ABSTRACT: 429

POSITIONAL CLONING AND CHARACTERIZATION REVEAL THE MOLECULAR BASIS FOR SOYBEAN MATURITY LOCUS *E1* THAT REGULATES PHOTOPERIODIC FLOWERING

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The maturity locus *E1* has a large impact on flowering time in soybean, but the molecular basis for the *E1* locus is largely unknown. Through positional cloning, we delimited the *E1* locus to a 17.4-kb region containing an intron-free gene (*E1*). The E1 protein contains a putative bipartite nuclear localization signal and a region distantly related to the B3 domain. In the recessive allele, a non-synonymous substitution occurred in the putative nuclear localization signal, leading to the loss of localization specificity of the E1 protein and earlier flowering. *E1* expression was significantly suppressed under short-day conditions and showed a bimodal diurnal pattern under long-day conditions, suggesting its response to photoperiod and its dominant effect induced by long day length. When a functional *E1* gene was transformed into the early-flowering cultivar Kariyutaka with low *E1* expression, transgenic plants carrying exogenous *E1* displayed late flowering. Furthermore, the transcript abundance of *E1* was negatively correlated with that of *GmFT2a* and *GmFT5a*, homologues of *FLOWERING LOCUS T* that promote flowering. The molecular identification of the maturity locus *E1* will contribute to our understanding of the molecular mechanisms by which a short-day plant regulates flowering time and maturity.

Positional cloning and characterization reveal the molecular basis for soybean maturity locus *E1* that regulates photoperiodic flowering

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Soybean is a short day (SD) plant

In the northern hemisphere (China, USA, Canada)

Southern cultivars → North Flowers late

Northern cultivars → South Flowers early

Photoperiod response

Vegetative Growth

Reproductive Growth

Temperature accelerates the speed

Current statues

E **serials**	***E1、E2、E3、E4***	→	**Cloned**
	E5、E6、E7、E8	→	**unknown**
	J	→	**unknown**
Florigens	***FT***	→	**Identified**
Growth Habit	***Dt1***	→	**Identified**

My involvement

E1 ***Pnas*** (2012)

E2 ***Genetics*** (2011)

E3 ***Genetics*** (2009)

E4 ***Genetics* (2008)**

DT1 ***Plant Physiology* (2010); *Pnas* (2010)**

FT ***Plant Physiology* (2010)**

Parents of mapping populations (*E2*, *E3*)

