Assignment of the *E4* locus to Soybean Classical Linkage Group 4

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ABSTRACT

Most soybean cultivars have a short-day requirement for floral induction, in which flowering is normally suppressed under long daylength conditions. Insensitivity of flowering to long daylength is thus an important character in order to develop cultivars adapted to the long daylength of high latitude regions. The present study was conducted to identify and map the maturity gene controlling insensitivity of flowering to incandescent long daylength (ILD) found in an early-maturing cultivar from Hokkaido, Japan. A cross was made between the ILD-insensitive cultivar, 'Ohyachi 2' (*e3e3e4e4*), and the ILD-sensitive line, 'Harosoy-*e3*' (*e3e3E4E4*). F_2 segregation data indicated an involvement of a single recessive gene (*e4*) in the ILD insensitivity of 'Ohyachi 2', and a recombination value of 14.9 ± 3.1 % was estimated between the gene and *Enp* (endopeptidase isozyme) located on linkage group 4. The present study thus confirmed that the maturity gene for the ILD insensitivity of 'Ohyachi 2' is a recessive allele (*e4*) at the *E4* locus, which is located on classical linkage group 4. The order is *E4*, *Enp*, *Ln* (narrow leaflet).

INTRODUCTION

Most soybean (*Glycine max*) cultivars have a short-day requirement for floral induction, and flowering is normally suppressed under long daylength conditions. Insensitivity of flowering to long daylength is thus an important character in the development of cultivars adapted to the long daylength of high latitude regions. Eight loci have been reported to control the time of flowering and maturity in soybean: *E1* and *E2* (Bernard, 1971), *E3* (Buzzell, 1971), *E4* (Buzzell and Voldeng, 1980), *E5* (McBlain and Bernard, 1987), *E6* (Bonato and Vello,

1999), *E7* (Cober and Voldeng, 2001), and *J* (Ray et al., 1995). Of these, *E1*, *E3*, *E4* and *E7* control responses of flowering to artificially induced long daylength (Buzzell, 1971; Buzzell and Voldeng, 1980; Saindon et al., 1989; Cober et al., 1996; Cober and Voldeng, 2001). The *E3* locus was characterized under fluorescent long daylength (FLD) by extending natural daylength to 20 h using cool white fluorescent lamps; *e3* singly controls the insensitivity to FLD (Buzzell, 1971). On the other hand, the *E4* locus was characterized under incandescent long daylength (ILD) by extending natural daylength to 20 h using incandescent lamps; *e4* combines with *e3* to control the insensitivity to ILD (Buzzell, 1971; Buzzell and Voldeng, 1980). Of the four loci, *E3* is linked to *Dt1* (determinate stem) with a recombination value of 27.5 % and is assigned to soybean classical linkage group 5 (CLG 5) (Cober and Voldeng, 1996), *E1* and *E7* are linked to each other at a distance of 6.2 cM on both sides of *T* (pubescence color) and is assigned to CLG 1 (Cober and Voldeng, 2001), but *E4* has not yet been assigned to any linkage group.

Abe et al. (1998) studied the genetic basis of the ILD insensitivity of 'Ohyachi 2', a cultivar adapted in cool-climate environments of Hokkaido, Japan, in a cross involving an ILD-sensitive cultivar, 'Tokachinagaha'. They found that the ILD insensitivity of 'Ohyachi 2' was controlled by a recessive gene tentatively designated *e* (t), which was linked to *Enp* (endopeptidase isozyme) and *Ln* (narrow leaflet), which are members of CLG 4. The recombination value of *e* (t) for the two markers was estimated as 8.3 % for *Enp* and 13.8 % for *Ln*, with the order of the three loci being determined as *E* (t) - *Enp* - *Ln*. 'Ohyachi 2' and some of its descendants have been used in the breeding for early maturity and chilling tolerance in Hokkaido. Two determinate cultivars, 'Kitamishiro' (PI 317334A) and 'Isuzu', were released from the cross between 'Ohyachi 2' and 'Tokachinagaha'. Both cultivars, like 'Ohyachi 2', were insensitive to ILD. Of these two cultivars, 'Kitamishiro' was used to assess the adapted genotype of maturity for determinate cultivars under high-latitude environments with short crop seasons, and was determined to have the genotype of *E1E1e3e3e4e4dt1dt1* (Saindon et al., 1990). Several lines of evidence thus suggest that the ILD insensitivity of 'Ohyachi 2', like that of 'Kitamishiro', is conditioned by *e3* and *e4*, and *e4* may be a candidate of *e* (t) because *e3* is located in CLG 5 (Cober and Voldeng, 1996), a different

linkage group from CLG 4 in which *Enp* and *Ln* are located (Muehlbauer et al., 1989).

