RESEARCH

A New Locus for Early Maturity in Soybean

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ABSTRACT

The genetic model for maturity in soybean [*Glycine max* (L.) Merr.] is a series of near-isogenic lines, but they do not span the natural variation for early maturity. The objectives of this study were to determine if a single gene in OT98-17 controls early maturity and if this is a new locus. A cross was made between 'Maple Presto' and OT98-17, an early-maturing Maple Presto–derived backcross line. A total of 201 F_3 progeny rows from this population and Maple Presto were grown at Ottawa, ON, in 1999. In 2000, F_4 progeny rows were grown and 150 late-maturing and 51 early-maturing families were observed to fit a 3:1 ratio (n = 201, X^2 = 0.01, P = 0.90). The early-maturing allele was transferred to a 'Harosoy' background, and isolines were grown from 2002 to 2006 at Ottawa, ON. The isolines were 9 and 6 d earlier maturing in Maple Presto and Harosoy backgrounds, respectively. To determine the independence of this locus, simple sequence repeat molecular markers were used to identify three candidate regions. The gene *E8* specifically mapped to linkage group C1 between Sat_404 and Satt136. No other maturity gene has been mapped to this region. The two other candidate regions were both related to maturity quantitative trait loci on molecular linkage group L and may be inadvertently selected along with early maturity. The gene symbol *E8e8* has been assigned by the Soybean Genetics Committee. *E8E8* results in later maturity and *e8e8* results in early maturity. The earliest Harosoy maturity isoline is now rated as maturity group 000.

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Abbreviations: MLG, molecular linkage group; QTLs, quantitative trait loci; SSR, simple sequence repeat.

In soybean [*Glycine max* (L.) Merr.], seven loci with two alleles at each locus have been reported to control time to flowering and maturity: *E1* and *E2* (Bernard, 1971); *E3* (Buzzell, 1971); *E4* (Buzzell and Voldeng, 1980); *E5* (McBlain and Bernard, 1987); *E6* (Bonato and Vello, 1999); and *E7* (Cober and Voldeng, 2001). Backcross-derived near-isogenic lines (isolines) are commonly developed to identify and study these genes using common genetic backgrounds with alternative alleles (Molnar et al., 2003).

Isolines of the cultivar Harosoy (genotype *e1e1 e2e2 E3E3 E4E4 e5e5 E7E7)* have been developed to identify and study these genes. A Harosoy isoline (OT94-47) containing early-maturity alleles at all known loci is classified as early-maturity group 0 when grown in short-season areas in Canada. Earlier maturing cultivars exist and a genetic model for this earlier maturity is not yet available.

Molecular markers have been identified for some of the flowering and maturing time loci and the *E* loci have been placed on molecular linkage maps. *E1* is located on molecular linkage group (MLG) C2 at position 113.0 cM on the 2003 composite map (Song et al., 2004). *E7* is linked to *E1* and also resides on MLG C2 (Molnar et al., 2003). *E2* is located at 136.3 cM on MLG O (www.soybase.org [verified 18 Sep. 2009]). *E3* is located on MLG L (Molnar et al., 2003). *E4* is located on MLG I (Abe et al., 2003; Molnar et al., 2003). Recessive alleles at *E6* condition delayed flowering under short days

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and were found in tropical soybean (Bonato and Vello, 1999). As a result, it is highly unlikely that *E8* is an allele at *E6*.

The objectives of this study were to determine if a single gene controls early maturity in OT98-17 and if a new locus is involved, as well as to determine maturity and some agronomic characteristics of *E8* isolines in the field.

