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Genetic Control of Photoperiod Response in Early-Maturing, Near-Isogenic Soybean Lines

E. R. Cober, J. W. Tanner, and H. D. Voldeng*

ABSTRACT

Photoperiod response is one factor responsible for the regional adaptation of soybean [*Glycine max* (L.) Merr.] cultivars. Few photoperiod response studies have been carried out with lines containing alleles for late maturity at only one or a few loci. An understanding of the photoperiod response of early-maturing soybean lines would facilitate cultivar development in short-season areas. The objectives of this study were to investigate the photoperiod response of early-maturing 'Harosoy' near-isogenic lines with indeterminate and determinate growth habit and to examine the genetic model for sensitivity to natural day length extended to 20 h with incandescent lamps [incandescent long day length (ILD)]. Harosoy near-isogenic lines were grown in the field under natural day length and ILD. The same lines were also grown in growth cabinets under 12- and 20-h photoperiods with cool white fluorescent plus incandescent lamps. Under natural day length, E_3 and E_4 alleles each delayed flowering 5 d and maturity 15 d while the E_1 allele delayed both flowering and maturity ≈ 16 d compared with the alternative early-maturing alleles. The E_3 and E_4 alleles each delayed flowering 30 d under ILD compared with natural day length. The E_1 allele did not delay flowering or maturity under ILD compared with natural day length. Under 12-h days in a growth cabinet, there were no differences among near-isogenic lines for flowering or maturity, but the loci responded differently in the 20-h photoperiods. The E_3 allele exhibited the largest photoperiod response, delaying flowering 24 d and maturity 84 d, compared with 12-h photoperiods. These photoperiod-sensitivity loci produced differential photoperiod responses that may be useful for short-season cultivar development.

PLANT BREEDERS and agronomists in North America continue to move soybean cultivation to more northern short-season long-day length areas. Soybean cultivars are generally adapted within a narrow north–south band (Scott and Aldrich, 1983). This regional adaptation is due primarily to photoperiod response, where southern cultivars respond to long days and are too late maturing in the north and northern cultivars respond to the shorter days and mature too early in the south. Elite southern germplasm is generally too late maturing to be easily utilized as parents in short-season breeding programs. An understanding of the photoperiod response conditioned by genes controlling flowering and maturity should allow northern soybean breeders to more efficiently include elite southern germplasm in their cultivar development programs.

Five loci have been reported to control time to flowering and maturity in soybean: E_1 and E_2 (Bernard, 1971); E_3 (Buzzell, 1971); E_4 (Buzzell and Voldeng, 1980); and E_5 (McBlain and Bernard, 1987). Bernard (1971) used a 'Clark' background to find that the E_1 allele delayed flowering 16 to 23 d but also shortened the reproductive period by delaying maturity only 15 to 18 d. The E_2 allele lengthened both the vegetative and reproductive periods by delaying flowering 7 to 14 d and maturity 14 to 17 d. Buzzell and Bernard (1975) reported that the E_3 allele delayed maturity by 6 d in a Clark background while Buzzell (1971) used two crosses to find that the E_3 allele delayed maturity 8 d. McBlain et al. (1987) studied the E_1, E_2, and E_3 alleles in Clark and Harosoy backgrounds and stated that flowering and maturity were delayed differently by alleles at each locus. The E_1 allele delayed flowering, but did not lengthen the reproductive period. The E_2 and E_3 alleles lengthened both the pre-flowering and post-flowering periods. E_2 delayed maturity 7 to 10 d, and E_3 delayed maturity 4 to 6 d. Saindon et al. (1989a) found the E_4 allele delayed flowering 1 to 6 d and delayed beginning maturity (R7) 8 to 20 d in a number of different genetic backgrounds. McBlain and Bernard (1987) reported that the E_5 allele was similar to the E_2 allele in delaying flowering and maturity.

