ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 110, No. 3 (2023), p. 225–234
DOI 10.13080/z-a.2023.110.026

Alloplasmic lines of beets with new cytoplasm from the wild genus *Beta patula*

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Abstract

The purpose of the study was to obtain new breeding genotypes containing a nuclear genome of a sugar beet sterility maintainer and sterile cytoplasm of wild beet (*Beta patula* L.). The donors of a new sterile cytoplasm of *B. patula* plants were selected by the indirect cytological traits of cytoplasmic male sterility (CMS), especially pollen sterility, abnormal development of the anthers, mosaic patterns of anthers and flowers. The anthers were examined for morphological traits of pollen and microspore degeneration in CMS. During reciprocal crosses, (i) the expression of recessive alleles of the nuclear genes of the CMS sterility maintainers was analysed, (ii) the peculiarities of hybridisation of the experimental genotypes with sterile *B. patula* cytoplasm were studied, and (iii) the selection of linked genes for hypocotyl colour *Rr* and one- or two-year growth cycle *Bb* was carried out. The obtained backcross offspring of BC_3S *B. patula* had 95% fertility, 100% CMS-0 type sterility, and sugar content between 19.5% and 21.5%. Recombination in the locus of linked genes of the one- and two-year growth cycles and hypocotyl colour *RB rb* in hybrids F_1, *B. patula* and backcross offspring was detected. At the same time, the one- and two-year growth cycle in seed plants was restored only in the 4th cycle of backcross. Recombinant genotypes with a red hypocotyl and two-year growth cycle BC_3S *B. patula* had multi-seed fruits, single or branched stems of seed plants and were chimerical by sterility (CMS-1 and CMS-0 types).

The obtained alloplasmic introduced lines containing the cytoplasm of *B. patula* have economically valuable properties as parent components for the development of new triploid hybrids. The experimental triploid hybrids had root sugar content from 19.5% to 20.5% and dry matter content from 25% to 29%. Alloplasmic lines with the new CMS of *B. patula* are recommended for sugar beet breeding.

Keywords: sugar beet, cytoplasmic male sterility, sterility maintainers, recessive alleles, wild beets.

Introduction

The wild beet (*B. patula* L.) is the oldest species of the *Vulgaris* section. It grows naturally on the island of Madeira and is considered a source of genetic variability that can be used in sugar beet breeding (Ascarini, Ragonezi, 2021). The systematic features of wild species *B. patula* were first described about 100 years ago. However, for a long time its attribution to *Vulgaris* was questionable. Only recently, the use of DNA technology and RAPT markers confirmed that this species belongs to the section *Vulgaris* and is derived from the wild species *Beta perenis* (Chesnokov et al., 2013). The chromosomes of *B. patula* were found to be the shortest (2n = 18). New sources of CMS from wild types of beets can be used to expand the genetic diversity of sugar beets and increase their resistance to diseases due to a high degree of cytoplasmic homogeneity (Bosemark, 2006; Chesnokov et al., 2013).

The CMS of sugar beet has a mutant character; it is caused by the interaction of two unlinked genes *Xx* and *Zz* and a mutation of the mitochondrial genome of the cytoplasm (Owen, 1945). According to the Owen genetic model, pollen-sterile plants have the genotype *(S) xxzz*, while other plants have the genotype *(S) XXZZ-(S) Xxzz* and are characterised by a significant variability in the number of fertile and sterile pollen grains and the morphological structure of pollen during the process of ontogenesis. Researchers that studied CMS (Maletskii et al., 2015; Levites, Kirikovich, 2017) also found convincing facts that cannot be explained by the hypothesis that CMS is controlled by two genes but can be explained by the mother type of inheritance and epigenomic variability of the CMS genotypes during the process of ontogenesis. Access to a wide range

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Kovalchuk N., Roik M., Zinchenko O. 2023. Alloplasmic lines of beets with new cytoplasm from the wild genus *Beta patula*. Zemdirbyste-Agriculture, 110 (3): 225–234. https://doi.org/10.13080/z-a.2023.110.026

of germplasm of wild species of the genus *Beta* L. is necessary to obtain new breeding genotypes resistant to future epiphytosis and to promote progress in sugar beet breeding (Chesnokov et al., 2013). In addition, the complete cytoplasmic homogeneity resulting from the use of only one source of CMS significantly reduces the efficiency of sugar beet use.

For a long time, theories of evolution and breeding focused on the importance of nuclear genes in the genetic variability of organisms and completely ignored the influence of cytoplasmic gene variability. New methods of molecular genetics and genetic engineering have made it possible to revise such previous decisions (Moritani et al., 2013; Arakawa et al., 2019). The question of interspecies cytoplasmic variability was studied by Gavrilenko and Yermishin (2017) and Iefimenko et al. (2018). The scientists have created several alloplasmic lines that combined the genome and cytoplasm of two different species. The adaptivity of interspecies cytoplasmic variability was proved by Riungu and McVetty (2004).