MATERIALS AND METHODS

Field Experiments

Isolines from two genetic backgrounds, 'Maple Presto' (Voldeng et al., 1982) and Harosoy (Weiss and Stevenson, 1955), were used as parents or treatments in this study (Table 1). L62-667 was described by Bernard et al. (1991). The remaining lines were developed at the Eastern Cereal and Oilseed Research Centre, Ottawa, ON, Canada. OT98-17, a Maple Presto backcross-derived line, was developed by transferring early maturity from X824A-ve to Maple Presto. X824A-ve was selected from the cross PI 438477/2 × Evans//L62-667. We presume that PI 438477 (Fiskeby 840-7-3) was the source of early maturity in X824A-ve transferred to OT98-17. OT02-18, an early-maturing Harosoy isoline, was developed by crossing an early-maturing Maple Presto BC_2 line, which was also an intermediate parent of OT98-17, with OT89-5 and then OT94-47, the early-maturing Harosoy recurrent parents.

To develop a genetic test population, a cross was made between Maple Presto and OT98-17, an early-maturing Maple Presto backcross-derived line. Single F_2 plants were randomly selected and harvested in 1998. In 1999, 201 F_3 progeny rows were planted at the Central Experimental Farm, Ottawa, ON, Canada, on 14 May and maturity dates were recorded for each row. Maple Presto was planted in border rows as a maturity check. From each F_3 row, five random contiguous plants were harvested. F_4 progeny rows were planted on 20 May 2000, with the two parents planted every 20 rows as check rows. In both years, progeny and check rows were planted in 4-m rows with 50 cm between rows at a seeding rate of about 15 seeds m^{-1} of row. Date of maturity (95% of pods with mature color) was recorded for each F_3 and F_4 progeny row. A chi-square test was used to test the goodness of fit of observed-to-expected ratios.

Field trials, with three replicates, of Harosoy and Maple Presto isolines were grown at Ottawa from 2002 to 2006. Trials were planted between 20 and 30 May, during the 5-yr period, in four row plots, 1.6 by 5 m in area. Entire plots were combine-harvested and seed yield was determined. The mass of 100 seeds randomly selected from each plot was determined and used as a measure of seed size. For each plot in the field, days from planting to maturity and a single plant height measurement were recorded. The GLM procedure of SAS (Version 8, SAS Institute, Cary, NC) was used for an analysis of variance. Environments were considered random, while all other effects were considered fixed.

Molecular Analysis

To identify candidate map locations for the *E8* locus, the maturity lines of interest were genotyped with a total of 78 microsatellite (simple sequence repeat [SSR]) markers selected from those designed and mapped by Cregan et al., (1999). The SSR markers were chosen to flank 25 maturity loci or maturity quantitative trait loci (QTLs) reported in Soybase (www.soybase.org [verified 24 Sept. 2009]) with additional SSR markers to provide genome-wide coverage. Standardized procedures for DNA isolation, polymerase chain reaction amplification, and gel analysis were reported earlier (Molnar et al., 2003). Maturity lines studied included the earliness donor PI 438477 (the early-maturing parent of X824A-ve); the isolines OT98-17 and Maple Presto; the Harosoy-based isolines OT02-18 and OT94-47; and Harosoy. In addition, from OT98-17 × OT94-47 (cross X4627, which was segregating for *E8* in a Harosoy background), one BC_7F_3 family (X4627-1) produced two late F_4 lines and four early F_4 lines, a second BC_7 F_3 family (X4627-3) produced two early F_4 lines, and the third BC_7 F_3 family gave three early F_4 lines. For preparing DNA for genotyping, leaves of early lines were collected and bulked from multiple F_5 plants because early lines are homozygous *e8e8*, whereas for each late line, leaves were collected from three F_5 individual plants that were separately genotyped. Thus, from the X4627 cross, nine DNA preparations representing bulked leaves from nine early lines and six DNA preparations representing three individual plants from each of two late lines were studied together with the parents.