Most work with maturity genes has used backcross-derived, near-isogenic lines to identify these genes and to evaluate the effects of alternative alleles at a single locus. This work has been done in germplasm of maturity group (MG) II or later where the two common cultivars used as recurrent parents in backcrossing to develop near-isogenic lines were Clark (MG IV) (Genotype e_1 E_2 E_3 E_4 e_5) and Harosoy (MG II) (Genotype e_1 e_2 E_3 E_4 e_5) (McBlain and Bernard, 1987; Bernard et al., 1991). To understand the genetic basis of earliness in natural field conditions in short-season areas, early-maturing near-isogenic lines that have late-maturing alleles at only one locus are desirable. Few of the maturity loci have been studied in the absence of late alleles at the other known maturity loci. This condition avoids potential interactions between late-maturing alleles.

Determinate growth habit (dt_1) also has an effect on time to flowering and maturity. Bernard (1972) reported that determinate near-isogenic lines matured 2 to 3 d earlier than indeterminate lines. Foley et al. (1986) reported similar results, with determinate lines flowering 1 d earlier and maturing 3.5 days earlier than indeterminate lines. There are no reports in the literature of testing the dt_1 allele for photoperiod responsiveness.

E.R. Cober and H.D. Voldeng, Plant Res. Ctr., Agric. Agri-Food Canada, Ottawa, ON, Canada, K1A 0C6; and J.W. Tanner, Crop Science Dep., Univ. of Guelph, Guelph, ON, Canada, N1G 2W1. Joint contribution from the Crop Science Dep., Univ. of Guelph, and the Plant Res. Ctr., Agric. Agri-Food Canada. PRC Contribution 1603. Received 22 Mar. 1995. *Corresponding author (voldenghd@em.agr.ca).

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Abbreviations: ILD, incandescent long day length; MG, maturity group; HSD, honest significant difference; LSD, least significant difference.

Insensitivity to natural day length extended to 20 h with incandescent lamps, which will be referred to as incandescent long day length (ILD) in this study, was controlled by the E_3 and E_4 loci (Buzzell and Voldeng, 1980; Saindon et al., 1989b). Saindon et al. (1989b) reported epistasis of E_3 on e_4 alleles, where only $e_3e_3e_4e_4$ genotypes were insensitive to ILD. Epistasis of E_4 on e_3 has not been reported. The reaction of the E_1E_1 genotype to ILD has not been reported in the literature.

The objectives of this study were to (i) investigate the photoperiod response of early-maturing, indeterminate, and determinate near-isogenic lines, particularly those with late-maturing alleles at only one locus; and (ii) examine the genetic model for ILD sensitivity, especially the hypothesis of epistasis of E_4 on e_3.

MATERIALS AND METHODS

Field Experiments

Harosoy near-isogenic lines with various maturity gene and growth habit combinations were developed with Harosoy as the recurrent parent in a backcrossing program with a number of different donor parents that served as sources of alternative alleles (Table 1). The near-isogenic lines with an L prefix were described by Bernard et al. (1991), and OT89-5 and OT89-6 were described by Voldeng and Saindon (1991). The remaining lines were developed at the Plant Research Centre, Ottawa, Ontario, Canada. Harosoy near-isogenic lines were grown in the field at Woodstock Research Station, Woodstock, Ontario, Canada (43° N lat.) in 1992 and 1993. Two photoperiod environments were provided: (i) natural day length, with the longest day about 15.4 h; and (ii) 20-h ILD. The ILD was provided by extending the natural photoperiod to 20 h with 150-W incandescent lamps placed 1.5 m above the soil surface. Ranges were 3 m wide, and lamps were spaced 3 m apart within ranges. This provided a mean photosynthetic photon flux of 3 μmol m^{-2} s^{-1} at the canopy surface or 1 μmol m^{-2} s^{-1} at ground level as measured at night with a LI-COR quantum/radiometer/photometer, Model LI185B (LI-COR Inc., Lincoln, NE). From planting to frost, lights were turned on at least 0.5 h before sunset to 2300 h and from 0300 h to at least 0.5 h past sunrise, resulting in a 4-h dark period from 2300 h to 0300 h.

Plots were single rows, 3 m long with 50 cm between rows. Plots were hand planted, with 30 seeds m^{-1}, on 27 May in 1992 and 1993. Fertilizer was applied according to soil test recommendations. Weeds were controlled with a post-emergence application of 0.42 L ha^{-1} Pursuit (240 g L^{-1} imazethapyr [(±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid]) plus 0.5 L ha^{-1} Agral 90 (non-ionic surfactant; nonylphenolethylene oxide condensate) and hand weeding.