Biotechnological methods of cultivating immature embryos in the process of interspecies hybridisation appeared to be promising for this purpose. Several studies have been carried out on alloplasmic lines of wheat with the cytoplasm of *Triticum*, *Aegilops*, *Secale*, and *Agropyron* (Reddy et al., 2015; Gavrilenko, Yermishin, 2017; Iefimenko et al., 2018; Kondratskaya et al., 2018). Alloplasmic lines of *Hordeum*, *Solanum*, *Gossipium*, *Brassica*, *Avena* and other genera containing the cytoplasm of relative species were obtained (Efremova et al., 2013; McGrath et al., 2015; Iefimenko et al., 2018). Research on the genetics of crop immunity has identified the prospects of using wild species in breeding to protect crops from pests and diseases and to enrich the adaptive potential of crops (Zaviryukha et al., 2017). New sources of resistance to rhizomania and cercosporosis were selected from the wild species of *Beta maritima* L. – 60 varieties originating in many regions from the Scandinavian Peninsula to the Mediterranean Sea (Chesnokov et al., 2013; Richardson, 2017; Leonova, 2019).

CMS in *Beta vulgaris* L. was first reported by Oldemeyer (1957), who analysed the collection of *B. patula* accessions and found two new sources of sterile cytoplasm: one originating from the natural populations of *Beta macrocarpa* L. and the other from the natural populations of *Beta cicla* L. in Turkey. The value of sugar beet germplasm enrichment was demonstrated by Doney (1998), who concluded that the *B. patula* genes can be easily transferred to the experimental breeding genotypes. The lines containing new germplasm from the wild species of *B. maritima* L. were classified as E, G, and H based on the mt-DNA nucleotide models and fertility-restoring properties (Moritani et al., 2013; Touzet et al., 2018). Not only Oldemeyer (1957) but Bosemark (2006) also found five new sources of male sterility originating from Morocco, Yugoslavia, and Turkey. The search for new sources of male sterility continued in the1960s: new sources of CMS were found in *B. maritima* L. populations from Turkey, India, Pakistan, and Manjuri. To determine the type of sterility, backcrosses with the sugar beet sterility maintainers were conducted (Bosemark, 2006). To obtain introduced alloplasmic lines, backcrosses, and genetic selective markers of the hypocotyl colour *Rr*, one- and two-year growth cycle *Bb*, monogermity *mm*, and cytoplasmic male sterility *XZ* were used. Morphological markers of anthocyanin colour genes *Rr*, *Yy*, one- and two-year growth cycle *Bb* were used during differentiation and selection of sugar beet sterility maintainers, as the fact of their linkage was previously confirmed by the results of genetic control of enzymes (Денисова и др., 2010).

The Laboratory of Cytogenetics of the Institute of Bioenergy Crops and Sugar Beet of Ukraine performed crosses between *B. patula* species with anthocyanin colour of the hypocotyl *R*, *Rr* and sterility maintainers that carry recessive homozygotes (identified by the green colour of the hypocotyl *rr*) (Ковальчук, 2013). As the issue of restoring fertility is still far from being resolved, a search for new ways of obtaining alloplasmic lines with a nuclear genome of sterility maintainer and sterile cytoplasm of wild species remains important (Kitazaki et al., 2015; Arakawa et al., 2020).

The purpose of this study was to obtain (i) a new sterile cytoplasm from wild beet (*B. patula* L.) using cytologic traits of male gametophyte degeneration in CMS, and (ii) introduced alloplasmic lines containing a nuclear genome of a sugar beet sterility maintainer and a new plasm of *B. vulgaris*.

Material and methods

Plant material. The study was conducted at the Institute of Bioenergy Crops and Sugar Beet of Ukraine in 2012–2021. The following plant material was used in the experiment. (1) Perennial species wild beet (*Beta patula* L.). The population of annual plants was created using seeds from the European Seed Bank. Wild beet plants were grown in pots. The seeds were sown at the end of April, and flowering was in June. *B. patula* plants had sedentary oval leaves, multi-flowered (6–8 flowers per cluster) and well-branched. The flowering was not simultaneous (Figure 1). (2) As a sterility tester, a sugar beet sterility maintainer resistant to rhizomania was used (breeding line No. 677 from the seed collection of the Institute of Bioenergy Crops and Sugar Beet). (3) Experimental triploid hybrids based on new sources of sterility were obtained using tetraploid multigerm pollinators *Ya/DA 4x* (Yaltushkiv Experimental Breeding Station) and *7009 BT 4x* (Veselyi Podil Experimental Breeding Station).

Cytological methods for the selection of control plants, donors of new sterile cytoplasm from the population of B. patula seed plants. Cytological analysis was performed according to the method of Зайковская (1968). From the 45 accessions of *B. patula*, the plants that had: fertile and sterile pollen grains (up to 20%), sterile pollen grains with a well-developed exine, and a mosaic pattern of the anthers in flower, i.e., with fertile and sterile pollen grains were isolated. Analysis of the

