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Features of embryonic development in sugar beet under apospory and cytoplasmic male sterility

Abstract. The aim of the study was to evaluate the theoretical foundations of the phenomenon of high seed self-reproduction in substituted lines and to develop a methodology for studying embryonic development in sugar beet breeding materials with cytoplasmic male sterility (CMS) of different genetic origins. Experimental investigations included field methods, pollen-free seed production in CMS breeding materials, laboratory methods, cytological analysis of embryo

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development during apozygoty over 34 days after flowering fixation, and a cytophotometric method for assessing genome-level ploidy variability in materials with different terms of apozygotic seed reproduction. The methodology for studying embryonic development during apozygoty in materials of different CMS source origins was improved, incorporating flowering marking, shoot isolation, and embryo fixation from 5 to 34 days until complete embryo development. It was established that apozygoty in sugar beet is characterised by delayed genetically determined development of 4, 8, or 10 days within the 28-day developmental cycle, depending on the origin of the material, as well as by polyembryony and a high percentage of degenerated embryos. Adventitious embryogenesis, apospory, and simultaneous embryo development from integuments and nucellus in both micropylar and chalazal regions were characteristic of male-sterile lines of different genetic origins. It was found that substituted lines based on new CMS sources derived from wild species of the genus *Beta* L. exhibit the phenomenon of high seed self-reproduction under pollen-free conditions. The methodology for studying embryo development synchrony was developed to investigate the nature of apozygoty (generative and somatic embryogenesis) in substituted lines with introduced sterile cytoplasms, compared with monoecious male-sterile lines carrying the F. Owen S-vulgaris cytoplasm. The type of apozygoty manifested through gametophytic and sporophytic embryogenesis and determined the genetic heterogeneity (variability in quality) of apozygotic seeds. Apomixis using substituted lines and new sterile cytoplasms derived from wild species of the genus *Beta* L., combined with a high percentage of apomictic seed reproduction, may provide significant advantages for the production of new crop hybrids, particularly through the fixation of heterosis and preservation of the maternal plant genotype

Keywords: adventitious embryogenesis; apospory; new sources of cytoplasmic male sterility; *Beta maritima*; *Beta patula*; cytoplasmic male sterility phenotypes

INTRODUCTION

Apomixis is a process of asexual plant reproduction that yields seeds in the absence of meiosis and fertilisation. Since apomictic plants can produce clonal offspring, preserving the genotype of the maternal plant through seeds, this process offers numerous agronomic advantages for crop production, namely the fixation of heterosis, the introduction of new germplasm from wild species into the breeding process, the simplification of hybrid seed production, and positive variation in vegetatively propagated varieties. In sugar beet breeding, the application of apomixis using substituted lines with new sources of sterile cytoplasms from wild species of the genus *Beta* L., *Beta maritima* and *Beta patula*, consolidates the positive characteristics of the maternal component controlled by the cytoplasmic genome, such as disease resistance, early carbohydrate accumulation, high adaptive potential for the development of stubble-free seed production, the creation of energy hybrids with a high monosaccharide content, and the simplification of sterility maintainer breeding.

F. Krahulec *et al.* (2020) indicated the existence of various modes of seed reproduction as intra-population polymorphism of reproductive traits. Y. Xu *et al.* (2022) note that apomixis can duplicate genotypes corresponding to the maternal plant, and that it has important applications for genotype fixation and acceleration of the breeding process. D. Li *et al.* (2021) concluded that apomixis occurs in more than 400 plant species but remains insufficiently studied among major agricultural crops. However, A. Schmidt (2020) indicates that achieving practical application of apomixis in breeding requires in-depth theoretical investigation of the epigenetic regulation of genes using methods for analysing the genetic basis of their expression. P. van Dijk *et al.* (2020) investigated the dominance of the parthenogenesis allele in dandelions and the complex nature of autonomous perisperm formation. Based on experimental results, K. Wang *et al.* (2021) concluded that the developmental characteristics of embryos during apozygoty remain insufficiently

studied, as in some cases they may clone the genotype, or have a recombinant basis and develop from embryo sac cells. Based on the research of M. Roik *et al.* (2024), high economic interest and prospects for profit are already determining competition among the world's leading agricultural corporations (Syngenta, Pioneer Hi-Bred International, Rijk Zwaan Zaadteelt B.V., Partec GmbH, and others). Embryological investigations play a primary role in establishing the nature of embryos – specifically gametophytic reduced and unreduced parthenogenesis – but require the refinement of methodologies for studying the synchrony of apomictic and hybrid embryo development using genetic selective markers. Using embryological investigations, the authors established that, depending on the mode of embryo sac formation, the nature of apozygotic embryo development is determined by the type of apomixis: diplospory, apospory, adventitious embryogenesis, and parthenogenesis. In diplospory, the embryo sac develops from an unreduced megasporocyte. In some cases, meiosis may be replaced by mitosis or the second division may be absent. Cytoembryological investigations showed that in aposporous embryo sacs of this type, parthenogenetic embryos form and develop to the globular stage, after which further development does not occur due to the absence of endosperm.