Table 1. Harosoy near-isogenic lines used in the study of photoperiod sensitivity and growth habit loci.

Line name	Line designation	Donor	Genotype
Harosoy 63†	L59-731		$e_1E_3E_4Dt_1$
Harosoy-dt_1‡	L67-153	Higan (dt_1)	$e_1E_3E_4dt_1$
Harosoy-e_3	L62-667	T204 (e_3)	$e_1e_3E_4Dt_1$
Harosoy-e_3dt_1	OT94-37§		$e_1e_3E_4dt_1$
Harosoy-e_4	OT94-41§		$e_1E_3e_4Dt_1$
Harosoy-e_4dt_1	OT94-39§		$e_1E_3e_4dt_1$
Harosoy-e_3e_4	OT89-5 (PI 546.043)	PI 438.477 (e_4) T204 (e_3)	$e_1e_3e_4Dt_1$
Harosoy-$e_3e_4dt_1$	OT89-6 (PI 546.044)	PI 438.477 (e_4) T204 (e_3) Higan (dt_1)	$e_1e_3e_4dt_1$
Harosoy-$E_1e_3e_4$	OT93-28¶		$E_1e_3e_4Dt_1$
Harosoy-E_1e_3	L71-802	PI 196.166 (E_1)	$E_1e_3E_4Dt_1$

† Harosoy 63 has the genotype $e_1e_2E_3E_4e_5Dt_1$.
‡ Substituted alleles only are indicated in the name.
§ Selected from the cross OT89-5/L67-153.
¶ Selected from the cross OT89-5/L71-802.

Natural day length plots were managed similarly to ILD plots, except in 1993, where natural day length plots were 1.5 by 5.5 m with four rows. Some of these near-isogenic lines were also grown at the Plant Research Centre, Ottawa, ON (45° N lat.) in 1993. Four-row plots, 1.6 by 5 m, were planted 30 May 1993. No fertilizer was recommended or applied. Weeds were controlled with 2.25 L ha^{-1} Dual (960 g L^{-1} metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide]) plus 2 kg ha^{-1} Lorox (500 g kg^{-1} linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea]). The maximum day length at Ottawa is 15.7 h.

Days to first flower (R1) and maturity (R8) were recorded according to Fehr and Caviness (1977). The reproductive period was calculated as R8 − R1. Within each photoperiod environment, entries were planted with two replications in a randomized complete-block design. For analyses of variance, genotypes and photoperiod treatments were considered fixed effects. Means within photoperiod treatments were compared with Tukey's honest significant difference (HSD) test. When there were only three treatments, the least significant difference (LSD) test was used to compare means. The LSD test was also used to compare treatment means for the Ottawa site. Genotypes were compared across photoperiod treatments with *t*-tests.

Growth Cabinet Experiments

Harosoy near-isogenic lines were grown in 6-L pots filled with potting mix (Metro-mix 245, W.R. Grace and Co. of Canada, Ajax, ON, Canada) and covered with 2 cm of clay chips (Turface, Applied Industrial Materials, Deerfield, IL). Pots were overseeded and thinned to one plant per pot. Fertilizer (20–4.37–16.6, N–P–K) was applied once per week with irrigation water. Days to R1 and beginning maturity (R7) were observed according to Fehr and Caviness (1977). The reproductive period was calculated as R7 − R1. R7 (beginning maturity) was easier to score than full maturity (R8) especially under 20-h conditions in the growth cabinet.

Twelve- and 20-h photoperiods were provided by cool white fluorescent lamps (Sylvania, Mississauga, Ontario, Canada, CW Very High Output F96T12) and 40-W incandescent lamps, which provided a total photosynthetic photon flux of 230 μmol m^{-2} s^{-1}. Air temperature was measured 75 cm from the lamps and maintained at 25°C during both light and dark periods. Each photoperiod experiment was grown in a growth cabinet in a randomized complete-block design with four replications. At R1, in the 20-h photoperiod, the genotypes were subdivided with two replications placed in a 12-h photoperiod and two replications remaining in the 20-h photoperiod. Means within photoperiod treatments were compared with Tukey's HSD test. Genotypes were compared across photoperiod treatments with *t*-tests.