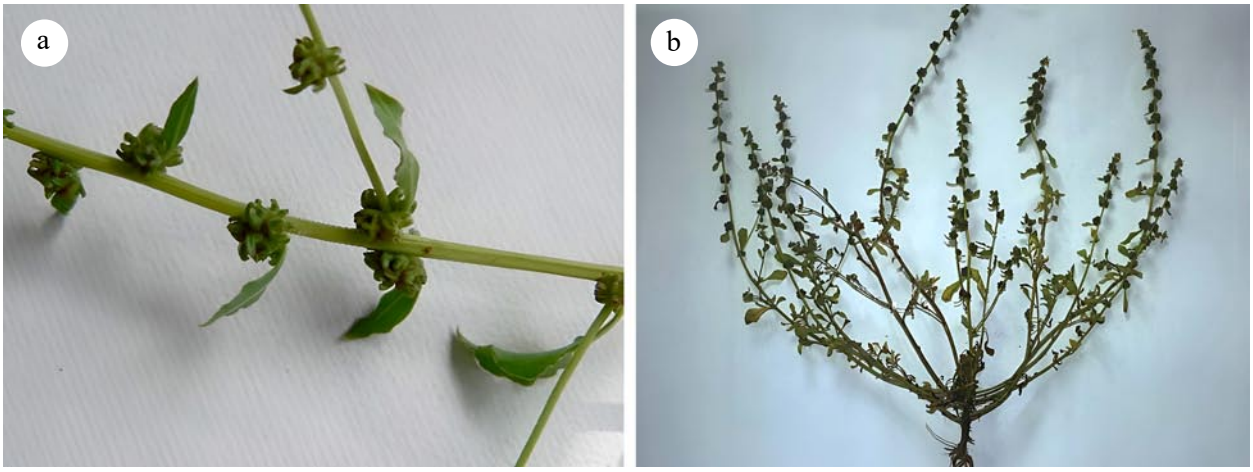


Figure 1. Morphological traits of *Beta patula* introduced in Ukraine: a) multigerm (6–8 seeds), b) stem

anther structure in terms of pollen fertility/sterility was performed using staining (4% acetocarmine and 0.09% methylene blue) and examining under the Carl Zeis microscope at a magnification of 12 × 20 and 12 × 40. During the cytological analysis, attention was paid to the development of the fibrous layer of the anthers, the level of degeneration of microspores and pollen in microsporogenesis and gametogenesis, and the morphological features of the anther development at the stages of the beginning and active flowering of the seed plants.

Clonal micropropagation for the preservation of breeding-valuable genotypes in the backcrossing. Control annual plants with a high percentage of sterile pollen and abnormally developed fibrous layer of anther was introduced into *in vitro* culture. As primary explants for clonal micropropagation, generative shoots of seed plants were used. For micropropagation, a modified medium (Ильенко, 1983) supplemented with the composition of macro- and micro-salts according to Gamborg et al. (1968) with the addition of sucrose 30,000 mg L^{-1}, BAP 0.2–0.5 mg L^{-1}, and gibberellin 0.1–0.2 mg L^{-1} was used. The breeding lines BC_2 Nos. 3, 4, 5, 7, and 9 of the two-year growth cycle (determined by the phenotypic traits of the CMS), obtained from clones and replicated under artificial nutrient media conditions were transferred to a hormone-free medium for 14 days and to a medium supplemented with 0.15–0.2 mg L^{-1} NAA (naphthyl acetic acid) and 0.15 mg L^{-1} IAA (indoleacetic acid).

Obtaining new plasm types with sterile cytoplasm of B. patula. Hybrid F_1 seeds were obtained in the pot experiment from the rooted clones of annual control plants selected by cytological traits according to the following genetic model: F_1♀ *B. patula S BBRr MM* × ♂ *B. vulgaris N xxzz bb rr mm*. Backcross was performed with sterility maintainers *B. vulgaris N xxzzmm* (No. 667) with recessive homozygotes for the genes *rr* and *bb* (one-year growth cycle and anthocyanin colour).

Selection of recessive homozygous lines by alleles (rr) and two-year growth cycle (bb) with B. patula cytoplasm. The seeds of backcross offspring BC_1 were obtained from interspecies hybrids by the following test crossing scheme: BC_1♀F_1CS *B. patula Xxzz Rr Bb Mm* × ♂ *B. vulgaris N xxzz rrbbmm* (No. 667). The next cycle of backcrosses was performed with sugar beet sterility maintainers with the red colour of the hypocotyl *R + r-* by the following scheme: BC_2S♀BC_1 *B. patula S Xxzz rrBbMm* × ♂*B. vulgaris N xxzz Rrbbmm* (No. 667). To obtain the offspring of seed plants BC_3 resistant to lodging, the crosses were made using a sterility maintainer with the dominant allele *R* of anthocyanin colour by the genetic model: BC_3 ♀ BC_2 *S B. patula Mm rrbb Xxzz* × ♂ *B. vulgaris N xxzzmm Rrbb* (No. 667). The roots were selected for their shape and high sugar content. They were used to obtain experimental hybrids and to multiplicate the seeds for the subsequent backcross cycles.

Variability in the root shape, one- and two-year growth cycles of the offspring with sterile cytoplasm of the wild species of B. patula. In the case of interspecies hybrids containing *B. patula* cytoplasm, only the backcross offspring of the 2nd cycle had two- and one-year growth cycles, while monogermity and the two-year growth cycle were restored using new sterile *B. patula* cytoplasm only in the seed plants BC_4. The variability in root shape of interspecies hybrids BC_2S *B. patula*, BC_3S *B. patula*, and BC_4S *B. patula* after selection for recessive traits of anthocyanin colour and a two-year growth cycle is shown in Figure 2.

