk 11–14 maya 1983, 272. Nauka, Leningrad.
- SVIRSHCHEVSKAYA A, and DOLEŽEL J. 2000. Production and performance of gynogenetic sugarbeet lines. *Journal of Sugar Beet Research* 37: 117–133.
- SZKUTNIK T, PRUSIŃSKA E, and CZERWCZAK U. 2001. Uzyskanie agamospermicznych potomstw u męskosterylnych roślin buraka cukrowego (*Beta vulgaris* L.). *Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin* 217: 249–261.
- ŚLIWIŃSKA E. 1997. Analiza cyklu komórkowego buraków cukrowych o różnym stopniu ploidalności za pomocą cytometrii przepływowej. *Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin* 202: 35–40.
- TASKIN KM, TURGUT K, and SCOTT RJ. 2004. Apomictic development in *Arabis gunnisoniana*. *Israel Journal of Plant Sciences* 52: 155–160.
- VALLEJOS CE. 1983. Enzyme activity staining. In: Tanksley SD and Orton TJ [eds], *Isozymes in Plant Genetics and Breeding, Part A*, 469–516. Elsevier Science Publishers, Amsterdam.
- VAN GEYT JPCF, and SMED E. 1984. Polymorphism of some marker enzymes of the sugarbeet (*Beta vulgaris* L.) investigated by polyacrylamide gel electrophoresis and starch gel electrophoresis. *Zeitschrift für Pflanzenzüchtung* 92: 295–308.
- WENDEL JF, and STUBER CW. 1984. Plant isozymes: Enzymes studied and buffer systems for their electrophoretic resolution in starch gels. *Isozyme Bulletin* 17: 4–11.
- YAN G, GUANGCHUN H, ZHIWEI W, DEDONG G, RUI Q, and RONGTIAN L. 2007. GISH and BAC-FISH study of apomictic *Beta* M14. *Science in China Series C: Life Sciences* 50(2): 242–250.
- ZHU H, BI YD, YU LJ, GUO DD, and WANG BC. 2009. Comparative proteomic analysis of apomictic monosomic addition line of *Beta corolliflora* and *Beta vulgaris* L. in sugar beet. *Molecular Biology Reports* 36(8): 2093–2098.