Prospects of exploiting of photoperiod sensitivity gene E_7 in early soybean breeding and revealing of its sources with SSR-markers

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Abstract
Recently described gene E_7 for photoperiod sensitivity in soybean is prospective for the development of early cultivars. Field trials of isolines differing in • $_7$/e_7 revealed yield gain equal to 21% while maturity was delayed by 6 days. Weak effect (4-5 days of flowering delay) makes distinguishing of • $_7$ by means of hybridologic analysis somewhat complicated. Screening of germplasm collection for • $_7$ sources with SSR-markers allowed identification a number of cultivars possessing this allele within different maturity groups (00 to II).

Introduction
Genetic system of photoperiod response in soybean includes over 10 known loci (Bernard 1971, Buzzell, Voldeng 1980, McBlain, Bernard 1987, Cober, Voldeng 2001, Destro et al. 2001). They contribute to fine adaptation of genotype to the certain latitude and climate. However, a few of these genes may be involved into the breeding programs for cool climates, in particular for maturity group (MG) 00. At least E_3, E_4, e_6, j_1, j_2, j_3 and j_4 are impossible to be used in MG 00, while an attempt to exploit E_1 did not result successfully (Cober et al. 1996, 2000). E_2 allele may be used for developing late 00 MG, late flowering cultivars, and the effects of E_5 in northern latitudes are not studied yet.E_7 locus was described on 'Harosoy' isolines. It is tightly linked to E_1 and *T* (pubescence color) (Cober, Voldeng, 2001). E_7 allele is the weakest among all known *E* loci delaying flowering by 4-5 days only. Previous investigations held in Russia and Belarus showed that in a wide range of moderate climates early flowering combined with an extended reproductive stage is preferable for soybean (Miroshnichenko 2005, Rosenzweig et al. 2003). From this point of view, E_7 seems to be the most attractive gene as well because of its weakest effect on flowering induction among all known loci. That is why an estimation of its agronomic value in early soybean gene pool background seems to be appropriate. Nevertheless, a search of additional sources E_7 allele using hybridological analysis (in case of its breeding value) is likely to be difficult because of its weak effect which sometimes can hardly be distinguished. Thus, a preferable way of searching for E_7 sources is the use of molecular markers. SSR-mapping of E_7 was carried by Molnar et al. (2003). These authors consider Satt319 and Satt100 to be the most reliable among the number of SSR-markers located close to or within $E_1 – T – E_7$ region of C2 linkage group.

Materials and methods
Field studies of E_7 influence on agronomic traits in early soybean were fulfilled on 'Harosoy' isolines developed at Eastern Cereals and Oilseeds Research Centre (ECORC, Canada) and provided by Cober. Mentioned isolines and other varieties of MGs 00 and early 0 were grown in the germplasm nursery of Soya-North Co., Ltd. in Luninets distr. (Belarus, 52^o N lat.), in 2005-2007. Plots were 2 m long, 1-row, with interrow spaces 45 cm. Stand density was 40-45 plants m^2. Phenological observations: registration of flowering start (R1) and maturity (R8), were carried according to common method. Correction factors for border effects in small-plot

nurseries (Rosenzweig, Goloenko 2007) were applied to increase the exactness of yield estimation. MGs 0 to II cultivars were maintained in a greenhouse. SSR markers to E_7 loci were chosen according to Molnar et al. (2003). Soybean SSR primers sequence were got from SoyBase (http://soybase.org/resources/ssr.php). 15-µL reaction mixture contained 30 ng DNA, 0.3 pmol of each primer, 250 µmol of each dNTP/L, 1,5 mmol MgCl2/L, 0,75 U Taq polymerase (Dialat Ltd, Moskow RF), and 1×PCR buffer (Dialat Ltd, Moskow RF) The thermocycler MJ Mini (BioRad) was programmed for PCR as follows: an initial denaturation step at 94 °C for 4 min; 35 cycles of denaturation at 94 °C for 40 sec, annealing at 58 °C for 1 min, and extension at 72 °C for 1 min 20 sec; followed by a final extension at 72 °C for 2 min. The SSR locus was resolved on an ALFexpressII (Amersham Biosciences Trading GmbH) after electrophoresis through 6% denaturing polyacrylamide gel.