RESULTS AND DISCUSSION

Under natural day length in the field, determinate (dt_1) lines usually flowered and matured earlier and generally had a reduced reproductive period compared with indeterminate (Dt_1) lines (Tables 2 and 3). The E_3 and E_4 alleles were equivalent, each delaying flowering ≈5 d

Table 2. Days to R1, days to R8, and the length of the reproductive period for Harosoy near-isogenic lines grown at Woodstock in 1992 under natural and 20-h incandescent long days.

Genotype	Days to R1		Reproductive period†		Days to R8	
	NAT‡	ILD‡	NAT	ILD§	NAT	ILD§
	d					
$e_1E_3E_4Dt_1$	70 a¶	98 a	82 bc	–#	152 a	–
dt_1	58 b	95 a	87 ab	–#	144 ab	–
$e_1e_3E_4Dt_1$	58 b	86 b	92 a	–#	150 a	–
dt_1	55 b	85 b	78 cd	–#	133 c	–
$e_1E_3e_4dt_1$	56 b	86 b	77 cd	–#	133 c	–
$e_1e_3e_4Dt_1$	53 b	57 d	80 bc	76 ab	133 c	133 b
dt_1	52 b	56 d	70 d	69 b	122 d	125 b
$E_1e_3e_4Dt_1$	69 a	65 c	81 bc	82 a	150 a	147 a
CV, %	3.6	1.9	0.6	2.9	2.6	1.4

† Days between growth stages R1 and R8.
‡ NAT = natural day length at Woodstock; ILD = natural day length extended to 20-h photoperiod with incandescent lamps.
§ LSD (P = 0.05) is used to compare three means.
¶ Means, within a column, followed by the same letters are not significantly different (P = 0.05) according to Tukey's HSD test.
# These lines did not mature before a killing frost.

Table 3. Days to R1, days to R8, and the length of the reproductive period for Harosoy near-isogenic lines grown at Woodstock under natural and 20-h incandescent long days and at Ottawa under natural days in 1993.

Genotype	Days to R1		Reproductive period†		Days to R8		
	NAT‡	ILD‡	NAT	ILD§	NAT	ILD§	OTT‡§
	d						
$e_1E_3E_4Dt_1$	52 c¶	83 b	81 a	–#	133 a	–	138 a
dt_1	47 d	78 bc	69 bc	–#	116 b	–	128 cd
$e_1e_3E_4Dt_1$	46 de	70 cd	74 ab	–#	120 b	–	134 b
dt_1	43 def	62 d	64 c	–#	107 c	–	128 cd
$e_1E_3e_4Dt_1$	46 de	79 bc	75 ab	–#	120 b	–	131 bc
dt_1	45 de	60 d	64 c	–#	109 c	–	126 d
$e_1e_3e_4Dt_1$	42 ef	44 e	62 c	70 ns	104 c	114 b	120 e
dt_1	41 f	43 e	53 d	65	94 d	108 b	108 f
$E_1e_3e_4Dt_1$	57 b	61 d	70 bc	70	127 a	130 a	–††
$E_1e_3E_4Dt_1$	64 a	104 a	–#	–#	–	–	–††
CV, %	1.9	4.3	2.8	4.2	1.3	2.6	1.4

† Days between growth stages R1 and R8.
‡ NAT = natural day length at Woodstock; ILD = natural day length extended to 20-h photoperiod with incandescent lamps; OTT = natural day length at Ottawa.
§ LSD (P = 0.05) is used to compare means.
¶ Means, within a column, followed by the same letters are not significantly different (P = 0.05) according to Tukey's HSD test.
# These lines did not mature before a killing frost.
†† These lines were not grown at Ottawa.

and maturity 15 d. These two loci appeared to act additively in a Harosoy background for both flowering and maturity. The E_1 allele resulted in a large delay in flowering (16 d) and maturity (20 d), compared with the e_1 allele. This long delay in flowering and maturity is similar to other reports for the E_1 allele (Bernard, 1971; McBlain et al., 1987). The $E_1e_3e_4Dt_1$ (Harosoy-$E_1e_3e_4$) isoline was equivalent to the $e_1E_3E_4Dt_1$ isoline (Harosoy) for days to maturity.