Productivity of the experimental triploid hybrids of sugar beet with new sources of B. patula sterility. The best alloplasmic introgressive lines were used for hybridisation with tetraploid multi-seed pollinators. Triploid hybrids were obtained at the Yaltushkiv Experimental Breeding Station according to the hybridisation scheme using tetraploid multi-seed pollinator *Ya/DA 4x* and at the Veselyi Podil Experimental Breeding Station using pollinators *7009 BT 4x* ♀ BC_4S *B. patula xxzz mm* × ♂ *Ya/DA 4x Mm* and ♀ BC_2S *B. patula xxzz mm* × ♂ *7009BT 4x Mm*. The obtained seeds of triploid hybrids were characterised by a high germination rate and a 99–100% stable genome *3x*. Three experimental breeding lines of triploid hybrids obtained in the crosses with a tetraploid pollinator *Ya/DA* were examined (Ivanina et al., 2018). The seeds were sown on April 28, and the sugar content was evaluated on September 15. The duration of vegetation was 4.5 months. The data

a – roots of the two-year growth cycle BC_2S *B. patula rr bb*; b – variability in the shape of roots BC_3S *B. patula* in comparison with BC_2S *B. patula rr bb*; c – roots of BC_3S *B. patula* with a sugar content of up to 22.5%; d – selection by the shape of roots in the alloplasmic line BC_4S *B. patula rr bb* for the use in the breeding

Figure 2. Roots of hybrids BC_2S *B. patula*, BC_3S *B. patula*, and BC_4S *B. patula*

obtained during the experiment included root weight, leaf weight, and sugar content.

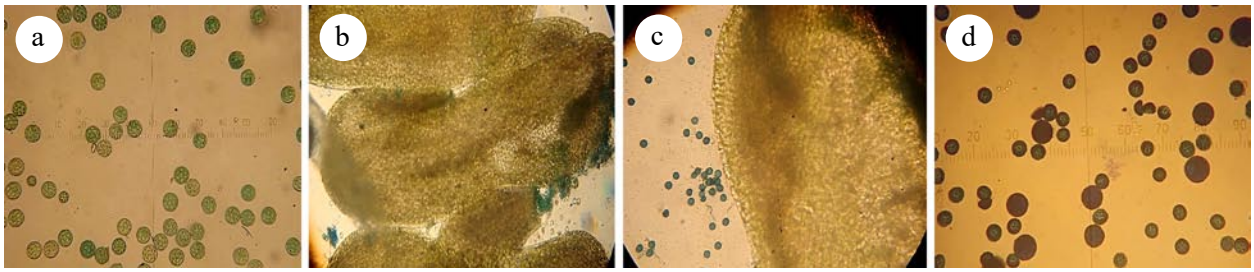
Sugar beet roots quality. The sugar content in the roots was determined by the cold extraction method (Ковальчук и др., 2010). The dry matter of roots was determined by weighting the sample before and after complete drying (Ковальчук и др., 2010). Ash content was determined as described by Ковальчук и др. (2010).

Statistical analysis of the sterility nature data of backcross offspring according to the variability of the CMS-0 and CMS-1 types was performed by the χ^2 method (Присяжнюк та ін., 2016).

Results

Among the studied biotypes, only one plant of *B. patula* was identified with the mosaic pattern of anthers, i.e., the anthers with fertile and sterile pollen grains in one flower (Figure 3).

Annual seed plants of interspecies hybrids F_1 were grown in pots and had various anomalies in development. Seed plants without flowers, pollen-sterile plants with many thin stems, multi-flower (5 flowers) clusters and sterile pollen grains with a developed exine were selected for use in further backcross cycles. The obtained seeds of B_1CS *B. patula* hybrids were sown the following year. From them, only annual multi-seed



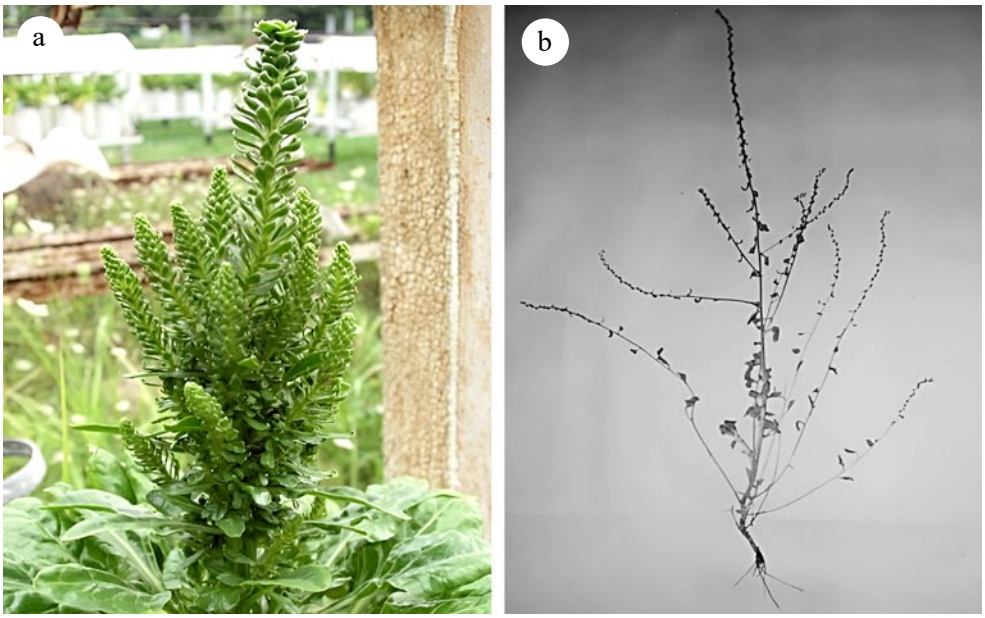
a – sterile pollen grains with a developed exine in *B. patula* seed plants; b – sterile pollen grains in the CMS donor *B. patula*; c – sterile pollen and degeneration of pollen grains at the single nucleus stage of gametogenesis; d – sterile and fertile pollen grains of the sterile cytoplasm donor *B. patula* No. 5 (magnification 12 × 40)

