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IDENTIFICATION OF PHOTOPERIOD SENSITIVITY GENE *E7* IN SOYBEAN CULTIVARS AND BREEDING LINES USING SSR MARKERS

Abstract. Soybean (*Glycine max*) is a short-day plant and its different genotypes start to flower when the day length is less than their critical day length. Southern regions of Kazakhstan are the main sowing area for this crop, and it is crucial to develop soybean cultivars adapted to conditions in North Kazakhstan. In this article, soybean cultivars and their breeding lines was tested to detect *E7* locus determining photosensitivity, SSR markers Satt100 and Satt319 linked to *E7* locus were used in order to distinguish promising cultivars and breeding lines for further breeding plan. 37 soybean cultivars and 34 breeding lines of legume department in KazSRIA&PG were taken as a research object. As a result of *SSR* genotyping 16 soybean cultivars and 7 hybrids have the recessive alleles corresponds to photoperiodic recessive alleles, these cultivars and breeding lines with *e7* locus are recommended to our breeders as a genetic resource to develop photoinsensitive varieties.

Keywords: soybean, *E7* gene, SSR markers, photoperiod insensitivity.

Introduction. Soybean (*Glycine max* (L.) Merr.) is one of the most economically important oil crops. It is a rich source of vegetable oil and protein feed, macronutrients and minerals, soybean also contains secondary metabolites [1], saponins, phytic acid, oligosaccharides, goitrogens [2], and phytoestrogens [3]. It is not only an important food, feed (protein-oil), technical culture in the world but also in Kazakhstan [4]. Soybean is a more profitable crop. Profitability of production reaches close to 120% in the southeast of Kazakhstan [5]. In 2017, soybean cultivated area in the world was about 123 million hectares [6]. Kazakhstan also pays more attention to increasing soybean production. Sowing area in 2018 reached 126 thousand hectares [7]. In the Republic of Kazakhstan according to the program for diversification of agricultural crops, sowing area of soybean will be reached 400,000 hectares by 2020, this would enable Kazakhstan to produce 1 million tons of soybean seeds in the country [8].

The main region of soybean cultivation is south and south-east of Kazakhstan, however, cultivating soybean in northern part of Kazakhstan is projected where soybean has not previously been a traditional culture (Kostanay, North Kazakhstan and East Kazakhstan regions). Extension of soybean cultivated area in the northern and eastern regions of the republic is a target indicator of the State program for the development of the agro-industrial complex in the Republic of Kazakhstan for 2017-2021. For a large variety of soil and climatic conditions of Kazakhstan, early maturing and resistant to various stresses of soybean cultivars are required. In terms of this, the Kazakh Research Institute of Agriculture and Plant Growing started developing soybean varieties, early maturing, photoperiodic insensitivity and drought resistant, are suitable for sowing in the northern ecotype.

The crop is a short-day plant and its different genotypes start to flower when the day length is less than their critical day length. It is grown worldwide from equator to 50◦N and 35◦S latitudes [9]. The photoperiod insensitivity is a major criterion that determines the latitudinal adaptation of soybean cultivars [10].

It has adapted to such a wide range of latitudes due to its natural variation of major genes and quantitative trait loci (QTLs) which control flowering time and maturity. Till now, 10 major genes controlling flowering time and maturity have been identified in soybean: *E1* and *E2* [11], *E3* [12], *E4* [13], *E5* [14], *E6* [15], *E7* [16], *E8* [17], *E9* [18] and *J* [19]. Of these 10 genes, *E1*, *E3*, *E4* and *E7* have been reported as quantitative photoperiodic genes [14] with dominant alleles conferring photosensitivity. While dominant alleles at *E1*, *E2*, *E3*, *E4*, *E5*, *E7* and *E8* loci delay time to flowering, recessive alleles at *E6*, *E9* and *J* loci delay flowering time to different extents, interacting with the environment and with genotypes at other loci [9, 18].

The *E7* locus is associated with the response to light quality and it may be related to phytochrome. The study of mapping *E7* locus with the microsatellite markers Satt100, Satt319 and Satt460 on LG C2 (chromosome 6) Satt442 (H), and Satt497 (L) revealed that Satt 100 and Satt 319 are the most effective markers to detect *E7* locus [20].