The $e_1e_3e_4$ (Harosoy-e_3e_4) and $e_1e_3e_4dt_1$ (Harosoy-$e_3e_4dt_1$) isolines were relatively unaffected by the ILD photoperiod. In 1992, flowering was slightly delayed, while in 1993, maturity was significantly delayed (Table 4). These genotypes were selected for insensitivity to a 20-h ILD regime (Voldeng and Saindon, 1991). While determinate lines matured earlier than indeterminate lines under natural day length, most determinate and indeterminate lines were unable to mature under ILD. The E_3 and E_4 alleles were most responsive to ILD with each late allele delaying flowering $\approx$30 d under ILD. In combination, the loci delayed flowering less than the additive effect of the two alleles. Genotypes with E_3 or E_4 alleles failed to reach maturity before frost under ILD. While the $E_1e_3e_4Dt_1$ isoline was much later to flower and mature than the $e_1e_3e_4Dt_1$ isoline under natural day length, the E_1 allele did not respond to ILD with a further delay in flowering or maturity (Table 4). Thus, it appeared that in the $E_1e_3e_4Dt_1$ isoline, the photoperiod response reached a maximum at or before a photoperiod of 15.4 h. The E_3 and E_4 alleles showed a much different response; they both responded only slightly to natural day length as seen in the slight flowering delay compared with the e_3 and e_4 alleles, but both E_3 and E_4 alleles produced a large flowering delay under ILD. Since the E_1 allele did not respond to extended day length, $E_1E_1e_3e_3e_4e_4$ genotypes may show consistent maturity or temperature requirements across a wider range of latitudes than is usually the case for soybean cultivars. If the photoperiod response (flowering delay) of E_1 remains constant after a specific photoperiod is reached, day length will become unimportant to adaptation at higher latitudes and temperature requirements will become important in determining the adaptation of these genotypes. Shortening days in the autumn will allow rapid reproductive development. The day length that elicits the maximum photoperiod response will provide the southern limit to the zone of adaption. Perhaps cultivars could be developed that would mature in a given number of days or thermal units within a wide range of latitude.

In the growth cabinet study (Table 5), determinate growth habit did not significantly affect flowering or maturity under either 12- or 20-h days. The early-maturing $e_1e_3e_4Dt_1$ and $e_1e_3e_4dt_1$ isolines were used as a baseline, and a photoperiod response was noted if there was a difference between the genotype in question and the early-maturing line with appropriate growth habit. The E_3 allele delayed flowering under long days. The

Table 4. Comparison of natural and 20-h incandescent long day length effects on days to R1, days to R8, and the length of the reproductive period for Harosoy near-isogenic lines grown at Woodstock in 1992 and 1993.

Genotype	Days to R1		Reproductive period†		Days to R8	
	1992	1993	1992	1993	1992	1993
$e_1E_3E_4Dt_1$	***	***	–§	–§	–	–
dt_1	***	***	–§	–§	–	–
$e_1e_3E_4Dt_1$	***	***	–§	–§	–	–
dt_1	***	***	–§	–§	–	–
$e_1E_3e_4Dt_1$	–‡	***	–‡	–§	–	–
dt_1	***	***	–§	–§	–	–
$e_1e_3e_4Dt_1$	*	ns	ns	**	ns	**
dt_1	*	ns	ns	**	*	**
$E_1e_3e_4Dt_1$	*	ns	ns	ns	ns	ns
$E_1e_3E_4Dt_1$	–‡	***	–‡	–§	–	–

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively; NS = nonsignificant (P > 0.05) with t-tests.
† Days between growth stages R1 and R8.
‡ These lines were not grown in 1992.
§ These lines did not mature under 20-h incandescent long day length.

Table 5. Days to R1, days to R7, and duration of the reproductive period in 12-h photoperiod, 20-h photoperiod, and 20-h transferred at R1 to 12-h photoperiod (20-12) for Harosoy near-isogenic lines.