D. Li *et al.* (2021) and K. Wang *et al.* (2021) demonstrated that haploid parthenogenesis occurs in reduced embryo sacs whose cells were formed as a result of meiotic division. They showed that haploid embryos, if diploidisation does not occur, are not viable; their proportion amounts to only 1-2%, and this phenomenon has been described in a range of species (*Datura*, *Nicotiana*, *Triticum*, *Oryza*, *Hordeum*, *Brassica*, *Crepis*, *Solanum*, *Zea*, *Gossypium*, and others). The proportion of haploid parthenogenesis and haploid embryos in certain species, such as the alloplasmic line of *Triticum aestivum*, can reach 90%. It has been established that seeds of sugar beet breeding materials formed without pollination, by virtue of polyembryony, are characterised by genetic heterogeneity and the presence of two or three seedlings in certain schizocarpic fruits. B. Heidemann *et al.* (2025) concluded that low seed productivity is characteristic of many other cultivated plant species, including sugar beet, regardless of the type of apozygoty, and represents one of the unresolved challenges for the broad application of this new mode of seed reproduction in breeding. Y. Xu *et al.* (2022) note that in most species, nucellar and integumental embryogenesis is combined with generative parthenogenesis and with embryos that penetrate the embryo sac from outside. The simultaneous presence of a haploid embryo derived from reduced cells and an adventitious embryo derived from somatic cells is entirely possible. Among their principal differences are: differing ploidy levels of embryo sac cells (aposporous and meiotic); differences in embryo genome structure (with the maternal genome, as in adventitious embryogenesis, or recombinant, derived from reduced and unreduced embryo sac cells). The majority of studies on apozygoty in sugar beet are ambiguous and contradictory, and require additional experimental data using methods of genetics, embryology, and cytogenetics.

The aim of this article was to investigate the characteristics of embryonic development of embryos under pollen-free conditions in sugar beets with different sources of sterility derived from wild species of the genus *Beta* L., *Beta maritima*, and *Beta patula*, and to refine the methodology for analysing the synchrony of embryogenesis stages in maternal components with sterile cytoplasms of different genetic origins, with a view to identifying breeding accessions with a high rate of apozygotic seed set.

MATERIALS AND METHODS

The investigations were conducted during the period 2016-2023. Experimental material for embryological studies was selected on the basis of phenotypic traits: monoecism, sterility under apozygoty, and genome ploidy level. The following source material was used: the best apozygotic progenies A_4 of sugar beet for embryogenesis analysis and characterisation of apozygoty types – 12-136MS, 12-157MS, 12-222MS, 12-138MS, 12-153MS; substituted sugar beet lines based on sterile cytoplasms of wild species *Beta maritima* and *Beta patula* with apozygotic seed reproduction and high seed

productivity, comprising the following breeding accessions: No. 15/1BC_2S (Greece), No. 15/2BC_2S (Greece), No. 15/3BC_2S (Greece), No. 16/1BC_2S (Greece), No. 16/2BC_2S (Greece), No. 16/3BC_2S (Greece), No. 3/1 dihaploid, F_1S clones (Turkey) *in vitro*, No. 3/2 dihaploid, F_1S clones (Turkey) *in vitro*, No. 1/17BC_3S *patula*, No. 2/7BC_3S *patula*; substituted lines of the third, fourth, fifth, and sixth backcross cycles were studied under pollen-free conditions in comparison with the development of hybrid embryos of *Beta vulgaris* cmS 0-type; breeding accessions: BC_6S maritima (Turkey) – c.2/1, c.4/1, c.5/1; BC_6S *patula* (Portugal, Madeira Island) – c.1/4, c.3/4, c.4/4, c.3/7; BC_6S *maritima* (Turkey) – c.5/1, c.7/2, c.6/3, c.7/5, for studying the seed productivity of new plasmotypes.

The characteristics of apozygotic seed reproduction in substituted lines were determined using a modified methodology for evaluating embryogenesis in sugar beet, adjusted with respect to flowering timing (Kovalchuk *et al.*, 2019). Selection based on sterility and monoecism traits was carried out in group isolators and in the plant-breeding glasshouse complex at Yaltushkivska Research Breeding Station, in accordance with the classification of F. Owen (1945). Monoecism was determined by characterising fruits on central shoots. Microscopic examination of preparations was performed using Carl Zeiss (Germany) and Polam (Poland) microscopes at a magnification of 5 × 12.5, and MBS-11 stereomicroscopes. The ploidy level of experimental apomictic materials was stabilised using the flow cytophotometry method and a Partec ploidy analyser.