The objective of this study was to identify and map the gene for ILD-insensitivity (*e*(t)) observed in the cross between 'Ohyachi 2' and 'Tokachinagaha' (Abe et al., 1998), using a cross segregating for *e4* of 'Ohyachi 2' with an ILD-sensitive line 'Harosoy-*e3*' whose genotype was *e1e1e2e2e3e3E4E4e5e5*.

MATERIALS AND METHODS

A cross was made between 'Ohyachi 2' and 'Harosoy-*e3*' (L62-667) in 1996. An F_1 plant was grown in an experimental field of Hokkaido University, Sapporo, to produce F_2 seeds in 1997. After a small piece of cotyledon was sampled for an isozyme analysis, the three-day-old seedlings were transplanted individually into Jiffypot strips (JIFFY A/S, Denmark) filled with a nursery soil (N: 200 mg, P: 1,000 mg, K: 200 mg per liter) on 28 May 1998. The seedlings were grown in a greenhouse where natural daylength was extended to 20 h by means of 200-W incandescent lamps placed 1.6 m above the soil surface at intervals of 0.7 m. Two weeks later, the seedlings were transplanted to an experimental field with a facility for extending daylength. The ILD was provided by extending the natural photoperiod to 20 h by means of 500-W incandescent lamps placed 2 m above the soil surface at intervals of 4 m. From seedling emergence to July 31, lights were turned on from 0200h to 0600h and from 1800h to 2200h. The natural daylength including twilight at Sapporo reached a maximum of 16.5 h. Under incandescent lamps, the red to far red quantum ratio (R:FR; 660:730) was 0.72, and the average photosynthetic photon flux at the canopy surfaces was 1 μ mol photon sec^{-1} m^{-2}, as measured at night using a LI-COR quantum sensor (Model LI-1800C, LI-COR Inc., Lincoln, NE). The plants were checked every other day for the first appearance of an open flower (R1) till the end of ILD treatment (31 July). Analysis for *Enp* followed the method of Abe et al. (1992).

RESULTS AND DISCUSSION

Under the ILD treatment, 'Ohyachi 2', on average, reached R1 on 24 July, whereas 'Harosoy-*e3*' remained vegetative untill the end of treatment (31 July), although the latter

flowered about one week earlier than the former under natural daylength (our unpublished data). Plants that reached R1 by 31 July were thus classified as ILD-insensitive, and those that were vegetative were classified as ILD-sensitive.

Of 150 F_2 plants tested, 33 were classified as ILD-insensitive and 117 were classified as ILD-sensitive (Table 1), which fitted a 1:3 ratio expected for a monogenic inheritance ($\chi^2 = 0.72$, df = 1, $0.25 < p < 0.50$). A linkage test further revealed that the gene for ILD insensitivity was linked with *Enp* of CLG 4, as observed in the cross of 'Ohyachi 2' with 'Tokachinagaha' (Abe et al., 1998) (Table 1). With the maximum likelihood method of Allard (1956), the recombination value was estimated as 14.9 ± 3.1 %, which was slightly higher than the value 8.3 % reported in the cross of 'Ohyachi 2' with 'Tokachinagaha' (Abe et al., 1998). However, the chi-square value of heterogeneity between the two recombination values was not significant ($\chi^2 = 3.70$, df = 1, $0.05 < p < 0.10$). Because the ILD-insensitivity of 'Ohyachi 2' was assumed to be conditioned by *e3e3e4e4* as 'Kitamishiro' and the genotype of 'Harosoy-*e3*' is *e3e3E4E4*, the results obtained in this study and our previous study (Abe et al., 1998) suggest that the gene responsible for ILD insensitivity segregating in F_2 of the crosses of 'Ohyachi 2' with 'Harosoy-*e3*' and with 'Tokachinagaha' is a recessive allele (*e4*) at the *E4* locus, which is located in CLG 4. The order is *E4*, *Enp*, *Ln*.

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Table 1. Segregation of ILD insensitivity and its association with *Enp* in F_2 of the cross

between ‘Ohyachi 2’ and ‘Harosoy-*e3*’

Marker genotype	No. of F_2 plants		
	ILD-insensitive	ILD-sensitive	Total
Enp-a/a	20	6	26
Enp-a/b	12	74	86
Enp-b/b	1	37	38
Total	33	117	150

‘Ohyachi 2’ has the genotype of *Enp*-a/a, and ‘Harosoy-*e3*’ has the genotype of *Enp*-b/b.