Genotype	Days to R1		Reproductive period†			Days to R7		
	12 h	20 h	12 h	20-12	20 h	12 h	20-12	20 h
	d							
$e_1E_3E_4Dt_1$	26 b‡	66 a	51 ns	62 ns	123 a	76 ns	130 a	188 a
dt_1	25 b	62 ab	52	65	115 a	77	123 ab	180 a
$e_1e_3E_4Dt_1$	27 ab	39 c	50	67	59 b	77	108 ab	97 c
dt_1	25 b	37 cd	52	61	69 b	77	99 ab	105 bc
$e_1E_3e_4Dt_1$	27 ab	58 ab	49	60	117 a	76	119 ab	174 a
dt_1	26 b	54 b	54	70	110 a	80	125 ab	163 a
$e_1e_3e_4Dt_1$	26 ab	34 cd	51	66	54 b	77	101 ab	86 c
dt_1	27 ab	30 d	52	56	56 b	79	88 b	84 c
$E_1e_3e_4Dt_1$	27 ab	32 cd	51	62	55 b	78	97 ab	84 c
$E_1e_3E_4Dt_1$	28 a	57 ab	50	66	73 b	78	123 ab	130 b
CV, %	3.4	8.1	3.9	12.3	10.1	2.6	8.4	6.0

† Days between growth stages R1 and R7.
‡ Means, within the same column, followed by the same letter are not significantly different ($P = 0.05$) according to Tukey's HSD test.

E_4 allele did not significantly delay flowering under long days. The E_1, compared with the e_1, allele did not respond to long days in the growth cabinet; this was seen in the similar responses of the $E_1e_3e_4Dt_1$ and $e_1e_3e_4Dt_1$ isolines. The $E_1e_3e_4Dt_1$ isoline did, however, show a significant (5 d) difference in the 12- vs. 20-h comparison for days to flowering (Table 6). This small response to long days for the E_1 allele in growth cabinets was in sharp contrast to field results where the $E_1e_3e_4Dt_1$ isoline flowered and matured much later than the $e_1e_3e_4Dt_1$ isoline. The E_1 and E_4 loci acted nonadditively for days to flowering and maturity under long days; the E_1E_4 combination delayed flowering much more than the individual alleles. Saindon et al. (1990) also reported similar nonadditive action for these two loci.

In the growth cabinet experiment, reproductive development was observed under short and long days following vegetative development under long days. This photoperiod regime of long photoperiods followed by short photoperiods is more characteristic of field conditions where naturally shortening days occur during the reproductive period. The comparison of 20-12 vs. 20-h (Table 6) detected differences in post-flowering photoperiod responses among the genotypes. Only the E_3 allele responded to post-flowering long days, compared with post-flowering short days; the E_1 and E_4 alleles did not appear to lengthen the reproductive period under long days when compared with treatments transferred to short days. This lack of post-flowering photoperiod response is not consistent with the work of Saindon et al. (1989b) in which the E_4 allele lengthened the post-flowering period under long days.

Table 6. Comparison of 12-h, 20-h, and 20-h transferred at R1 to 12-h (20-12) photoperiod effects on days to R1, days to R7, and the length of the reproductive period in Harosoy near-isogenic lines.

Genotype	Days to R1 12- vs. 20-h	Reproductive period† 20-12 vs. 20-h	Days to R7 20-12 vs. 20-h
$e_1E_3E_4Dt_1$	***	***	***
dt_1	***	***	***
$e_1e_3E_4Dt_1$	***	ns	ns
dt_1	***	ns	ns
$e_1E_3e_4Dt_1$	***	***	***
dt_1	***	***	***
$e_1e_3e_4Dt_1$	***	ns	ns
dt_1	ns	ns	ns
$E_1e_3e_4Dt_1$	*	ns	ns
$E_1e_3E_4Dt_1$	***	ns	ns

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively; NS = nonsignificant ($P > 0.05$) with t-tests.
† Days between growth stages R1 and R8.

In this study, there was a marked difference in response, for the E_1 and E_3 alleles, between field and growth cabinet experiments. Under natural day length in the field, the $E_1e_3e_4Dt_1$ isoline was much later to flower and mature than the $e_1e_3e_4Dt_1$ isoline, whereas these two genotypes flowered at the same time under long days in the growth cabinet. A discrepancy between field and growth cabinet experiments also was noted for the E_3 allele. Under long days in the growth cabinet, the E_3 allele delayed flowering $\approx$20 d compared with the e_3 allele, while the E_4 allele did not significantly delay flowering. Under natural day length in the field, both the E_3 and the E_4 allele acted similarly and did not significantly delay flowering compared with the e_3e_4 combination; however, maturity was delayed significantly. The E_3 and E_4 alleles both significantly delayed flowering under ILD. The two late-maturing alleles acted differently in the growth cabinet studies but similarly in both field studies. Light quality studies were undertaken to explore these phenomena (Cober et al., 1996).