The efficacy of embryo staining on embryological preparations was assessed on days 16 and 28 after flowering marking. Stains applied included: 0.03% methylene blue, iodine solution, and bromophenol orange. Seed set under pollen-free conditions in substituted lines was studied over a 10 cm segment in 5 replications per seed-bearing plant. Breeding accessions were evaluated in relation to one another by origin. Data were processed according to statistical analysis methods (Prysiazhniuk *et al.*, 2016). For each seed-bearing plant, the mean value of embryo development per 10 cm segment was determined using formula (1):

$$X = \frac{\sum \bar{M} - x}{n}, \quad (1)$$

where X is the mean number of developed seeds per 10 cm segment under apozygoty; x is the number of seeds per 10 cm segment for each breeding accession; $\bar{M}$ is the mean number of developed seeds for each seed-bearing plant across 5 replications per 10 cm segment; n is the total number of seeds from each seed-bearing plant across all 5 replications.

The standard error of the arithmetic mean is determined by formula (2):

$$m = \frac{\delta}{\sqrt{n}}, \quad (2)$$

where ð is the standard deviation of the arithmetic mean for each measurement variant, namely the number of fruits per 10 cm segment; *n* is the total number of fruits for each breeding accession (seed-bearing plant).

The investigations were conducted in accordance with the ethical standards of the Convention on Biological Diversity (1992) and the Convention on the Trade in Endangered Species of Wild Fauna and Flora (1973).

RESULTS AND DISCUSSION

To refine the methodology for differentiating early stages of embryogenesis during apozygoty, fixation of ovules in Carnoy's solution (3:1) was applied, along with various maceration and staining techniques for studying embryo sacs. Embryo development in sugar beet is genetically determined (Kovalchuk *et al.*, 2019). The characteristics of embryonic development of apomictic embryos in male-sterile lines of sugar beet at Yaltushkivska Research Breeding Station were studied. Thus, in apomictic embryos of the second cycle of apomictic reproduction (A_2), degeneration on day 28 after fixation occurred in 16.3% of cases. By day 28, a normal hybrid embryo should have been fully developed. Such development was observed in only 3.3% of ovules. Instead, ovules with embryo development at the 1/2 ES stage accounted for 23.3%, and at the 1/4 ES stage for 24.3%. This indicates a developmental delay of 8-10 days. The phenomenon of polyembryony – the development of two embryos – was also observed; the proportion of such plants was high and amounted to 32.7% (Fig. 1).

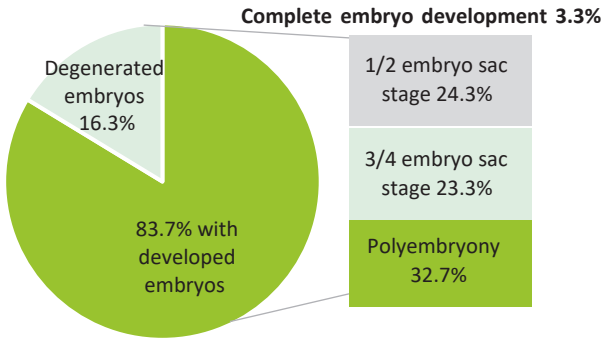

Figure 1. Embryogenesis of embryos from apozygotic progenies, day 28, A_2
Note: A_2 – apomictic pollen-free development of the second reproduction cycle
Source: compiled by the authors

The results of apomictic embryo development in the best A_4 progenies of sugar beet obtained under pollen-free conditions are presented in Table 1.

Table 1. Analysis of embryogenesis and characterisation of apozygoty types in the best apozygotic progenies A_4 of sugar beet

Breeding accession	Embryos examined, n	Unfertilised, %	Number of embryos with developmental delay, %	Anomalous embryo position, %		Type of apozygoty
				From nucellus	From integuments	
12-136MS	67	4.5	55.2	40.3	–	adventitious embryogenesis
12-157MS	75	6.7	66.7	26.6	–	adventitious embryogenesis
12-222MS	80	12.5	43.8	–	43.7	apospory
12-4652-12-12-2-4MS c.52	69	11.6	40.4	–	48.0	apospory
12-138MS	70	8.6	48.4	43.0	–	adventitious embryogenesis
12-153MS	72	12.5	45.8	41.7	–	adventitious embryogenesis

Note: A_4 – apozygotic progeny of the fourth reproduction cycle
Source: compiled by the authors

