

**ANALYSIS OF MOLECULAR GENETIC POLYMORPHISM OF
SUGAR BEET BREEDING MATERIAL WITH SIGNS OF SELF-
FERTILITY AND SELF-STERILITY**

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The article describes the study of molecular and genetic polymorphism of self-fertile and self-sterile forms of sugar beet. 149 RAPD-locus in self-fertile and self-sterile lines were analyzed, and 54% of them were polymorphic. The absence of interlinear of polymorphism self-fertile lines that may be a criterion for the presence of this trait was found.

Key words: *sugar beet, self-fertility forms, polymorphism, RAPD-locus.*

Self-sterility is a characteristic feature of the type of *Beta vulgaris L.*, which is genetically determined and prevents to self-pollination. Self-fertility for sugar beet is a contrast as compared with the condition of self-sterility. These phenomenon are opposite conditions of one process – reproduction of forms within the same species [1].

Self-fertility of sugar beet is realized as self-pollination that occurs between the flowers of the same plant, or within the same flower.

The genetics of self-fertility sign was investigated by F. Owen in the 60 years of the last century. Phenotypic it is shown in high degree of seed-bud at self-pollination among several generations, that doesn't differ from the same indicator at free pollination of plants. Level of self-fertility in such plants is so

high that even with excessive amounts of pollen strange the share of cross-pollination is low [2]. Self-fertility of beets is controlled by gene S^f [1].

Using DNA markers allows with high precision and in the reduced terms to conduct the assessment and selection of genotypes that combine certain qualities. Analysis of the literature indicates a relatively low number of articles devoted to the issue of molecular genetic polymorphism of sugar beet compared to other crops, such as wheat, barley and corn [4]. Investigation of molecular genetic polymorphism of sugar beet lines with self-fertility and self-sterility characteristics is essential not only theoretically, but also acquire practical importance that is why it was the **purpose of our research**.

Materials and methods. For research of molecular and genetic polymorphism of breeding materials of sugar beet was selected on 30 plants previously the defined three self-fertile lines of the seventh and the seventeenth inbreeding and three self-sterile lines of O-type.

DNA was extracted from axillary buds of selected materials, with each plant individually. Abjection was performed according to methods using cationic detergent CTAB. The concentration of DNA was determined in 0.8% agarose gel under standard solution.

For research of molecular and genetic polymorphism used modification polymerase chain reaction such as RAPD-method. The amplification reaction was performed under the following temperature conditions: denaturation – 3 min. at 94°C and 33 cycles that included three temperature regimes: 1 min. denaturation at 93°C , 2 min. annealing at the optimum temperature for each primer within $47\text{--}55^{\circ}\text{C}$ and elongation 2 min. at 72°C [3].

The main structural characteristics and the optimum temperature of annealing primers are provided in tab. 1.

1. The characteristic of the used RAPD primers

№	Primer	Nucleotide sequence 5' 3'	Number of nucleotide, units.	CG-composition, %	Annealing temperature, °C
1	P815	ggC ATC ggC C	10	80	55
2	P816	CCC AAg ATC C	10	60	50
3	P817	CCA Cgg gAA g	10	70	50
4	P818	TCA gAg CgC C	10	70	50
5	P819	gTC TCg TCg g	10	70	50
6	P820	gTg TAg ggC g	10	70	50
7	P821	ggC CTT CAg g	10	70	55
8	P822	gCT CTC ACC g	10	70	55
9	P823	CATCCTACTTTCTTCGTT	18	39	50
10	P824	CAA CAT TCC AAg TAA TCA ACA T	22	32	50

After PCR was carried out electrophoretic separation of the products in 2% agarose gel with ethidium bromide addition in 1^x TBE at a constant voltage of 2.6 V/cm og gel for three hours [3].

On the basis of the received electrophoretic spectrum it is constructed matrix in which the presence / absence of allele for each separate locus was displayed as 1/0 respectively. On a matrix was counted the percent of polymorphic loci and index of a polimorfnost of a locus percentage of polymorphic loci and index of polimorfphity of locus.