Figure 3. Development of male gametophyte in *Beta patula*

plants with both red and green colour of the hypocotyl were obtained. Only morphological mutants BC_1Rr with cup-shaped leaves and without flowers were obtained (Figure 4a). From the experimental genotypes with the green colour of the hypocotyl *rr*, only recombinant single-stem annual seed plants *Bb* with three-flower pollen sterile CMS-1 and CMS-0 type clusters were selected (Figure 4a, b).

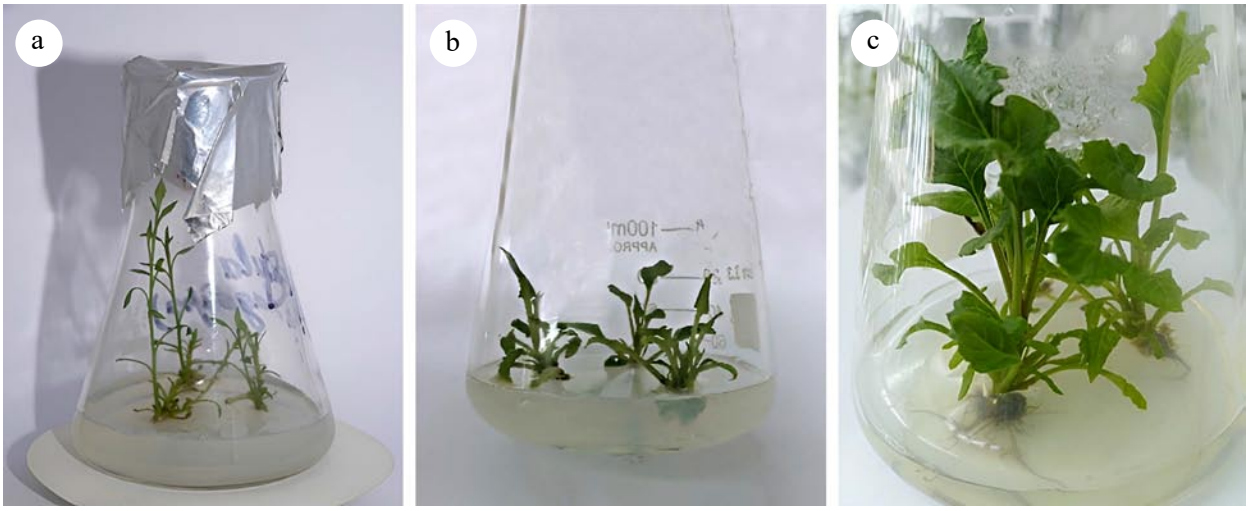
The stages of clonal micropropagation of the breeding-valuable genotype *B. patula* No. 5 are shown in Figure 5.

The seeds of BC_2S *B. patula* obtained from plants grown in the pots had a 14-day delay in germination compared to *B. vulgaris* seeds. From the seed plants of the 2nd backcross cycle, recombinant genotypes with the red colour of the hypocotyl *Rr*, good branching, one-year growth cycle *bb*, monogermity *mm* and 100% separate flowers were selected (Figure 6a). Seed plants BC_2S *rrbbMm* had branched stems and recessive hypocotyl colour and were chimeric for fertility (1 to 3 germs per cluster) and mosaic for anther sterility (CMS-0 and CMS-1 types) (Figure 6b).



a – recombinant genotype with the absence of generative shoots BC_1S *B. patula rrBb*; b – annual seed plant BC_1S *B. patula rrBbMmXxzz* with three-flower CMS-1 and CMS-0 type clusters

Figure 4. Morphological traits of seed plants of the BC_1S *B. patula* interspecies hybrids



a – in sterile culture; b – clonal micropropagation of BC_2S *B. patula rrbb Mm*; c – rooting of valuable genotypes BC_2S *B. patula* for the use in hybridisation and genetic analysis

Figure 5. *Beta patula* and backcross offspring BC_2S *B. patula*, deposited and rooted in artificial nutrient media

Though hybrids obtained using *B. patula* species of the *Vulgaris* section set seeds, they are characterised by uneven flowering, one-year growth cycle, and branched and woody roots (Chesnokov et al., 2013). The genetic model of the test crosses and selection by the recessive linked traits of sugar beet sterility maintainers *rb* showed recombinant events within the loci of the hypocotyl coloration, one- and two-year growth cycle, monogermity and sterility in the hybridisation with the wild species of *B. patula*. The expression of CMS against the background of homozygosity of recessive genes *rr* and genes of nuclear control of CMS of sterility maintainers of *B. vulgaris Nxxzz* differed in the ratio of phenotypes of the CMS-0 and CMS-1 types (Table 1).