According to Table 1, the materials under study were characterised by a high proportion of embryos with anomalous positioning (up to 48.0%). The anomalies observed were represented by embryos of integumental (26.6% to 43.0%) and nucellar (up to 48.0%) origin. The number of underdeveloped embryos varied between accessions in this group from 40.4% to 66.7%. Unfertilised embryos ranged from 4.5% to 12.5%. Embryos with polyembryony were not observed in A_4, which may be associated with the stabilisation of monoecism over four cycles of reproduction. Optimisation of the embryogenesis methodology under apozygoty was carried out using substituted lines with a high apozygotic seed set rate, with new cmS sources derived from the wild species *Beta maritima* and *Beta patula* L., propagated as clones under an in vitro system. Ovules were fixed on day 10. Using the modified methodology, embryo sacs were studied from day 10 to day 16, and cytological investigations of embryo development in the micropylar, chalazal, and central regions, as well as perisperm development, were conducted from day 16 to day 38 after flowering marking. Data are presented in Table 2.

Table 2. Indicators of embryonic development of apomictic embryos under pollen-free conditions on day 16 after flowering marking in backcross progenies with new sterile cytoplasms from wild species *Beta maritima* and *Beta patula*

No.	Breeding accession	Developmental stages, fixation date, %										Number with polyembryony	
		Degenerated		club stage		sphere stage		heart stage		heart = globular			
		n	%	n	%	n	%	n	%	n	%	n	%
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**15**	**16**
1	No. 15/1B_2CS (Greece)	5	16.7	4	13.2	10	33.4	16	20	5	16.7	5	16.7
2	No. 15/2B_2CS (Greece)	7	23.3	2	6.7	7	23.3	11	36.7	3	10.0	4	13.2
3	No. 15/3B_2CS (Greece)	10	33.4	1	3.2	9	30.0	10	33.4	–	–	3	10.0
4	No. 16/1B_2CS (Greece)	6	20.0	–	–	10	33.4	10	33.4	4	13.2	5	16.7
5	No. 16/2B_2CS (Greece)	9	30.0	2	6.6	9	30.0	5	16.7	5	16.7	4	13.2
6	No. 16/3B_2CS (Greece)	5	16.7	5	16.7	10	33.4	10	33.4	–	–	3	10.0
7	No. 3/1 dihaploid, F_1S clones (Turkey) *in vitro*	4	13.2	–	–	2	6.8	24	80.0	–	–	–	–
8	No. 3/2 dihaploid, F_1S clones (Turkey) *in vitro*	4	13.2	–	–	–	–	26	86.8	–	–	6	20.0
9	No. 1/17B_3CS *patula*	4	13.2	2	6.7	14	46.7	10	33.4	–	–	12	40.0
10	No. 2/7B_3CS *patula*	2	6.7	4	13.2	19	53.4	5	16.7	–	–	10	33.4

Note: degenerated embryos as a proportion of 30 analysed per breeding accession
Source: compiled by the authors

Male-sterile seed-bearing clones obtained by in vitro deposition and rooted under pot conditions differed from one another in various anomalies of apomictic embryo development compared with hybrid embryos. Thus, for breeding accessions No. 15/1, No. 15/3, and No. 16/3 on the *Beta maritima* L. cytoplasmic background, originating from Greece, on day 16 the development of embryos at the "sphere" and "heart" stages was predominant, reaching a combined value of 53.4-60%, compared with 16.7-33.4% of degenerated embryos. For some breeding accessions, developmental delay on day 16 was considerable: embryo development at the "club" stage was observed in 16.7% of cases, and at the "sphere" stage in 33.4%. For dihaploid line No. 3 (Turkey), embryos were delayed in development by 8 days and were characterised by relatively stable values of 80% to 86.8% at the "heart" stage. The proportion of degenerated embryos differed between breeding accessions and substantially exceeded the values recorded for hybrid embryos. In certain backcross progenies on the sterile *Beta patula* cytoplasmic background, a particularly high proportion of embryos with polyembryony and developmental delay in the micropylar region compared with the chalazal and central regions were observed.

On days 10-12 from the onset of flowering, following maceration, washing, and staining, embryo sacs were obtained showing embryo development from synergids at the "club" stage, degeneration of the egg cell, and two unfused polar nuclei. Only by means of a methodology that involves shifting the embryo fixation date by 8-10 days under apozygoty is it possible to assess polyembryony and its progression throughout embryogenesis. The efficacy of embryo staining on days 16 and 28 after flowering marking is illustrated in Figure 2 (a, b, c). Based on embryological investigation results, polyembryony in the apomictic line No. 2/7 BC_3S *patula* A_1 was observed in the experimental material at a proportion of 33.4%.