Results and discussion

Results of field evaluation of 'Harosoy' isolines are presented in Table 1. E_7 isolines out yielded its neutral analog by 21% while ripening only 6 days later. Such yield gain is much greater than could be expected from the regression of yield on maturity. This regression coefficient in early soybeans is usually about 1.23-1.26 (Rosenzweig 2006; similar estimations may be obtained from Voldeng et al. 1997 and OOPSCC 2008). As seen from Table 1, increase was provided by cumulative effect of all elements of plant productivity. This allowed supposing that involving of E_7 allele into the gene pool of MG 00 may be desirable. There is no data, how widely allele E_7 is distributed within MG 00 and later MGs, since 'Harosoy' (or its isolines) is the only approved source. Search of additional sources of $\bullet_7$ with the means of classical genetics is somewhat complicated due to its weak effect which sometimes may not exceed statistical error. That is why 'Harosoy' isolines (OT 94-41, OT 89-5, OT 94-47) and 86 cultivars from the germplasm nursery were tested with SSR-markers to $\bullet_7$ (Table 2). Haplotypes differing in this locus, differed in fragment lengths amplified with SSR-markers. E_7 isolines possessed a 173 ± 1 b.p. fragment (designated as "A") amplified with Satt319, and 168 b.p. ("A"), with Satt100. In isoline OT 94-47 recessive in e_7, 176 b.p. fragment ("B") amplified with Satt319, and 133 b.p. ("B") fragment amplified with Satt100 were observed. In addition, both markers revealed multiple allelic forms. Satt319 produced also 179 b.p. fragment ("C"), while Satt100 amplified 115-116 ("C"), 143 ("D"), and 149 ("E") b.p. fragments in a number of cultivars (see Table 2). "A" fragments amplified with both SSR markers indicated presence of E_7 allele in studied cultivars. "C" allele (Satt100) was always (8 cases) combined with "A" fragment (Satt319) therefore presence of E_7 not be excluded in these cultivars. Most of them can be interpreted as E_7 judging from phenological observations. However, 'McCall' and 'AC Albatross' are likely rather to be neutral. They both require in average 37 days from germination to R1, like neutral varieties which have 36-37 days, and opposite to $e_1e_2e_3e_4e_5E_7$ genotypes requiring 39-44 days to first flower. Therefore, identification of "C" allelic form needs additional investigations. "C" fragment (Satt319), and "D" and "E" fragments (Satt100) were preliminarily treated as corresponding to e_7. "D" allele (Satt100) was combined with "C" allele (Satt319) in 10 cases of total 18, otherwise mostly with "B" allele (Satt319). "E" allele occurred in 2 cases, of which 'Fiskeby V' is obviously recessive in e_7. Totally, among 87 genotypes examined, 29, or 33 % were proved as $\bullet_7$. This allele appeared to be not rare within early cultivars. Certain genotypes were not adequately identified because of inconsistent data obtained with the used markers. Molnar et al. (2003) treated 'Evans' as possibly E_7 source while both SSR-markers used in our study allow supposing that this cultivar contains e_7 allele. 'Evans' (='Merit' / 'Harosoy' (Voldeng et al. 1997)) must have inherited e_7 from 'Merit' which is recessive in this locus as well. Morrison et al. (1997) supposed that tawny pubescence is preferable for soybean cultivar in a cool climate because it provides better accumulation of sunlight energy and

accelerates ripening by 2-3 days. According to Gass et al. (1996), one of the modes of cold tolerance is associated with additional racemes that start flowering 5-15 days later then main racemes in the given node. In case of cold stress and flower abortion, lateral racemes fulfill a compensatory function. Presence of lateral racemes was correlated with tawny pubescence and controlled by two genes, of which one is linked to *T* (Schori, Gass 1994). • $_7$ is tightly (4.4 cM) linked to *T* (Cober, Voldeng 2001) mostly in a position • $_7$ – *t*. Because of low frequency of crossovers, • $_7$• $_7$*TT* genotypes may be of a particular interest as parental forms for breeding. They are: 'Adoc', 'Soriano', 'Lada', 'Soer 3491', 'VNIIS-2', 'Oktyabr 70', 'Vega', 'Rassvet', 'Mria'.

Acknowledgements
We express our sincere thanks to Dr. E.R. Cober (Eastern Cereals and Oilseeds Research Centre, Canada) who kindly provided 'Harosoy' isolines for this study. This study was partly supported by Government scientific programs "Biotechnology" (assignment • 4.4.8)"

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Table 1: Agronomic traits of 'Harosoy' isolines differing in E_7 (Belarus, 2005-2007 mean)

Isoline	Genotype	Days to		Yield, % [1, 2]	Height, cm	Main stem nodes	Pods per node	Seeds per pod	TSW, g	Lodging, score [3]
		R1	R8							
OT 94-47	$e_1e_2e_3e_4e_5e_7$	36	117	86	64	10.8	1.8	1.5	226	1.5
OT 89-5	$e_1e_2e_3e_4e_5E_7$	40	123	104	81	11.4	2.3	1.9	245	1.5

[1] compared to check 'Yaselda'; 100% = 2.8 t ha^{-1}

[2] difference significant at $\alpha < 0.05$ by Mann – Whitney *u*-test

[3] considered as: 1 = absent, to 5 = complete

Table 2. Genotypes of soybean cultivars in E_7 / e_7 locus identified with SSR markers