The effectiveness of detecting *E* alleles for the early diagnosis of the reaction of soybean plants to the photoperiod has been shown in numerous publications [21–24], but these markers have not yet been used in the practical soybean breeding in Kazakhstan. Domestic breeders, developing varieties for various climatic zones of Kazakhstan, deal with a mosaic of forms, groups of maturity and varieties with varying degrees of photosensitivity. Breeders need information on the genetic variability that determines this diversity, as well as a real tool to diagnose the adaptive potential of a plant to a certain longitude of the day in the early stages of the selection process.

The aim of this study is to identify the allelic composition of parental forms and their breeding lines of soybean by the *E7* photoperiod sensitivity gene and select photoinsensitivity forms.

Materials and methods. 37 soybean cultivars and 34 breeding lines of legume department in KazSRIA&PG were taken as research objects. Early maturing and ultra-early maturing accessions were used to develop early maturing soybean varieties for the eastern and northern regions of Kazakhstan. [Table 2, Table 3].

Simple sequence repeat (SSR) markers Satt 100 and Satt 319 were selected from those designed and mapped by Molnar *et al.* [20] (table 1).

Table 1 – SSR markers used for detecting polymorphism at *E7* locus.

SSR name	Primer sequences (5'→3')
Satt 100	F: ACCTCATTTTGGCATAAA
	R: TTGGAAAACAAGTAATAATAACA
Satt 319	F: CAACTCAGTAGGGGTCAATAACAA
	R: TGAAATAGGGAAAATAAGGGAACA

DNA was isolated using the CTAB method [25] from the first true leaves of individual seedlings grown in greenhouse conditions. Fresh leaf samples, about 100 mg each, were transferred to 2-ml test tubes with 600 µl of CTAB extraction buffer containing 1.0% polyvinylpyrrolidone (PVP40) and homogenized using a stainless steel pestle. After that the mix was incubated in water bath for 1-1.5 hours, then added chloroforum/octanol (24:1), 96% ethanol added to the supernatant to precipitate and washed several times with 70% ethanol. Isolated DNA was then dissolved in 100 µl of sterile water. The concentration and quality of DNA samples was determined at 260 and 280 nm using a spectrophotometer Jenway 6715 (Jenway, Staffordshire, UK). DNA samples were diluted with sterile water to a concentration of 100 ng/µl for use in further experiments.

Polymerase chain reaction was curried in Eppendorf Mastercycler (Geremany). The PCR conditions for both primers were as follows: 1×PCR buffer, 2.5 mM MgCI2, 150 µM each of dNTPs, 0.35 µM 1 of each forward and reverse primers, 1 µl BSA (2 mg/ml) and 1 U of Taq DNA polymerase (Biosan, Novosibisk, Russia) and 100 ng of template DNA in 15 µl. Thermo-cycling conditions were initial denaturation at 95 ^{0}C for 5 min, followed by 35 cycles of 20 s at 95 ^{0}C, 1 min at 53 ^{0}C, 80 s at 72◦C, and with a final extension of 10 min at 72◦C. The amplification products were separated in polyacrylamide gel

(8% acrylamide, 1×TBE buffer), and gels were stained with ethidium bromide for digital imaging by the QuantumST4 Gel documenting system (Vilber, Collégien, France), as indicated above. The dimensional characteristics of PCR products were determined using the computer software 'QuantumCapt' (Vilber, Collégien, France) to determine the length and intensity of DNA fragments.

Results and discussion. In our experiment, three alleles of microsatellite were identified using both of Satt100 and Satt319 markers, the amplified fragments were named as A, B and C. According to Rosenzweig et al. [26] A and B corresponds to E7 and e7 genes respectively, an unknown allele also was revealed with a fragment of 154 bp corresponds to C at the locus satt100. The study curried on 37 soybean cultivars showed that 18 cultivars have *E7* locus and 16 varieties have recessive *e7* locus which are associated to photoperiodic insensitivity (table 2). 3 cultivars, 346-271-92, Annushka and Brianskaia, have unknown locus that needs to conduct further investigation to detect the locus.