Each line's genotype at the *E8* locus was predicted based on pedigree and family relationships and on maturity phenotype. On this basis, seven allelic relationships among the lines were predicted for the *E8* locus, most of which should also occur for tightly linked SSR loci:

1. OT98-17 (*e8e8*) has a different allele from Maple Presto (*E8E8*) because they are isolines contrasting for *E8*;
2. OT02-18 (*e8e8*) has a different allele from OT94-47 (*E8E8*) because they are isolines contrasting for *E8*;
3. OT98-17 (*e8e8*) has same allele as OT02-18 (*e8e8*) because there is only one *e8e8* donor;
4. X4627 early lines (*e8e8*) have the same allele as early parent OT98-17 (*e8e8*);
5. X4627 early lines (*e8e8*) have different allele from late parent OT94-47 (*E8E8*);
6. X4627 late plants (*E8E8* or *E8e8*) are probably homozygous because they are $BC_7F_{4:5}$ plants, in which case they have the same allele as late parent OT94-47 (*E8E8*);
7. Earliness donor line PI 438477 (*e8e8*) has the same allele as the derived early line OT98-17 (*e8e8*).

Observed SSR allelic relationships were compared with those predicted for the *E8* locus to identify clusters of SSRs that might be tightly linked to the *E8* locus.

To determine the correct candidate region for the *E8* locus, a small selected population of 15 clearly early- plus 23 clearly late-maturing F_2 lines derived from OT98-17 (*e8e8*) × OT94-47 (*E8E8*) (cross X4957-e8) was genotyped with 15 SSR markers derived from the candidate regions. Given the limited number of nonrandom lines available, rigorous recombination mapping was not possible and so molecular marker order was assumed to be the same as in the consensus soybean recombination map (Song et al., 2004) and the best placement of the *E8* locus into a marker interval was determined using MapMaker Macintosh V2.0 (Lander et al., 1987).

RESULTS AND DISCUSSION

Over 5 yr on the Central Experimental Farm, Maple Presto matured in 97 d and OT98-17, a Maple Presto isoline, matured in 88 d (Table 2). In 1999, 201 randomly derived F_3 progeny rows, from a cross between Maple Presto and OT98-17, were scored for maturity and separated into 156 late-maturing or segregating and 45 early-maturing lines (Fig. 1a). This population fit a 3:1 ratio ($n = 201$, $\chi^2 = 0.73$, $P = 0.39$). Because 1999 was a dry season, Maple Presto matured almost 2 wk earlier than usual.

The maturity rating of F_3 rows was confirmed in 2000 using five random F_4 progeny rows derived from each F_3 row. The 2000 season was wet and Maple Presto maturity was delayed about 10 d compared to the 5-yr average. Flooding in part of the field resulted in greater delay in maturity in these areas; however, maturity classification of progeny rows was possible because both parents were planted every 20 rows throughout the nursery. The maturity variability is reflected in the large standard error for the parental means and the overlap of the maturities of the early and late families (Fig. 1b). In 2000, there were 51 early-maturing, 57 segregating, and 93 late-maturing families. Using the information from the $F_{3:4}$ lines, there were a total of 22 errors made in the 1999 classification of F_3 rows; two early-maturing-classified lines were late maturing, six early-maturing-classified lines were heterozygous, and 14 late-maturing-classified lines were early maturing. Since only five rows were grown per F_3 family, the probability of finding a recessive early-maturing F_4 row in a group of five rows from a heterozygous F_3 row was approximately 75%. This resulted in the segregating class being underrepresented and the late-maturing homozygous class overrepresented in the observations. Because F_3 derived families could not be accurately separated into heterozygous and homozygous late-maturing, the two groups were pooled together, resulting in a ratio of 150 late-maturing and 51 early-maturing families. These observations fit a 3:1 ratio ($n = 201$, $\chi^2 = 0.01$, $P = 0.90$).

The *e8e8* genotypes were 9 and 6 d earlier maturing in Maple Presto and Harosoy genetic backgrounds, respectively. Across the two backgrounds, *e8e8* isolines yielded about 60%, were about 80% as tall, and had smaller seed compared with the *E8E8* isolines (Table 2).

