# Inheritance of Genes Controlling Photoperiod Insensitivity and Flowering Time in Soybean

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# Abstract

The objective of this research was to study the inheritance of genes controlling photoperiod insensitivity and flowering time in soybean. Two single-cross populations, IX132 (PI 317.336 X 'Corsoy'), and IX136 (PI 317.334B X 'Corsoy') were developed for this purpose. The populations were inbred to obtain 101 and 100  $F_{677}$  lines, respectively, using a modified single seed descent. Flowering time (days to R1) of the RI lines from each population was observed in the growth chamber at 12 h and 20 h photoperiods using fluorescent and incandescent lamps. Results show that the RI lines have dramatically different responses to day length. A normal distribution of flowering times was observed when the lines were grown in growth chamber with 12 h photoperiod. When the lines were grown in growth chamber with 20 h photoperiod, however, a discontinuous distribution was observed. This suggested that the insensitivity of the RI lines on long day length may be controlled by few major genes. The time of flowering was delayed in almost all lines when grown in growth chamber with 20 h photoperiod compared to those grown in the growth chamber with 12 h photoperiod. The flowering delays were 5 to 75 days in population IX132 and 0 to 75 days in population IX136. Chi-square tests show that the segregation data fit a 1:6:1 ratio in population IX132 and IX136. Based on these tests a minimum of three genes are proposed to control photoperiod insensitivity in both populations. .

## Introduction

Soybean [*Glycine max* (L.) Merr.] is recognized as a short day plant (Garner and Allard 1920; Kenworthy et al. 1989). Most soybean genotypes require short day exposure to initiate flowering. Shanmugasundaram and Tsou (1978) reported that for photoperiod sensitive genotypes, 27 short days (10 h photoperiod) were required for flowering induction and that anthesis was observed 9 days after the completion of the induction. They also reported that the critical time of short day exposure was 9 days after emergence.

Photoperiod insensitivity also has been reported in soybean (Yoshida 1952; Criswell and Hume 1972; Guthrie 1972; Nissly et al. 1981). Two insensitive lines include PI 317.336 ('Sinshei') and PI 317.334B ('Kitami-Shiro'); two genotypes that were introduced from Japan. In addition, it has also been reported that the early-maturing genotypes are less affected by changes in photoperiod than later maturing genotypes (Johnson et al. 1960; Byth 1968; Criswell and Hume 1972; Kenworthy et al. 1989).

In this study we use segregating progeny of two single-cross populations segregating for photoperiod insensitivity to estimate the number of genes controlling this trait and to evaluate the segregation distribution frequencies of the populations. This study was conducted in preparation for a QTL mapping study.

## **Materials and Methods**

#### **Population development**

Two single-cross populations were developed. The first population, IX132, was developed by crossing PI 317.336 ('Shinsei') and 'Corsoy'. The second population, IX136, was developed by crossing PI 317.334B ('Kitami-Shiro') and 'Corsoy'. Both PI parents were reported to be photoperiod insensitive with regard to flowering time and maturity and are classified as maturity group 0 (MG 0) (PI 317.336) and MG III (PI 317.334B) (Guthrie 1972; Nissly et al. 1981). Both PI parents also display strongly determinate stem morphology (Metz et al. 1985). Corsoy, on the other hand, is classified as MG II, has indeterminate stem morphology, and is photoperiod sensitive.

The populations were advanced separately by a modified single seed descent. Between 10 and 20  $F_1$  seeds were made for each cross.  $F_1$  plants were bulk harvested to obtain  $F_2$  seed. The  $F_2$  seed bulk was divided into a portion to reserve in cold storage and a portion to plant in bulk. The populations were advanced to the  $F_6$  generation by pod bulking (Fehr 1987a, b). A two-to-three-seeded pod was harvested from each plant in the  $F_2$  through  $F_5$  generation, and the seeds were bulked. At each generation, the seed bulk was divided into a portion to reserve in cold storage and a portion to plant in bulk. The  $F_{6:7}$  seeds from these plants were grown for evaluation in growth chamber. A total of 101 lines from population IX132 and 100 lines from population IX136 were evaluated for days to first flower (days to R1).

#### Growth chamber observation

The parents and progeny of each cross were grown in growth chamber (gc) under two different day lengths, 12 h and 20 h. Three seeds were planted in each pot then thinned to one plant at the two open leave stage. Each line consisted of three pots and was replicated

twice. The R1 stage (Fehr and Caviness 1977) was observed as the number of days after emergence when the first bloom appears in a plant. Flowering time (days to R1) was recorded separately for each day length treatment. The difference in flowering time between 12 h and 20 h day length was used to determine the number of days flowering time was delayed by treatment of a 20 h photoperiod.

### Data analysis

Flowering delay due to long day treatment was determined for each line by subtracting the number of days to R1 when grown in growth chamber with 20 h photoperiod (gc20), from the number of days to R1 when grown in a growth chamber with 12 h photoperiod (gc12). Based on frequency distribution of the data for days to R1 (gc20) and the number of days flowering was delayed, we then classified the plant phenotypes into two or three phenotypic classes (Table 1). A Chi-square test was performed to test the goodness of fit of each proposed segregation ratio.

## **Results and Discussion**

The distribution frequencies for days to R1 in gc20 and days that flowering was delayed are shown in Figure 1. Flowering delay of each RI line was determined by subtracting the number of days to R1 when grown in the gc20, from the number of days to R1 when grown in gc12.