Origin	Cultivar	MG	Fragment length amplified with		Allele
			Satt319	Satt100	
USA	'Harosoy' ($e_1e_2E_3E_4e_5E_7$)	II	A	A	E_7
	OT 94-41 ($e_1e_2E_3e_4e_5E_7$)	0	A	A	E_7
	OT 89-5 ($e_1e_2e_3e_4e_5E_7$)	00	A	A	E_7
	OT 94-47 ($e_1e_2e_3e_4e_5e_7$)	00	B	B	e_7
	'Amsoy 71'	II	A	A	E_7
	'Evans'	0	C	D	e_7

	‘Lambert’	0	A	A	E_7
	‘Agassiz’	0	C	D	e_7
	‘MN 0901’	0	C	D	e_7
	‘McCall’	00	A	C	?
	‘Ada’	00	A	B	?
Canada	‘Merit’	0	C	D	e_7
	‘Maple Arrow’	0	B	B	e_7
	‘Maple Donovan’	0	A	A	E_7
	‘Korada’	0	B	B	e_7
	‘Hudson’	0	B	B	e_7
	‘Maple Ridge’	00	B	B	e_7
	‘AC Albatross’	00	A	C	?
	‘AC Colibri’	00	A	A	E_7
	‘OAC Erin’	00	C	D	e_7
	‘OAC Atwood’	00	A	A	E_7
	‘KG 20’	00	B	B	e_7
	‘PS 3008-1’	00	B	B	e_7
	‘Im 55-2’	00	A	A	E_7
	‘Maple Presto’	000	B	B	e_7
China	‘Xiaobaidou’	II	A	E	?
	‘Huang li’	0	A	A	E_7
	‘Heihe 3’	0	A	A	E_7
	‘Heihe 54’	0	A	C	?
	‘Dong nong 55-6012’	0	A	C	?
	‘Ke shuang’	0	A	A	E_7
	‘Dong nong 36’	000	B	B	e_7
Japan	‘Nagaha Hadaka 1’	I	B	D	e_7
	‘Ohyachi 2’	I	B	B	e_7
France	‘Adoc’	I	A	A	E_7
	‘Sakura’	I	A	B	?
	‘Soriano’	I	A	A	E_7
	‘Solano’	0	A	A	E_7
	‘Armour’	0	B	B	e_7
	‘Effi’	0	C	D	e_7
	‘Major’	00	B	B	e_7
	‘Kalmit’	00	A	C	?
Russia	‘Vilana’	I	A	A	E_7
	‘Delta’	0	A	A	E_7
	‘Lira’	00	C	D	e_7
	‘Lada’	00	A	A	E_7
	‘Belgorodskaya 6’	00	B	D	e_7
	‘Belgorodskaya 48’	00	A	C	?
	‘Veidelevskaya 17’	00	A	C	?

Table 2. continued

Origin	Cultivar	MG	Fragment length amplified with		Allele
			Satt319	Satt100	
Russia	‘Soer 3491’	00	A	A	E_7

	‘Gribskaya 30’	00	A	D	?
	‘VNIIS-2’	00	A	A	E_7
	‘Oktyabr 70’	00	A	A	E_7
	‘Vega’	00	A	A	E_7
	‘Rassvet’	00	A	A	E_7
	‘Dauria’	00	A	A	E_7
Ukraine	‘Podilska 416’	0	A	B	?
	‘Yug 30’	00	C	D	e_7
	‘Zolotysta’	00	C	D	e_7
	‘Kyivska 27’	00	B	B	e_7
	‘Chernyatka’	00	B	B	e_7
	‘Ustia’	00	B	B	e_7
	‘Niva’	00	B	B	e_7
	‘Belosnezhka’	00	A	C	?
	‘Romantyka’	00	B	B	e_7
	‘Mria’	00	A	A	E_7
	‘Osobliva’	00	A	B	?
	‘4346-1-84’	000	B	B	e_7
Moldova	‘Aura’	0	A	A	E_7
	‘Timpuria’	00	C	D	e_7
Hungary	‘BS 31’	00	B	D	e_7
Belarus	‘Yaselda’	00	B	B	e_7
	‘Pripyat’	00	B	B	e_7
	‘Ros’	00	A	D	?
	‘Vilia’	00	B	D	e_7
	‘Stviga’	00	B	B	e_7
	‘Berezina’	00	C	D	e_7
	‘Yanina’	00	B	B	e_7
	‘Snezhok’	00	A	A	E_7
	‘SN 54-11’	00	A	D	?
	‘SN 62-19’	00	A	A	E_7
	‘SN 1470-20-1’	0	B	D	e_7
Netherlands	‘Line # 12’	0	A	A	E_7
Lithuania	‘DSS 2527’	0	A	A	E_7
Poland	‘LF 19’	00	A	A	E_7
Sweden	‘Fiskeby III’	000	B	B	e_7
	‘Fiskeby V’	000	B	E	e_7