Results and discussion. Molecular genetic polymorphism of self-fertile and self-sterile lines of sugar beet was investigated with use of 10 RAPD-primers. The result is 149 loci, 76 of which were polymorphic. The level of polymorphism was 54%. Mass researches take a lot of time and are expensive therefore it is advisable to pay attention to the analysis with concrete primers which detect high level of polymorphism. Thus, the highest level of

polymorphism 71% was obtained using primers P 822 and P 824, which identified 10 polymorphic loci from 14. With primers that detect low levels of polymorphism it is inexpedient to carry out analyses. The lowest this indicator was for P 819 – 35%, although with its using was investigated 20 loci, however, polymorphic were only 7. Index of polymorphity of locus for research sampling primers varied in the range from 0.25 to 0,31 (tab. 2).

2. Polymorphism of RAPD-loci identified in self-fertile and self-sterile lines of sugar beet

№	Primer	Total loci	Polymorphic loci	% of polymorphism	Index of polimorphity of locus
1	P 815	17	8	47	0,3
2	P 816	14	7	50	0,31
3	P 817	15	8	53	0,3
4	P 818	14	9	64	0,3
5	P 819	20	7	35	0,25
6	P 820	12	5	42	0,3
7	P 821	16	10	62	0,3
8	P 822	14	10	71	0,3
9	P 823	13	6	46	0,31
10	P 824	14	10	71	0,3
		149	76	54*	0,3*

- * –Average.

Most of the used primers allow detecting polymorphism from 50% to 71%. It should be noted that so high for a lineal material, the level of polymorphism and consequently so large proportion of polymorphic loci, obtained mainly due to the self-sterile O-types.

As seen from the Figure polymorphism correlation between self-fertile forms is small and it was not observed inside the lines № 1 I₇ (first and second samples) and № 3 I₁₇ (fifth sixth and seventh samples). However, for plants of line

№ 2 I₇ (third and fourth samples in Figure) using primer P 816 polymorphic fragments were found. Among the two samples presented in Figure, the third pattern is typical for this line, and the fourth – no. According to the electrophoretic spectra of amplicons received by means of other primers, internal linear polymorphism for self-fertile lines was not revealed.

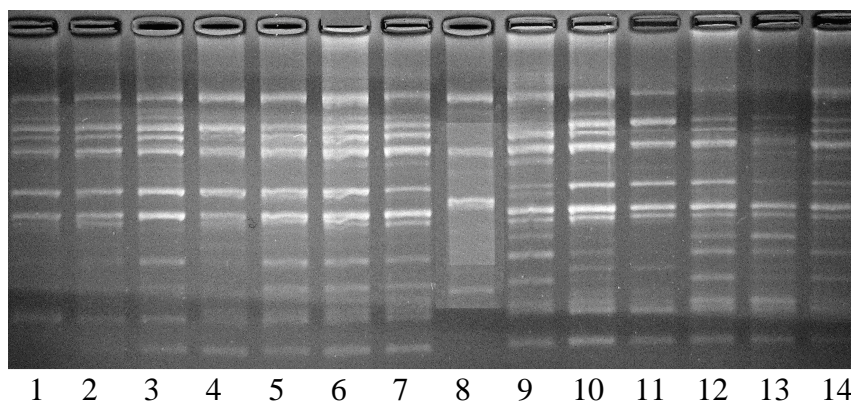


Fig. Electrophoretic spectrum of amplicons obtained with primer P 816 for self-fertile and self-sterile forms of sugar beet.

1–7 – self-fertile forms (1, 2 - № 1 I₇, 3, 4; - № 2 I₇, 5, 6, 7 - № 3 I₁₇), 8 – molecular weight marker, 9–14 – self-sterile O-types (9 - № 1, 10, 11 - № 2, 12, 13, 14 - № 3).

Considering the received results, the specificity of feature of self-sterility and the level of inbreeding of investigated lineal material it is possible to assume that existence of internal linear polymorphism of self-fertile line is the result of mechanical transfer to the genotype sample of other line.