To determine whether this early-maturity gene was at a new locus, molecular markers were used to compare parental isolines and derived lines, in an effort to determine the genetic map location of the *E8* locus. The lines were genotyped with a total of 78 SSR markers, which were selected from the soybean consensus map (Song et al., 2004) to ensure markers closely linked to 25 previously reported maturity loci or QTLs as well as markers well dispersed throughout the genome. Since earliness (*e8e8*) is recessive, it was possible based on pedigree and on observed maturity to predict the genotype at the *E8* locus for the lines under study. The predicted genotypes suggested seven independent allelic relationships at the *E8* locus (details in Materials and Methods), many of which would also be expected at closely linked SSR markers. A review of the SSR genotypes of the maturity lines obtained with the 78 SSR markers identified three candidate regions for the *E8* locus. On linkage group C1, SSR marker Satt361 (passes six of seven [6/7] test relationships) is flanked by Satt399 (5/7), Satt139 (5/7), Sat_077 (5/7), and Sat_042 (5/7). A second candidate region is on MLG L near Satt313 (5/7), Satt284 (5/7), and Satt497 (5/7). The third candidate region is also on MLG L near Satt166 (4/6). The second and third candidate regions are believed to be discreetly different because of low correlations for intermediate markers such as Satt481 (1/7), Satt156 (1/7), and Satt527 (2/6). To differentiate between the three candidate regions, 15 clearly early- and 23 clearly late-maturing F_2 lines were selected from a population developed from OT98-17 × OT94-47 and genotyped with 12 SSR markers located in the MLG C1 candidate region

Table 1. Soybean isolines used as parents to develop early-maturing isolines and isolines used in this study of early maturity.

Line	Genotype†	Pedigree
Harosoy	*e1e1 E3E3 E4E4 E7E7*	Mandarin (Ottawa) × 2/AK (Harrow)
L62-667	*e1e1 e3e3 E4E4 E7E7*	Harosoy × 6/T204
OT89-5	*e1e1 e3e3 e4e4 E7E7*	PI 438477/2 × Evans/2/7 × L62-667
X2749-K1	*e1e1 e3e3 E4E4 e7e7*	PI 196529/6 × L62-667
OT94-47	*e1e1 e3e3 e4e4 e7e7*	OT89-5/X2749-K1
X824A-ve	*e1e1 e3e3 e4e4 e7e7*	PI 438477/2 × Evans/2/L62-667
OT02-18	*e1e1 e3e3 e4e4 e7e7 e8e8*	X824A-ve/3 × Maple Presto/2/3 × OT89-5/3/3 × OT94-47
Maple Presto	*e1e1 e3e3 e4e4 e7e7*	Amsoy/Portage/2/PI 438477
OT98-17	*e1e1 e3e3 e4e4 e7e7 e8e8*	X824A-ve/7 × Maple Presto

†Harosoy and all the Harosoy isolines also have the genotype *e2e2 e5e5* (Bernard et al., 1991). Maple Presto is also presumed to have the same *e2e2 e5e5* genotype.

Table 2. Maturity and agronomic characteristics of soybean maturity isolines grown at Ottawa, ON, Canada, over 5 yr, 2002 to 2006.

Isoline	Background†	Genotype‡	Maturity	Seed yield	Plant height	Seed weight
			d	kg ha^{-1}	cm	g 100^{-1}
Maple Presto	MP	*E8E8*	97	1846	63	16.8
OT98-17	MP	*e8e8*	88	994	53	15.4
OT94-47	Har	*E8E8*	104	2228	71	19.7
OT02-18	Har	*e8e8*	98	1471	54	17.5
$LSD_{0.05}$			3	486	15	1.0

†Genetic background of isolines; MP = Maple Presto; Har = Harosoy.

‡*E8E8* has a late-maturing and *e8e8* has an early-maturing phenotype.