The data in Table 1 show that the percentage of CMS-0 type containing *B. patula* germplasm in the offspring of BC_2S *B. patula* hybrids varied from 34.1% to 46.3% (sampling was from 34 to 56 seed plants). In the 2nd and 5th sampling, the value of χ^2 was 0.32 and 0.96, respectively. The value of the probability showed agreement between experimental and theoretically expected data ($0.05 \geq P \geq 0.01$ and $0.8 \geq P \geq 0.5$, respectively). Two variants indicated the correspondence of the expected and obtained frequencies of the phenotype splitting into the CMS-0 and CMS-1 types. Analysis of the offspring of seed plants BC_2S *B. patula rrbb* from the seed plants obtained from clones BC_2 No. 3, 5, and 7 for three years showed that the results did not correspond to the theoretically expected splitting: $\chi^2 = 5.12$, 2.28, and 7.68.

a – BC_2S *B. patula RrBbmm* of the one-year growth cycle, 100% monogermity and sterility of the CMS-0 type; b – seed plant BC_2S *B. patula rrbbMm* with branching stems of the CMS-1 type

Figure 6. Recombinant biotype BC_2S *B. patula* of one- and two-year growth cycles

Table 1. The ratio of phenotypic and genotypic types of CMS in the offspring of recessive homozygotes obtained in crosses ♀BC_2S *B. patula Xxzz rrbbMm* × ♂ *B. vulgaris Nxxzz Rrbbmm*

Origin	Initial phenotypes, generation	Offspring	Year	Plant distribution by phenotype			χ^2	*P*
				CMS-0	CMS-1	Σ		
BC_2S *B. patula Xxzz rrbbMm*	CMS-1 BC_2	BC_2 No. 3	2014	17 (34.0%)	33 (66.0%)	50	5.12	$0.05 \geq P \geq 0.01$
		BC_2 No. 4	2015	25 (46.3%)	29 (53.7%)	54	0.32	$0.64 \geq P \geq 0.5$
		BC_2 No. 7	2015	20 (35.7%)	36 (64.3%)	56	2.28	$0.2 \geq P \geq 0.05$
		BC_2 No. 5	2016	15 (34.1%)	29 (65.9%)	44	7.68	$P \leq 0.2$
		BC_2 No. 9	2016	13 (38.2%)	21 (61.8%)	34	0.96	$0.8 \geq P \geq 0.5$
B. vulgaris S xxzz Rrbbmm[1] CMS0302	CMS-0	F_1	2016	30 (90.9%)	3 (9.1%)	33		

Note. [1] – analogue of the sterility maintainer with the Owen sterile cytoplasm of sugar beet; *P* – probability of obtained and theoretically expected CMS-0 and CMS-1 types splitting by phenotypic groups 1:1.

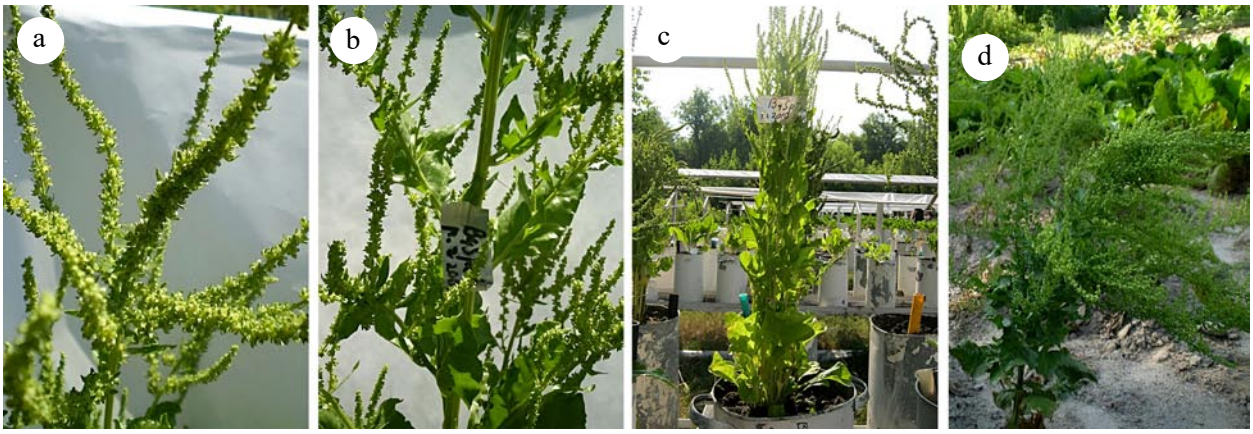
Two-year-old roots *bb* and recessive homozygotes by hypocotyl colour *rr* were selected from the offspring of the 2nd backcross cycle. The roots of BC_2S *B. patula* were thin, and their length reached 30 cm. A total of 310 plants were tested. The obtained splitting by phenotype corresponded to the theoretically expected 1:1 ratio. The hypocotyl colour genetic marker was inherited in BC_3S *B. patula* hybrids as dominant/recessive and served as an indicator of the positive effect of replacing the sterility maintainer genome of sugar beet with the sterile cytoplasm of *B. patula* (Table 2).