Misuzudaizu

MoshidouGong 503

Morphological differences between Parents

Misuzudaizu

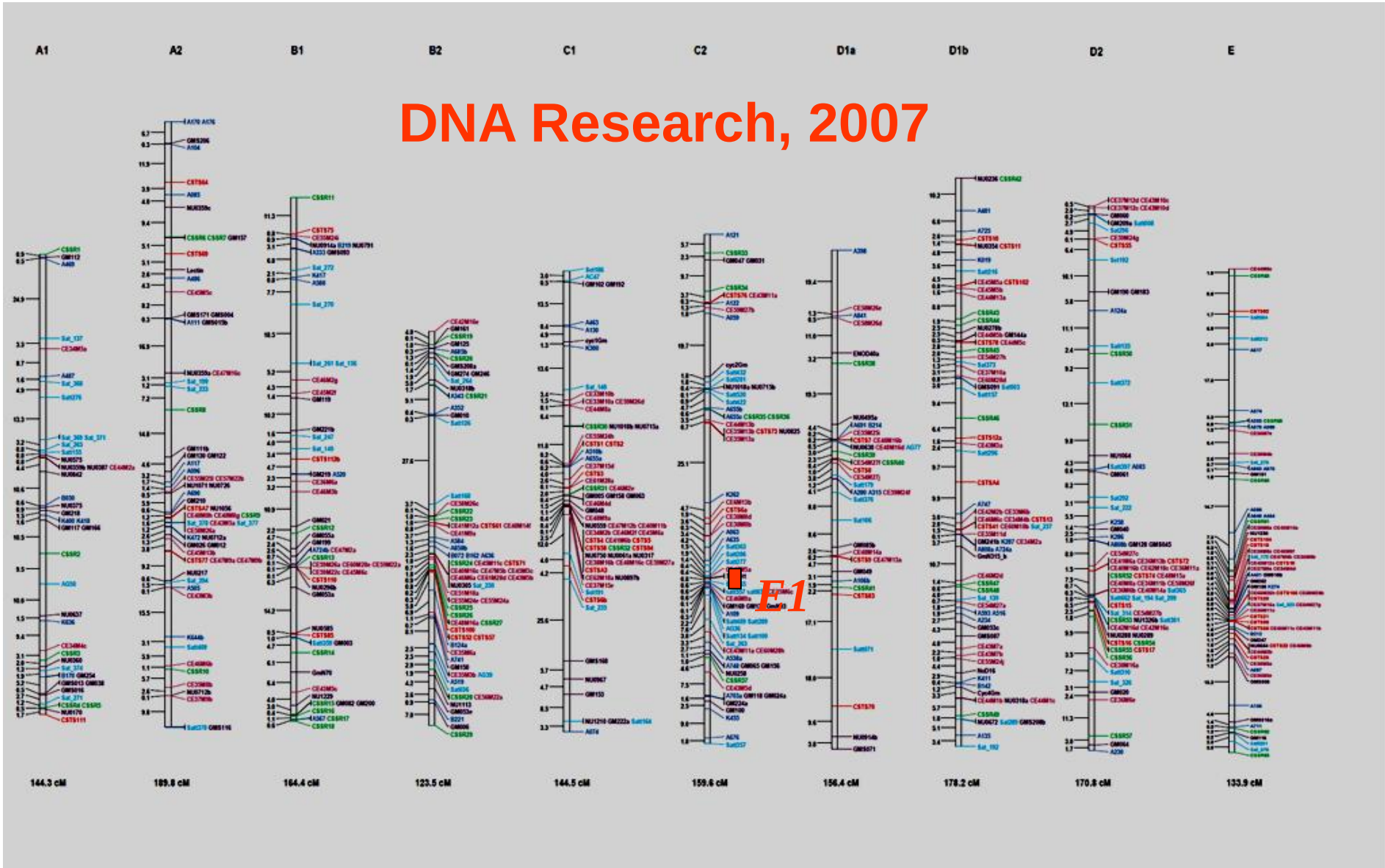