Table 2 – Results of SSR genotyping used for detecting polymorphism at *E7* locus in 37 soybean cultivars

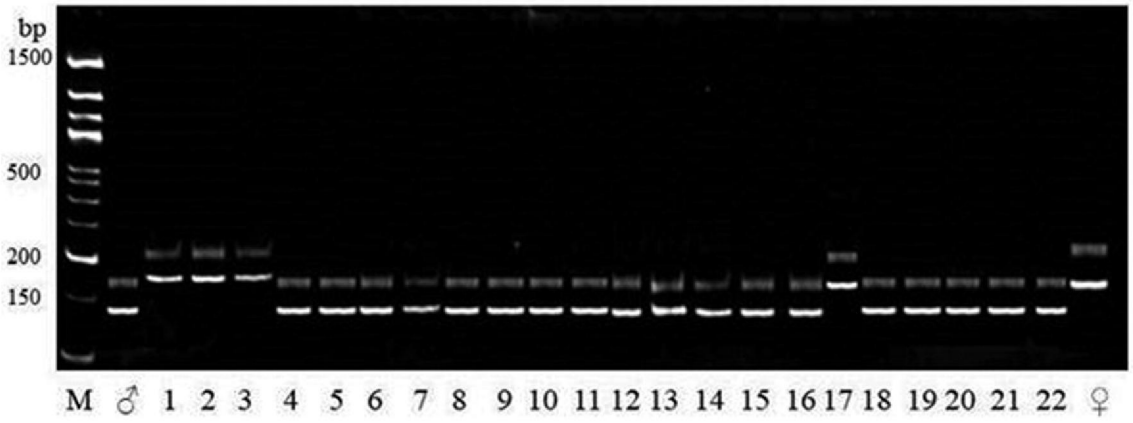
№	Accession/cultivar name	Origin/source	Alleles of loci/genes		
			Satt 100	Satt 319	*E7* Gene
1	HP -203	USA	A	A	*E7*
2	GilChin	China	A	A	*E7*
3	K3972	–	B	B	*e7*
4	K6932	–	B	B	*e7*
5	Rokujuunichi mame	Japan	B	B	*e7*
6	234	Russian	B	B	*e7*
7	347	–	A	A	*E7*
8	346-271-92	Russian	C	C	-
9	317-236-11	Russian	A	A	*E7*
10	211-124-206	Russian	B	B	*e7*
11	2 CH	–	B	B	*e7*
12	583583	–	B	B	*e7*
13	Fiskeby 111	Sweden	B	B	*e7*
14	LMF	Poland	B	B	*e7*
15	AC Brant	Canada	A	A	*E7*
16	Amour	France	B	B	*e7*
17	Sepia	France	B	B	*e7*
18	Amurskaia 401	Russian	B	B	*e7*
19	Annushka	Ukraine	C	C	-
20	Brianskaia	Russian	C	C	-
21	Birlik KV	Kazakhstan	B	B	*e7*
22	Vega	Russian	A	A	*E7*
23	VNIIS 1	Russian	A	A	*E7*
24	Zara	Kazakhstan	A	A	*E7*
25	Zakat	Russian	A	A	*E7*
26	Luchezarnaia	Russian	A	A	*E7*
27	Mriia	Ukraine	A	A	*E7*
28	Podiaka	Ukraine	A	A	*E7*
29	Rassvet	Russian	A	A	*E7*
30	Soer 3	Russian	A	A	*E7*
31	Soer 4	Russian	A	A	*E7*
32	Soer 5	Russian	A	A	*E7*
33	Ustia	Ukrain	B	B	*e7*
34	Khorol	Canada-Ukraine	B	B	*e7*
35	Jaselda	Belarus	B	B	*e7*
36	Lastochka	Kazakhstan	A	A	*E7*
37	Pamiat UGK	Kazakhstan	A	A	*E7*

Table 3 – Results of SSR genotyping used for detecting polymorphism at *E7* locus in 36 hybrids and their parent cultivars