Table 2. Splitting in hybrid population BC_3S *B. patula* by the presence of hypocotyl colour alleles

	R+	*r*−	Total
Experimental (*p*)	158	152	310
Expected (*g*)	155	155	310
Deviation $d = g - p$	−3.0	0.3	0
d^2	9	9	
d^2 / g	0.058	0.058	$\chi^2 = 0.116$

According to the data given in Table 2, splitting by the presence/absence of the dominant allele of the hypocotyl colour *R*+ in BC_3S *B. patula* corresponded to the theoretically expected ratio 1:1: $\chi^2 = 0.116$ at $0.99 > P > 0.95$. The red colour of the hypocotyl corresponded to the presence of the *R*+ allele in the genotype of the backcross hybrids of the 3rd backcross cycle, while the absence of red colour indicated recessive homozygotes *rr* according to the genetic model of the test crosses. Among BC_3S *B. patula* roots with the *R*+ allele, three forms of seed plants were selected. The seed plants derived from roots with the dominant *R* allele had good branching, branched stems and multigerm seeds. They were classified as the CMS-1 and CMS-2 types. Multi-flower seed plants with branching stems and the CMS-0 type were also selected.

Among the seed plants with only recessive alleles of the colour of the hypocotyl *rr*, two forms were obtained: with monogerm seeds (Figure 7a) and single-stem plants with thin flower-bearing shoots, monogerm (100%) and sterile seeds (100%) (Figure 7b, c, and d).



a – annual seed plants of the CMS-0 type with one- and two-seed fruits *Rr*; b – two-year single-stem seed plants *Rr*; c – the shape of the seed plant BC_3S *B. patula rr* with 100% monogermity and 100% sterility; d – seed plant BC_3S *B. patula rr* of the CMS-0 type with branching stems and monogerm fruits

Figure 7. Different forms of seed plants of BC_3S *B. patula* hybrids with the red and green colour of the hypocotyl

The best breeding genotypes of the new BC_4S *B. patula* plasmotypes were evaluated for monogermity, sterility, and root shape. The seeds *of* BC_5S *B. patula* were obtained in crosses with sterility maintainers of *B. vulgaris Nxxzz* (No. 667). The analysis was performed based on phenotypic traits of the nuclear sterility genes in new alloplasmic lines. As shown in Table 3, high and stable sterility rates were obtained in monogerm pollen-sterile lines with the cytoplasm of *B. patula*.

The breeding value of sterile cytoplasm from wild species of the genus *Beta* L. as a possible alternative to the new source of sterility by Owen (1945) is currently unknown. Sugar accumulation in selected breeding genotypes in early September and mid-October is shown in Table 4.

According to the data given in Table 4, a high sugar content from 19.5% to 21.5% was determined in all variants of the experiment at the 0.5 mineral fertilisation

Table 3. Analysis of the components of backcross to produce seeds of pollen sterile lines with the sterile cytoplasm of *Beta patula*

Group insulator No.	Components of the cross[1]	Number of planted roots	Number of plants	of them	
				monogerm *mm*	fertile *xxzz*
1	*B. vulgaris* L. *Nxxzz* 5–25	10	15	7	15
2	BC_4S *B. patula* No. 2	10	3	3	–
	B. vulgaris Nxxzz r1	20	13	7	13
3	BC_4S *B. patula*, r 40–60	10	6	5	–
	B. vulgaris Nxxzz r1	20	12	8	12
4	BC_4S *B. patula*, r 63–83	10	7	7	–
	B. vulgaris Nxxzz r1	20	12	8	12
5	BC_4S *B. patula*, r 88–108	10	9	8	–
	B. vulgaris Nxxzz r2	20	13	9	13
6	BC_4S *B. patula*, r 111–131	10	5	4	–
	B. vulgaris Nxxzz r2	20	18	14	18
	BC_4S *B. patula*, r 142–162	10	10	7	–

Note. [1] – further backcross cycle was carried out according to the scheme: ♀BC_4S *B. patula xxzzmm* × ♂*B. vulgaris Nxxzzmm*.

Table 4. Productivity of experimental triploid hybrids with the sterile cytoplasm of *Beta patula*

Origin of experimental hybrids, 3x	Growing season	Root weight g	Ash content %	Dry matter content %		Sugar content %
				leaves	roots	
BC_4S B. patula × 2 × *Ya/DA 4x*, 1/1	2804–1010	380	0.528	19.4	26.8	19.7
1/2	2804–1509	325	0.382	21.2	29.4	20.6
1/3	2804–1010	465	0.401	21.0	28.9	21.5
1/4	2804–1010	415	0.377	19.8	26.5	19.5
1/5	2804–1010	450	0.328	19.8	29.1	20.9
1/6	2804–1010	440	0.362	22.4	29.5	20.7
1/7	2804–1010	410	0.401	21.4	28.5	21.2
BC_4S B. patula r1 × 7009 *BT 4x*, 2/1	2804–1010	500	0.401	21.2	30.2	21.0
2/2	2804–1509	425	0.377	16.6	25.3	20.1
2/3	2804–1010	380	0.391	18.0	27.7	20.2
2/4	2804–1010	355	0.401	20.6	28.7	20.0
2/5	2804–1010	440	0.381	20.8	28.9	21.5
2/6	2804–1010	430	0.386	22.0	29.0	20.5
2/7	2804–1010	480	0.377	17.4	28.9	20.5
3/7	2804–1010	430	0.416	21.4	28.1	20.5
	$LSD_{0.05}$	23	0.012	1.3	1.5	0.2

rates both in September and at the end of the vegetation period in October. The content of dry matter in some roots reached 30.2%. The content of conductometric ash varied from 0.328% to 0.528%. The root weight of the experimental triploid hybrids obtained in the process of hybridisation of alloplasmic lines with the sterile cytoplasm of *B. patula* with tetraploid multigerm pollinators *Ya/DA 4x*, 1/1, and *7009 BT 4x*, 2/1 during the vegetation period (4.5 months) varied from 325 to 500 g. The best experimental hybrids significantly differed in productivity.