The alleles at the three loci under study, E_1, E_3, and E_4, had no effect under short-day conditions. Under a 12-h photoperiod, there was generally no significant difference in time to flower, length of the reproductive period, or days to maturity among the near-isogenic lines. These three loci affected flowering and maturity by conditioning sensitivity to photoperiods >12 h, and they should be regarded as photoperiod-sensitivity genes.

Saindon et al. (1989a) presented a model to explain sensitivity to ILD. Two loci were proposed, E_3 and E_4, each with two alleles, plus epistasis of the E_3 allele on e_4 alleles. Saindon et al. (1989a) concluded that the E_3 locus was of primary importance in the ILD response. The results of the present study show that E_4 is epistatic to e_3 alleles. Dominant alleles at either the E_3, E_4, or at both loci resulted in a flowering delay of 25 to 30 d under ILD, compared with natural day length. Contrary to the conclusion of Saindon et al. (1989a), this study demonstrated that the E_4 allele is just as important in ILD sensitivity as the E_3 allele. The E_1 locus did not appear to be involved in the ILD response, since there was no delay in flowering or maturity for the $E_1e_3e_4Dt_1$ isoline under ILD compared with natural day length.

Saindon et al. (1989b) proposed ILD selection in combination with a backcrossing program to limit the number of backcrosses required to recover early-maturing genotypes when late-maturing parents are used. The current study has shown that lines with the genotype $E_1E_1e_3e_3e_4e_4$ could be selected under ILD conditions. This would allow the use of southern determinate cultivars as parents in a short-season breeding program in combination with an early-maturing source of e_3 and e_4 alleles. Saindon et al. (1989b) suggested that ILD be terminated at R5

to avoid losing some late-maturing ILD-insensitive germplasm. However, the ILD selection technique could be used to provide a rigorous test for the early-maturing $e_3e_3e_4e_4$ genotype because flowering would be delayed about one month in segregants with late-maturing alleles at either the E_3 or E_4 locus. If lamps were kept on throughout the season as in this study, late-maturing segregants would fail to reach maturity before frost. Breeders could select $e_3e_3e_4e_4$ genotypes under ILD and not be concerned with alleles at the E_1 locus because the E_1 allele did not respond to ILD. It should be possible to intermate ILD-insensitive F_2 plants from early-maturing lines by southern determinate crosses and develop populations adapted to short season areas but that contain $\approx 50\%$ southern genes. The large delay in maturity under ILD, of ILD-sensitive segregants, would make this scheme feasible.

In conclusion, photoperiod sensitivity appeared to be the function of the genes studied in this work. The E alleles responded to long days with delayed flowering and maturity. In the absence of long days, the E alleles had no effect on flowering or maturity, acting similar to e alleles. The E_3 and E_4 alleles each delayed flowering a few days but delayed maturity to a greater extent in the field. The E_1 allele had a large delaying effect on flowering in the field. These loci acted in an additive fashion with the exception of E_1 and E_4, which acted nonadditively. The model for ILD sensitivity has been shown to be one of duplicate dominant epistasis, where dominant alleles at either the E_3 or E_4 loci resulted in a large flowering delay. The E_1 allele delayed flowering and maturity under natural days compared with the e_1 allele. However, extending the natural day length to 20 h with incandescent lamps did not result in a further flowering delay for E_1. The $E_1E_1e_3e_3e_4e_4$ genotype may show more consistent days-to-maturity across a wider range of latitudes, compared with current cultivars, because of this lack of response to further lengthened photoperiod. A determination of the minimum photoperiod that results in maximum delay in flowering would help to estimate the range of adaptability of this genotype. A photoperiod response experiment to determine this photoperiod may need to use natural light, because artificial light sources did not elicit a photoperiod response in the $E_1E_1e_3e_3e_4e_4$ genotype in this study.

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