Simultaneous embryo development in both the micropylar and chalazal regions is shown in Figure 2 (d). During the course of investigations, degeneration of one of the embryos was frequently observed – more often that of the adventitious one (Fig. 2 (e)). Within the embryo

sac, alongside the development of an apomictic embryo from the egg cell, an embryo from the synergids develops, lagging behind the hybrid by 8 days (Fig. 2 (f)). In line 1-16 BC_2S (Greece) A_1, alongside normal embryo development corresponding to the hybrid pattern, ovules were present with embryo development in both the micropylar and chalazal regions (Fig. 2 (g)). A developmental delay of embryos from generative cells of 8-10 days relative to the zygotic embryo is shown in Figure 2 (h). Complete development of the apomictic embryo in lines 1/16 BC_2S (Greece), corresponding to the zygotic pattern, lags by 10 days (Fig. 2 (i)).

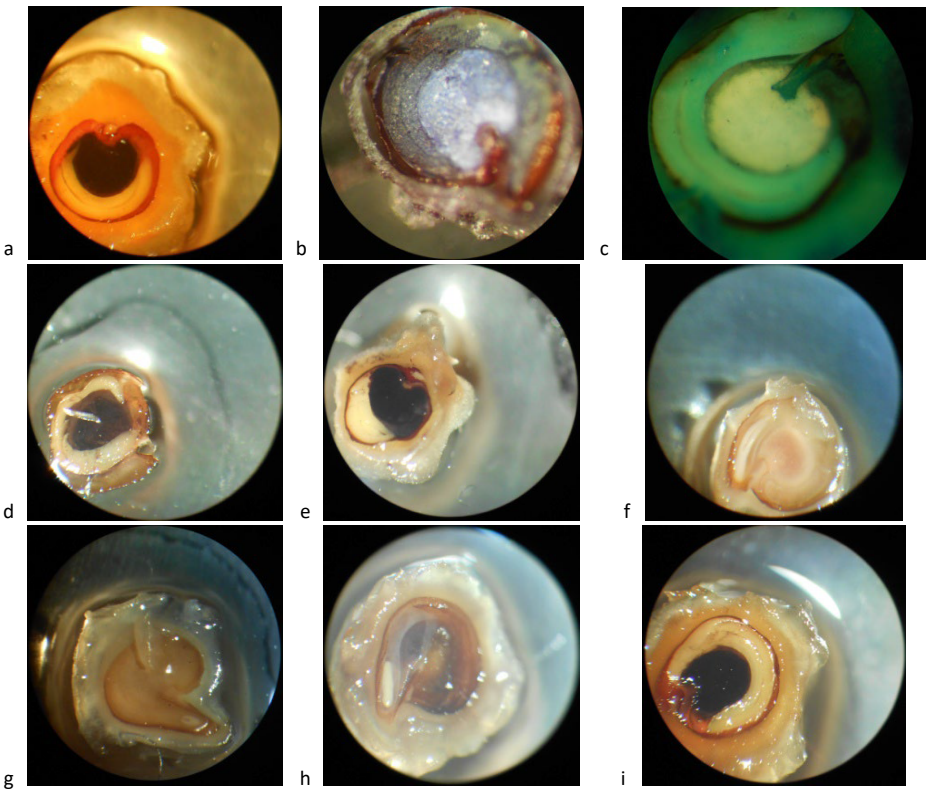

Figure 2. Study of embryonic development of apozygotic embryos using lines with high apozygotic seed self-reproduction. Magnification: 5×12.5

Note: a) embryo staining with 0.3% iodine solution; b) staining of apomictic embryos with bromophenol orange; c) response of apomictic embryos to methylene blue staining; d) development of apomictic embryos with polyembryony in line BC_3S *patula*; e) degeneration of one of the embryos in line BC_3S *patula*; f) developmental delay of embryos on days 16-20 after flowering marking; g) development of haploid embryos at the "club" stage from synergids; h) developmental delay of an embryo in line 1/15 BC_2S (Greece) A_1; i) normal embryo development in line 1/16 BC_2S (Greece) A_1, corresponding to the zygotic pattern on day 32 after flowering fixation

Source: photo by the authors

The characteristics of embryonic development during apozygoty were studied in substituted lines BC_3, BC_4, BC_5, and BC_6 under pollen-free conditions, in comparison with the development of hybrid embryos of *Beta vulgaris* cmS 0-type (Table 3). The analysis of embryo development presented in Table 3 was conducted taking into account the previously established developmental delay in sugar beet, with the fixation period extended to 10 days. Based on the results of cytological analysis, polyembryony in the majority of the lines under study was observed both on day 24 after flowering marking and on day 38, depending on the genetic origin of the breeding material.