Discussion

New sources of CMS in sugar beet were first reported by Oldemeyer (1957) and then later by Bosemark (2006); however, to date, no new germplasm has been used in the sugar beet breeding. Nowadays, a problem of cytological homogeneity of sugar beet hybrids arises. New sources of CMS in sugar beet were identified using a genetic model of crossbreeding based on marker genes for one- and two-year growth cycles, hypocotyl colour and monogermity, and monogerm pollen-sterile lines of the two-year growth cycle were selected from F2 hybrids (Ковальчук, 2013). In the populations obtained by crossing selected *B. maritima* lines from different geographical areas with the DNA technologies, a new germplasm from wild species of *B. maritima* was classified as E, G, and H based on mt-DNA nucleotide models and fertility maintenance characteristics (Moritani et al., 2013; Touzet et al., 2018). To introduce alloplasmic lines with the new germplasm from wild species of *Beta* L. into the breeding process, long-term field testing of sterile forms is needed for the traits of one- and two-year growth cycles, fertility, sterility, and productivity.

Great attention is paid to the research of genetic information localised in the organelles and mitochondria. The significance of cytoplasmic genes and their localisation in chloroplasts, which are related to the herbicide tolerance mechanism and the yield potential are investigated (McGrath et al., 2015; Richardson et al., 2019; Stognienko, Stognienko, 2019). The methods of somatic hybridisation were used to induce new plasmotypes of crops. Gamma irradiation was applied to combine protoplasts and inactivate the nucleus in donor plants, and chemical mutagens are used to induce mutations in the cytoplasmic DNA of chloroplasts (McGrath et al., 2015; Iefimenko et al., 2018).

To enrich the adaptive potential of sugar beet, interspecies hybridisation studies are focused on the isolation of new sources of cytoplasmic sterility from the wild species of *Beta maritima* L. and obtaining alloplasmic lines at the Institute of Bioenergy Crops and Sugar Beet (Ковальчук, 2013). The cytological traits of pollen degeneration in CMS phenotypes were studied by Зайковская (1968). In the population of beet plants, N- and S-cytoplasms interact and the response of the male gametophyte depends on mitochondrial DNA heteroplasm (Heng et al., 2014). Using indirect cytological traits of CMS, pollen sterility, mosaic development of the anthers, the peculiarity of the anther fibrous layer, degeneration of pollen grains at the single-nucleus gametogenesis stage, a control genotype − a donor of a new sterile cytoplasm – was isolated from the wild species of *B. patula* and multiplied *in vitro*.

As a result of test crosses and selection by recessive genes of sugar beet sterility maintainers, two-year *bb* biotypes were selected only from breeding genotypes of the 2nd backcross cycle of *BC_2S B. patula bb rr,* which indicates the presence of recombinant events in the structure of loci of linked genes. Only monogerm phenotypes were selected.

The indicators of monogermity of *BC_4S B. patula* given in Table 3 required additional rejection of multigerm seed plants. This indicates a more complex structure of the fertility locus of studied wild beets of the

genus *Beta* L. and a special nuclear genome expression of sterility maintainers with a new sterile cytoplasm. From 30 seed plants (control plants of *B. vulgaris S xxzz*, breeding line No. 677), only three plants of the CMS-1 type were selected at flowering, which can be explained by the stability of the expression of CMS phenotypes and epigenomic variability of traits determined by the genetic information of the cytoplasm (Maletskii et al., 2015; Levites, Kirikovich, 2017).

The clonal micropropagation and rooting *in vitro* methods were used to deposit selected control genotypes BC_2S *B. patula Xxzz rrbbMm* of a two-year growth cycle. New breeding genotypes were selected, and their productivity was tested at the level of experimental triploid hybrids obtained according to the scheme ♀BC_4S *B. patula* × 2 × ♂*Ya/DA 4x*, 1/1. Our findings were in accordance with the studies of French researchers Doney (1998) and Bosemark (2006), who also reported the probability of expression of additional sugar alleles under the conditions of newly introduced cytoplasm that ensure the extension of the growing season.

It can be concluded that obtained new genotypes can be used for the breeding of energy beets hybrids, which are used as a raw material for the bioethanol production.

Conclusions

1. Alloplasmically introduced lines carrying sterile wild beet (*Beta patula* L.) plasma were obtained during the study. Expression of nuclear genes in the BC_4S *B. patula* offspring was characterised by high sterility of the CMS-0 type. However, the indicators of monogermity rates indicated the need for additional rejection of multigerm seed plants.

2. The sugar content of alloplasmic lines with the new CMS (at the level of experimental triploid hybrids) was 19.5% and 20.5%; therefore, these lines could be recommended for the energy beet breeding.

Received 30 05 2023
Accepted 21 08 2023

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