№	Name of hybrids and parent cultivars	Allele of Satt 100 locus	Allele of Satt 319 locus	*E7* gene
1	Lastochka	A ♀	A	*E7*
2	234	B ♂	B	*e7*
3	LT44/11	B	B	*e7*
4	LT44/12	B	B	*e7*
5	LT44/2	A	A	*E7*
6	Zara	A ♀	A	*E7*
7	Jaselda	B ♂	B	*e7*
8	N 8	A	A	*E7*
9	Zara	A ♀	A	*E7*
10	Khorol	B ♂	B	*e7*
11	N 10/1	A/B	A/B	*E7/e7*
12	N 10/2	A/B	A/B	*E7e7*
13	Zara	A♀	A	*E7*
14	234	B♂	B	*e7*
15	L 8/31	A	A	*E7*
16	L 8/32	A	A	*E7*
17	Pamiat UGK	A♂	A	*E7*
18	Birlik KV	B♀	B	*e7*
19	M 15/1	A	A	*E7*
20	M 15/2	B	B	*e7*
21	M 15/3	B	B	*e7*
22	M 15/4	B	B	*e7*
23	M 15/5	A/B	A/B	*E7/e7*
24	M 20	B	B	*e7*
25	Zara	A♀	A	*E7*
26	583583	B♂	B	*e7*
27	L1/1	A	A	*E7*
28	L1/21	A	A	*E7*
29	L1/22	A	A	*E7*
30	L1/23	A	A	*E7*
31	Zara	A♀	A	*E7*
32	Ustia	B♂	B	*e7*
33	L4/1	A	A	*E7*
34	L4/31	A	A	*E7*
35	L4/32	A	A	*E7*
36	L4/33	A	A	*E7*
37	L4/34	A/B	A/B	*E7/e7*
38	Zara	A♀	A	*E7*
39	2 CH	B♂	B	*e7*
40	L11/21	A	A	*E7*
41	L11/22	A	A	*E7*
42	L11/23	A	A	*E7*
43	L11/24	A	A	*E7*
44	L11/25	A	A	*E7*
45	L11/26	A	A	*E7*
46	L11/27	A	A	*E7*
47	L11/28	A	A	*E7*
48	L11/4	B	B	*e7*
49	LT44/2	A	A	*E7*
50	LT44/3	A	A	*E7*

PCR analysis related to detecting *E7* locus in hybrids is shown in table 3. All hybrids developed from Zara x Jaselda, Zara x 234, Zara x 583583 and Zara x Ustia possess *E7* locus, hybrids N10/1 and N10/2 from Zara x Khorol have both *E7* and *e7* loci. 11 derivate lines were developed by crossing Zara x 2 CH, among them only line L11/4 has a recessive allele linked to photoperiod insensitivity.

Hybrids LT44/11 and LT44/12 derived from the cross Lastochka x 234 have the fragment with a size of 145 bp at Satt100 locus that corresponds to *e7* gene. 4 out of 6 lines (M15/2, M15/3, M15/4 and M20) derived from Pamiat UGK x Birlik KV (figure) also contain recessive *e7* gene while others have photosensitivity gene.



M-marker; ♂- Birlik KV; ♀ - Pamiat UGK;
M15/1 (1-3); M15/2 (4-7); M15/3 (8-11); M15/4 (12-15); M15/5 (15-19); M20 (20-22)

PCR products of parental forms and hybrid population of Birlik KV and Pamiat UGK

The variety of domestic breeding Zara was highly preferred by plant architectonics and a high percentage of hybrid pod abscission in designing the hybridization scheme. Zara variety was developed on the basis of KazSRIA&PG and recommended for adapting in the East Kazakhstan region

However, DNA identification showed that Zara and all hybrid combinations with its participation had photoperiod sensitivity gene *E7*, while sowing these accessions in eastern and northern Kazakhstan their vegetation period extended.

The use of the soybean variety as a maternal form developed in the conditions of eastern Kazakhstan and approved for production there - Birlik KV proved to be more productive. A high percentage of segregation with photoinsensitivity was recorded in hybrid populations with the use of Birlik KV as the maternal form.

Thus, the data of genetic analysis on the targeted interest are needed in designing hybridization schemes for the effective and fruitful work of the breeder

In conclusion, SSR analysis curried on 37 soybean cultivars and 34 breeding lines, developed from the 8 crosses to get new promising lines with the *e7* locus, showed that 16 cultivars and 7 hybrids out of 34 possess photoperiodic recessive alleles, these cultivars and breeding lines will be used in further breeding programs to develop new varieties adaptable to the conditions of Northern Kazakhstan.