The lines grown in gc20 clearly group into distinct phenotypic classes. For example, in population IX132 there are no plants observed in days 35 and 90 (Figure 1a). These are 'natural' break points that separate the extreme sensitive and insensitive phenotypes from those of moderate insensitivity. Break points were also observed for number of days to R1 delayed (Figure 1b), with no plants observed in days 15 and 65, thus again separating the three phenotypes. Natural break points are also observed in population IX136 (Figures 1c and 1d).

The number of genes that control photoperiod insensitivity was predicted using the segregation data for days to R1 from RI lines grown in gc20 and the data for days to R1 delayed due to long day treatment. Chi-square tests of the segregation data show that photoperiod insensitivity fits very well to a 1:6:1 segregation ratio (P=0.84) in population IX132 based on the gc20 data. In this population a 1:6:1 segregation ratio (P=0.91) based on the flowering delayed data (Table 1) is also acceptable. In population IX136, photoperiod insensitivity also fits a 1:6:1 segregation ratio (P=0.15) based on gc20 data as well as a 1:6:1 segregation ratio (P=0.07) based on the flowering delayed data (Table 1). A Chi-square test rejected the two-gene model segregation ratio of 1:2:1 for population IX132 (Table 1) for both gc20 data and data for flowering delay. A Chisquare test also rejected the two-gene model segregation ratio of 1:2:1 for the gc20 and flowering delay data for population IX136. These data suggest that photoperiod insensitivity is controlled by a minimum of three genes in populations IX132 and IX136. Results of our experiment are similar to the current soybean gene-model explaining the sensitivity to incandescent long day (ILD) reported by Saindon et al. (1989) and Cober et al. (1996). Therefore, based on this study, we find it plausible to accept that three or more genes control insensitivity of soybean to long day length, thus making this an acceptable target for QTL analyses.

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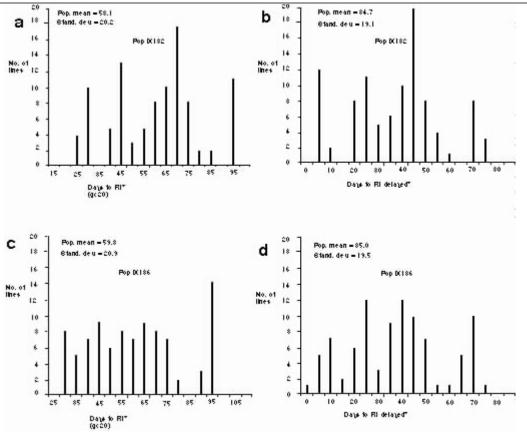
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Figure 1. Distribution frequency of flowering time (days to R1) of the F<sub>6:7</sub> RI lines in population IX132 and IX136 when grown in growth chamber with 20 h photoperiod (a and c) and number of days flowering time was delayed due to 20 h photoperiod (b and d).

Table 1. Chi-square analyses of segregation ratios for flowering time and number of days that flowering was delayed among RI lines from populations IX132 and IX136.Phenotypic classes were assigned based upon 'natural' break-points in the distribution frequencies within each population. Two-gene and three-gene models were tested.

Flowering tim	e (days to R1) <sup>a</sup>	a					
	Early (Ins)	Interm.	Late (Sens)	Total progeny	Ratio tested <sup>b</sup>	X <sup>2c</sup>	P-value
	25-38d <sup>d</sup>	39-80d <sup>d</sup>	>80d <sup>d</sup>	-			
	0-15d <sup>e</sup>	16-55d <sup>e</sup>	>55d <sup>e</sup>				
Population IX	132						
Based on gc20							
Observed	14	76	11	101	1:6:1	0.36	0.84
Expected	12.6	75.8	12.6	101			
Observed	14	76	11	101	1:2:1	26.14	< 0.001
Expected	25.3	50.4	25.3	101			
Based on flowe	ering delayed de	ata					
Observed	14	74	12	101	1:6:1	0.2	0.91
Expected	12.6	75.8	12.6	101			
01 1		7.4	10	101		24.1	< 0.001
Observed	14	74	12	101	1:2:1	24.1	< 0.001
Expected	25.3	50.4	25.3	101			
Population IX	136						
Based on gc20	data						
Observed	16	67	17	100	1:6:1	3.45	0.15
Expected	12.5	75	12.5	100			
Observed	16	67	17	100	1:2:1	11.58	0.015
Expected	25	50	25				
Dural (1							
Observed	ering delayed de 16	65	19	100	1:6:1	5.69	0.07
Expected	10	75	19	100	1:0:1	5.09	0.07
Барескей		15	12.3	100			
Observed	16	65	19	100	1:2:1	9.18	0.01

Expected	25	50	25	100					
<sup>a</sup> Ins=Insensitive to long day length; Sens=Sensitive to long day length; Interm.=intermediate phenotype.									
<sup>b</sup> Eight (three-gene model) and four (two-gene model) genotypic classes were tested in both populations.									
<sup>c</sup> The null hypothesis of the tests is that the progeny segregate in the ratios tested.									
<sup>d</sup> The range of values of days to R1 of RI lines grown in gc20 accepted for each phenotypic class.									
<sup>e</sup> The range of values of flowering delay accepted for each phenotypic class.									



\* = The upper class limit of days to R1.