Thus, the absence of internal linear polymorphism in self-fertile lines can serve as a criterion existence of a sign of self-fertility and conversely the high level of internal linear polymorphism in self-sterile lines of O-type – indicates the absence of sign of self-fertility. For mass analysis of breeding material should be used only primers that can detect the highest level of polymorphism.

Conclusions. According to the results of research of 149 RAPD-loci in self-fertile and self-sterile lines of sugar beet, polymorphic were 76, which is

54%. The majority of polymorphic loci it is identified for self-sterile O-types, and polymorphism is found both between various lines and in lines. For self-fertile lines is characteristic exclusively interline polymorphism, which can serve as criterion of existence of sign of self-fertility. For research of breeding materials it is enough to use only primers P 822 and P 824, allowing to reveal the greatest share of polymorphic loci – 71%. Internal linear polymorphism in self-fertile forms was found for one plant using primer R816. Considering that the self-fertile lines used in researches had a high degree of homozygosis, detected polymorphic genotype can be regarded as pollution.

Analyzing obtained results it's arguable that there is a possibility of self-fertile/self-sterile materials determination for the presence/absence of internal linear polymorphism.

BIBLIOGRAPHY

1. Балков І.Я. Проблеми одержання та використання самофертильних ліній цукрових буряків./ І.Я.Балков, М.О.Корнеєва / Збірник наукових праць. - К.: Аграрна думка. - 1997.

2. Роїк М.В. Генетичний контроль і фенотипові прояви ознаки самосумісності – самонесумісності у цукрових буряків. / М.В.Роїк, М.О.Корнеєва / Збірник наукових праць. - К.: ІЦБ УААН.- 2003, Випуск 5. С. 18-27.

3. Визначення молекулярно-генетичного поліморфізму роду *Beta L.* з допомогою полімеразної ланцюгової реакції. Методичні рекомендації / [М.В. Роїк, Ю.М. Сиволап, Г.П. Петюх, та ін.] – К. «ПоліграфКонсалтінг», 2007. – 27 с.

4. Сиволап Ю.М. Геном рослин і «Молекулярна селекція» / Ю.М. Сиволап / Селекція і насінництво. – 2008. Випуск 96.– С. 34-42.

**АНАЛИЗ МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКОГО ПОЛИМОРФИЗМА
СЕЛЕКЦИОННОГО МАТЕРИАЛА САХАРНОЙ СВЕКЛЫ С
ПРИЗНАКАМИ АВТОФЕРТИЛЬНОСТИ И АВТОСТЕРИЛЬНОСТИ**
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Представлены исследования молекулярно-генетического полиморфизма автофертильных и автостерильных форм сахарной свеклы. Проанализировано 149 RAPD-локусов в автофертильных и автостерильных линиях, из них полиморфными оказались 54%. Установлено отсутствие внутрелинейного полиморфизма автофертильных линий, что может быть критерием присутствия данного признака.

Ключевые слова: сахарная свекла, автофертильные формы, полиморфизм, RAPD-локусы.

**АНАЛІЗ МОЛЕКУЛЯРНО-ГЕНЕТИЧНОГО ПОЛІМОРФІЗМУ
СЕЛЕКЦІЙНИХ МАТЕРІАЛІВ ЦУКРОВИХ БУРЯКІВ З ОЗНАКАМИ
АВТОФЕРТИЛЬНОСТІ ТА АВТОСТЕРИЛЬНОСТІ**
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В статті наведено дослідження молекулярно-генетичного поліморфізму автофертильних та автостерильних форм цукрових буряків. Проаналізовано 149 RAPD-локусів у автофертильних та автостерильних ліній, з яких поліморфними виявились 54%. Встановлено відсутність внутрішньолінійного поліморфізму серед автофертильних ліній, що може слугувати критерієм наявності даної ознаки.

Ключеві слова: цукрові буряки, автофертильні форми, поліморфізм, RAPD-локуси.