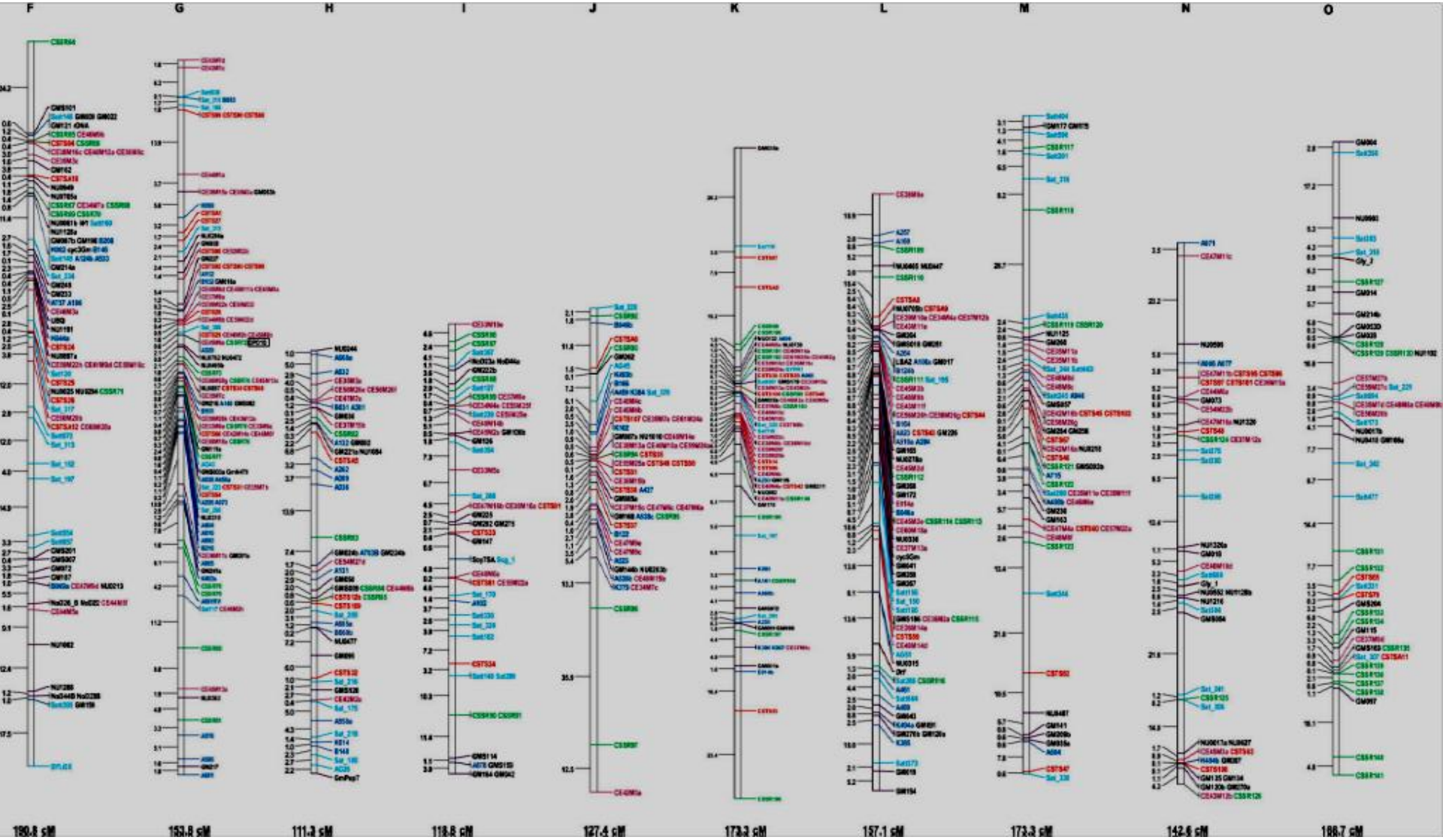


Mashidougong503

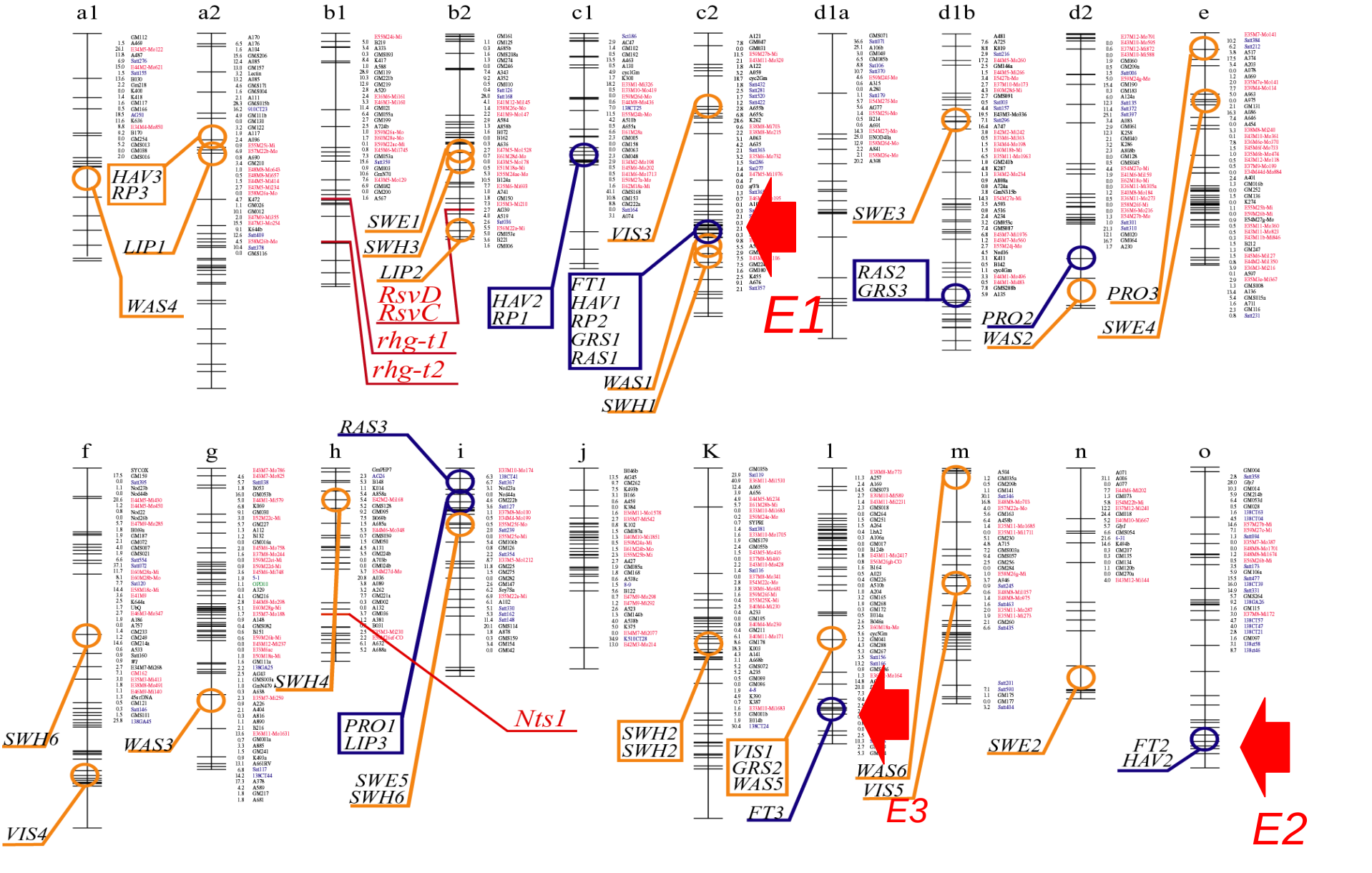
Linkage map (1)