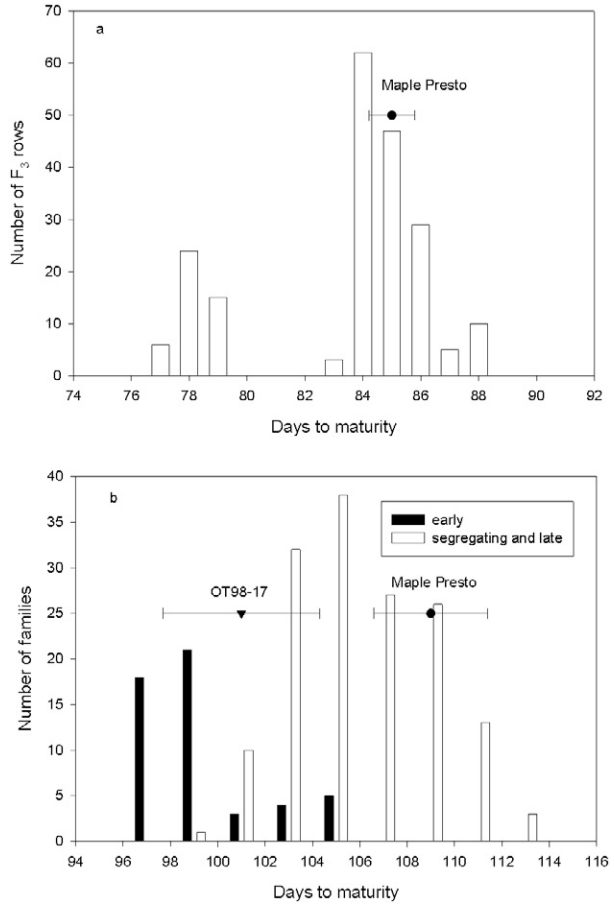

Figure 1. A frequency distribution of the maturity of (a) F_3 progeny rows and (b) mean of $F_{3:4}$ families (n = 5 F_4 rows) from the soybean cross Maple Presto × OT98-17 grown in the field at Ottawa, ON, Canada. Parental maturity is shown as mean ± 2 SE.

and three in the MLG L candidate regions. The *E8* locus grouped strongly (logarithm of odds 6.0) with the MLG C1 markers. Based on the consensus marker order reported by Song et al. (2004), we determined a locus order of Satt161, Satt718, Sat_404, *E8*, Satt136, Satt361, Sat_077, Satt399. The Sat_404 to Satt136 interval is approximately 1 cM and Satt136 is approximately 0.5 cM from Satt361, the marker showing the best correlation with *E8* in our initial analysis of the *E8* near-isogenic lines. However, the exact order of the markers flanking *E8* may differ slightly from the consensus order because fine mapping of such tightly linked markers would require large recombinant populations.

The *E8* genomic region on MLG C1 overlaps with reproductive period QTL 3-4 and possibly also 2-2, Soybase nomenclature for QTLs reported by (Orf et al., 1999), and *E8* may be a candidate gene for either QTL. Interestingly, the first alternate region on MLG L near Satt284 is one that we previously identified as having homology to the MLG C2 region containing *E1* and *E7* and we therefore predicted finding a new maturity locus in this region (Molnar et al., 2003). The second alternate region on MLG L near Satt166 corresponds to pod maturity QTLs 8-3 and 8-4 (Orf et al., 1999) as well as flowering time QTLs 4-3 and 4-4 (Orf et al., 1999). While these associations may be coincidental, it is also possible that these two maturity-related regions on MLG L are being inadvertently selected during breeding for *e8* early maturity.

In summary, *E8* is a new maturity locus and the gene symbol *E8 e8* has been assigned by the Soybean Genetics Committee. *E8E8* results in later maturity, while *e8e8* results in early maturity. The *e1e1 e2e2 e3e3 e4e4 e5e5 e7e7 e8e8* Harosoy isoline (OT02-18) extends the genetic model for maturity to MG 000 (equals the maturity of Maple Presto). The *e8* allele is presumed to have originated from PI 438477, which had been identified as day-neutral by Polson (1972).

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