It was established that in the 24 cross combinations of the Zara variety with *E7* allele showed dominance to the paternal forms with the *e7* allele in the hybrid populations. This variety is not desirable for crossing when developing soybean varieties that are insensitive to photoperiod.

It is recommended to use varieties of Khorol, 234 and Birlik KV for developing photoinsensitive lines possessing *e7* gene.

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SSR МАРКЕРЛЕРІН ПАЙДАЛАНА ОТЫРЫП, ҚЫТАЙ БҰРШАҚ СОРТТАРЫ МЕН СЕЛЕКЦИЯЛЫҚ ЛИНИЯЛАРЫН ФОТОПЕРИОДҚА СЕЗІМТАЛ *E7* ГЕНІ БОЙЫНША ИДЕНТИФИКАЦИЯЛАУ

Аннотация. Қытай бұршақ (*Glycine max*) – қысқа күндік өсімдік, қытай бұршақ генотиптері, күннің ұзақтығы олардың критикалық ұзақтығынан аз болған жағдайда гүлдену басқышына өтеді. Қазақстанның оңтүстік аймақтары қытай бұршақ өсірілетін негізгі өңір болып табылады, Қазақстанның сотүстік аймақтарына өсіруге бейімделген Қытай бұршақ сорттарын шығару - еліміз үшін маңыздылығы өте жоғары. Бұл мақалада фотопериодқа сезімталдығын бақылайтын *E7* локусын анықтау үшін, қытай бұшағының ата-аналық формалары мен селекциялық линияларға талдау жасалынды. Перспективті сорттар мен линияларды идентификациялау үшін, *E7* локусынмен тығыз байланысқан Satt100 және Satt319 SSR-маркерлері пайдаланылды. Зерттеу объектісі ретінде Қазақ егіншілік және өсімдік шаруашылығы ғылыми зерттеу институтының дәндібұршақ бөлімінен қытайбұршақ өсімдігінің 37 сорт үлгілері мен 34 селекциялық линиялары алынды. SSR генотиптеу нәтижесі бойынша, фотопериодқа сезімтал емес *E7* генінің рецессивті аллельдері бар қытай бұршағының 16 сорты және 7 селекциялық буданы анықталды, осы алынған сорттар мен линиялар фотопериодқа сезімтал емес сорттарды шығару үшін генетикалық ресурс ретінде селекциянерлерге пайдалануға ұсынылды.

Түйін сөздер: қытай бұршағы, *E7* гені, SSR-маркерлер, фотопериодқа сезімтал емес.

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ИДЕНТИФИКАЦИЯ ГЕНА ЧУВСТВИТЕЛЬНОСТИ К ФОТОПЕРИОДУ *E7* У СОРТОВ И СЕЛЕКЦИОННЫХ ЛИНИИ СОИ С ИСПОЛЬЗОВАНИЕМ SSR-МАРКЕРОВ

Аннотация. Соя (*Glycine max*) – растение короткого дня, и его различные генотипы переходят к цветению, когда долгота дня меньше их критической длины. Южные регионы Казахстана являются основным регионом возделывания для этой культуры, и для страны крайне важно создать сорта сои, адаптированные к условиям Северного Казахстана. В статье родительские формы сои и их селекционные линии были протестированы по выявлению локуса *E7,* контролирующего фоточувствительность. Для идентификации перспективных сортов и линий были использованы SSR-маркеры Satt100 и Satt319, тесно сцепленные с локусом *E7.* В качестве объекта исследования были использованы 37 сортов и 34 селекционные линии сои отдела зернобобовых культур Казахского НИИ земледелия и растениеводства. По результатам SSR-генотипирования выявлены 16 сортов сои и 7 гибридов, имеющих рецессивные аллели гена фотопериодической нейтральности локуса E7 и эти сорта и селекционные линии рекомендуются селекционерам в качестве генетического ресурса для создания нечувствительность к фотопериоду сортов.

Ключевые слова: соя, ген *E7*, SSR-маркеры, нечувствительность к фотопериоду.

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