Table 3. Embryonic development of embryos in breeding materials with apomixis of different genetic structure in substituted lines

No.	Breeding accessions	Fixation period, days	Number of seeds	Developmental stages							Number of cases with polyembryony, %
				Degenerated embryos, %	Polyembryony, %	Heart stage, %	"heart = globular" 1/4 embryo sac stage, %	1/2 embryo sac stage, %	3/4 embryo sac stage, %	Complete embryo development, %	
1	2	3	4	5	6	8	9	10	11	12	13
2	*Beta vulgaris* CMS-0302	16	30	–	–	–	–	30	–	–	–
		20	30	–	–	–	–	–	30	–	–
		24	30	–	–	–	–	–	–	30	–
		28	30	–	–	–	–	–	–	30	–
3	B_3CS No. 3 (Greece)	16	44	2.27	22.73	–	56.81	18.19	–	–	36.4
4	B_3CS No. 5 (Greece)	38	58	12.07	20.69	–	–	8.62	58.62	–	22.4
5	B_6CS No. 7 (Greece)	38	43	4.65	–	–	–	30.23	–	65.12	69.7
6	B_4CS No. 4 *patula*	20	40	10	–	–	60	–	30	–	40
7	B_4S No. 5 *patula*	28	41	26.83	–	–	43.90	29.27	–	–	48.7
8	B_5CS No. 11 (Turkey)	24	45	20	–	–	17.78	40	22.22	–	51.1
9	B_6CS No. 13 (Turkey)	28	50	28	–	–	32	–	40	–	40
10	B_4CS No. 1 (Turkey)	38	29	41.38	–	–	–	24.14	13.79	20.69	–
11	B_4CS No. 2 (Turkey)	38	30	56.67	–	16.67	–	–	10	16.66	–
12	B_3CS No. 5 (Turkey)	38	32	31.26	9.37	–	–	9.37	–	50	9.4

Note: BC_6S No. 7 (Greece) – polyembryony was observed in all studied ovules with complete embryo development in the chalazal region and a 1/2 embryo sac developmental delay in the micropylar region on day 38 after the onset of flowering

Source: compiled by the authors

A distinctive characteristic of breeding material with apozygoty, in comparison with hybrid embryos, is the high proportion of degeneration, amounting to 41.38% and 56.65%, at a fixation period of 38 days across all breeding accessions studied. However, low degeneration values of 4.65% and 12.07% were observed in backcross progenies at different embryo developmental stages in material on the *Beta maritima* (Greece) cytoplasmic background. The percentage values for complete embryo development at the adjusted fixation period of 38 days ranged from 16.60% to 65.12%, depending on the genetic origin of the material. The seed productivity of breeding accessions in substituted lines under apozygoty is presented in Table 4.

Table 4. Seed productivity of new plasmotypes under apozygoty A_1, based on introduced sterile cytoplasm of *Beta patula* and *Beta maritima* under pollen-free conditions

Breeding accession	Origin	Number of fruits per 10 cm segment, X ± m	Of these, %	
			Developed	Degenerated
1	2	4	5	6
BC_6S** *maritima* (Turkey), c.4/1	Amb. No. 17224	36 ± 0.22	92.5	7.5
BC_6S *maritima* (Turkey), c.5/1	Amb. No. 17224	32 ± 0.1	46.2	53.8
B_6CS *maritima* (Turkey), c.2/1	Amb. No. 17224	21 ± 0.2	39.1	60.9
BC_6S *patula* (Portugal, Madeira Island), c.1/4	Amb. No. 17222	32 ± 0.3	45.7	54.3
BC_6S *patula* (Portugal, Madeira Island), c.3/4	Amb. No. 17222	38 ± 0.42	51.3	48.7
BC_6S *patula* (Portugal, Madeira Island), c.3/4	Amb. No. 17226	37 ± 0.21	50.5	50.0
BC_6S *patula* (Portugal, Madeira Island), c.3/7	Amb. No. 17226	37 ± 0.29	68.8	31.2
BC_6S *maritima* (Turkey), c.5/1	Amb. No. 17224	28 ± 0	79.3	20.7

Table 4, Continued

Breeding accession	Origin	Number of fruits per 10 cm segment, X ± m	Of these, %	
			Developed	Degenerated
BC_6S *maritima* (Turkey), c.7/2	Amb. No. 17224	27 ± 0.32	96.4	3.6
BC_6S *maritima* (Turkey), c.6/3	Amb. No. 17224	24 ± 0.27	95.7	4.3
BC_6S *maritima* (Turkey), c.7/5	Amb. No. 17224	24 ± 0.29	88.5	11.5

Note: pollen-free conditions ensured by spatial isolation and the use of parchment isolators; BC_6S^{**} – substituted lines of the sixth backcross cycle
Source: compiled by the authors

According to Table 4, on day 28 from the onset of flowering, the highest and most stable seed set values under spatial isolation conditions were characteristic of seed-bearing plants of substituted lines with breeding accessions BC_6S *maritima* (Turkey) c.7/2, c.6/3. Seed set values in substituted lines per 10 cm segment ranged from 39.1% to 96.4%. Fruit set values varied from 21 ± 0.2 to 38 ± 0.42, depending on the origin of the breeding accessions. In the BC_6S *patula* Line, the number of apozygotic seeds set was characterised by stable values of 32 ± 0.3 to 38 ± 0.42, whilst the proportion of degenerated embryos ranged from 31.2% to 54.3%. For the BC_6S *patula* L. line, the number of fruits per 10 cm segment substantially exceeded the values recorded for seed-bearing plants on the sterile *Beta maritima* L. (Turkey) cytoplasmic background. Seed-bearing plants with high seed self-reproduction were identified, notably BC_6S maritima (Turkey) c.4/1 and c.5/1, as well as those on the *Beta patula* L. (Madeira Island) cytoplasmic background under breeding accessions BC_6S *patula* c.3/4 and c.3/7.

Embryo sacs formed from the archespore belong to the *Polygonum* type. The sugar beet seed reproduction system encompasses all types of reproduction. The frequency of apozygoty in free-pollinating sugar beet populations is unknown. According to D. Hojsgaard *et al.* (2014), the development of diploid plants under pollen-free conditions proceeds through adventitious embryogenesis or apospory. T. Szkutnik (2010) established that the appearance of tetraploid cells in apomictic progenies is conditioned by the formation of an embryo from an egg cell with an unreduced chromosome set and by tissue differentiation during ontogenesis through the phenomenon of endopolyploidisation. Based on embryological investigations by L. Seilova & A. Sedlovskiy (2000), apomictic embryos differ from sexual ones in their mode of formation and position within the embryo sac. Additional embryos arise from synergid or antipodal cells – apogamy. M. Roik *et al.* (2024) indicate that embryo formation via adventitious embryogenesis proceeds from cells of the integuments and nucellus. It was noted that some apomictic embryos perish during embryogenesis. Selection for ploidy level and apozygotic seed set contributes to enhanced apozygotic tendency and the identification of apomixis donors.

The influence of polyploidy on trait variation in *Amelanchier* (*Rosaceae*) was described by M. Burgess *et al.* (2014). M. Hand *et al.* (2015) investigated the evolution of apomixis loci in *Pilosella* and *Hieracium* (*Asteraceae*) using marker analysis in natural and experimental populations. Research by D. Rodriguez-Leal & J.-P. Vielle-Calzada (2012) emphasises that apomixis may be considered a spatiotemporal deregulation of the sexual process rather than a wholly independent mechanism. The authors analyse new evidence that the regulation of apomixis – particularly female gametogenesis and seed formation – is governed by the same epigenetic mechanisms as sexual reproduction. Similar approaches to interpreting the molecular regulation of apomixis are presented in the work of T. Niccolò *et al.* (2023). G. Gerashchenkov *et al.* (2015) identified fragments of genes controlling endosperm development without fertilisation in *Arabidopsis* using molecular genetics methods.

On cytological preparations, the genetic heterogeneity of seeds under apozygoty is determined by the development of embryos from embryo sac cells or from somatic cells of the ovule. In addition to cytoembryological characterisation, morphological marker traits – such as recessive hypocotyl colouration – are capable

of identifying embryos developed via generative reduced parthenogenesis from heterozygous apomictic plants. Using the embryo culture method, N. Kovalchuk *et al.* (2019) isolated haploid and dihaploid lines from apomictic progenies of male-sterile sugar beet lines. Embryological investigations of apozygoty across cmS sources of different origins highlighted several problematic issues: variability in phenotypic traits of monoecism and sterility in apozygotic progenies does not conform to Mendel's laws of genetics, but is determined by the genetic heterogeneity of seeds, which is linked to the differing nature of embryos developed from both somatic and generative cells (Matzk *et al.*, 2000). It was established that apozygotic lines from different genetic sources of sterile cytoplasms differed from one another in their embryonic development characteristics. Breeding materials based on *Beta patula* germplasm were characterised by greater capacity for polyembryony. The use of apomictic lines with a high apozygotic seed set requires the identification of source material using genetic markers for the differentiation of generative and somatic embryogenesis, which in one case leads to new genetic variability, and in another to the cloning of breeding material and the preservation of its principal traits in subsequent generations.

CONCLUSIONS

Experimental investigations of embryological development based on different cmS sources revealed several characteristics of uniparental seed reproduction in sugar beet. It was established that the embryonic development of apomictic progenies is characterised by various types of anomalies, a developmental delay of 8-10 days, simultaneous embryo development in both the micropylar and chalazal regions, adventitious embryogenesis, and apospory. Embryonic development under pollen-free conditions was studied in substituted lines BC_3, BC_4, BC_5, and BC_6, in comparison with the development of hybrid embryos of Beta vulgaris cmS 0-type. Polyembryony was observed both on day 24 after flowering marking and on day 38, and was dependent on the genetic origin of the breeding material. In the course of the cytological experiment, the synchrony of developmental stages of apomixis and sexual plant reproduction was compared, and the possibility of differentiating apozygotic progenies by apomixis type was demonstrated. In the BC_6S *patula* Line, the number of apozygotic seeds set was characterised by stable values of 32 ± 0.3 to 38 ± 0.42, whilst the proportion of degenerated embryos ranged from 31.2% to 54.3%. For substituted lines based on the *Beta patula* sterility source, the number of fruits per 10 cm segment substantially exceeded the values of seed-bearing plants on the sterile *Beta maritima* L. (Turkey) cytoplasmic background. Breeding accessions with high apozygotic seed reproduction values were identified, notably BC_6S maritima (Turkey) c.4/1 and c.5/1, as well as those on the *Beta patula* L. (Madeira Island) cytoplasmic background under breeding accessions BC_6S *patula* c.3/4 and c.3/7. Notwithstanding that polyembryony frequently determines the genetic heterogeneity of apozygotic seeds, generative reduced parthenogenesis in sugar beet may be identified in future investigations using morphological marker traits and ploidy stabilisation techniques. The cytoembryological method, employed for the first time for the detection of adventitious embryogenesis, remains to this day one of the most reliable approaches for studying both the type of apomixis and the characteristics of embryonic structure development. Prospects for further research lie in the investigation of the molecular-genetic mechanisms of apomixis, the identification of genetic markers of generative and somatic embryogenesis, and the development of methods for stabilising apozygotic progenies for practical application in the breeding of high-yielding sugar beet hybrids.

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CONFLICT OF INTEREST

None.

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Особливості ембріонального розвитку зародків заміщених ліній буряків цукрових при апозиготії і цитоплазматичній чоловічій стерильності

Анотація. Метою дослідження було оцінити теоретичні основи феномену високої саморепродукції насіння у заміщених ліній та розробити методику ембріонального розвитку у селекційних матеріалів буряків цукрових з цитоплазматичною чоловічою стерильністю (ЦЧС) різного генетичного походження. Для експериментальних досліджень були використані методи польові, безпилкове отримання насіння у селекційних матеріалів у ЦЧС, лабораторні,

цитологічний аналіз розвитку зародків при апозиготії впродовж 34 діб після фіксації цвітіння, цитофотометричний метод мінливості рівня геному за плоїдністю у матеріалів з різним терміном репродукції апозиготичного насіння. Було вдосконалено методику дослідження ембріонального розвитку при апозиготії у матеріалів різного походження джерел ЦЧС, а саме відмітка цвітіння, ізоляція пагонів, фіксація зародків за терміном від 5 до 34 діб, до повного розвитку зародка. Було проаналізовано, що при апозиготії у цукрових буряків спостерігається відставання генетично-детермінованого розвитку 28 діб на 4, 8, 10 діб залежно від походження матеріалу, поліембріонією, високим відсотком дегенерованих зародків. Адвентивна ембріонія, апоспорія та розвиток зародків з інтигументів і нуцелусу в мікропілярній і халазальній частині одночасно були характерними для пилкостерильних ліній різного генетичного походження. Було встановлено, що у заміщених ліній на основі нових джерел ЦЧС від диких видів роду *Beta* L. спостерігається феномен високої саморепродукції насіння у безпилкового режиму. Методика вивчення синхронності розвитку зародків розроблена для дослідження природи апозиготії (генеративний, соматичний ембріогенез) у заміщених ліній з інтродукційними стерильними цитоплазмами, в зрівнянні з роздільноквітковими пилкостерильними лініями з S-vulgaris цитоплазмою Ф. Оуена. Тип апозиготії проявлявся в гаметофітному та спорофітному ембріогенезі і визначав генетичну неоднорідність (різноякісність) апозиготичного насіння. Апоміксис з використанням заміщених ліній і новими стерильними цитоплазмами від диких видів роду *Beta* L. та високим відсотком апоміктичної репродукції насіння, може забезпечити багато переваг для виробництва нових гібридів сільськогосподарських культур, а саме фіксацію гетерозису і збереження генотипу материнської рослини

Ключові слова: адвентивна ембріонія; апоспорія; нові джерела цитоплазматичної чоловічої стерильності; *Beta maritima*; *Beta patula*; фенотипи цитоплазматичної чоловічої стерильності