Linkage map (2)



QTL mapping


a1
a2
b1
b2
c1
c2
d1a
d1b
d2
e
HAV3
RP3
LIP1
WAS4
SWE1
SWH3
LIP2
RsvD
RsvC
rhg-t1
rhg-t2
HAV2
RP1
FT1
HAV1
RP2
GRS1
RAS1
VIS3
WAS1
SWH1
E1
SWE3
RAS2
GRS3
PRO2
WAS2
PRO3
SWE4
f
g
h
i
j
K
l
m
n
o
RAS3
SWH4
SWH6
WAS3
VIS4
PRO1
LIP3
SWE5
SWH6
Nts1
SWH2
SWH2
VIS1
GRS2
WAS5
FT3
WAS6
VIS5
E3
SWE2
FT2
HAV2
E2

General information of BAC libraries

Library Name	GmMiB	GmWBb
Cultivars	Misuzudaizu	Williams 82
Vector	PBAC-LAC	pIndiogoBac526
Clones	53760	92160
Insert size	116.2Kb	150Kb
X	5.43	12
Restriction Enzyme	*Hind*III	*Bsty*I

3D pooling of BAC libraries

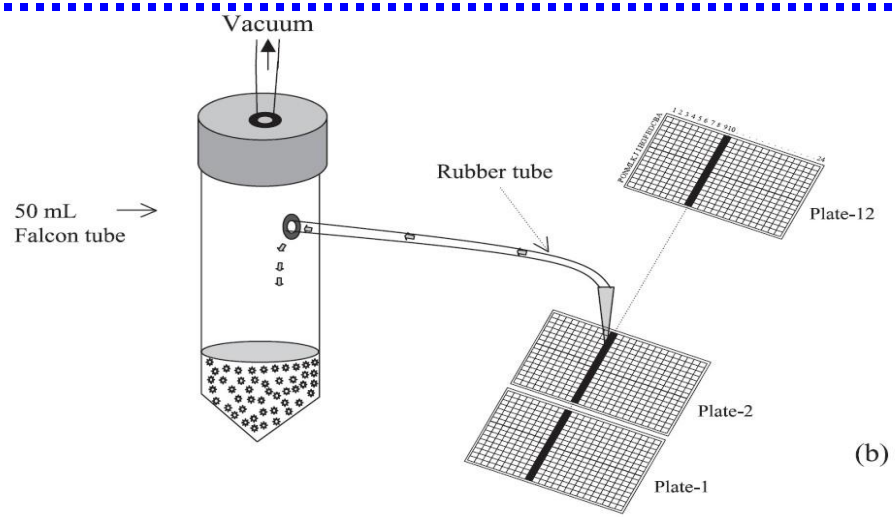
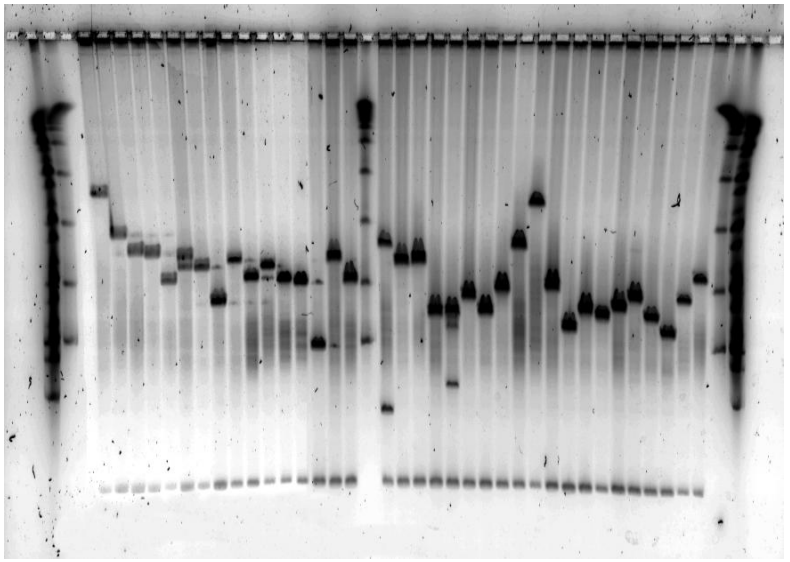
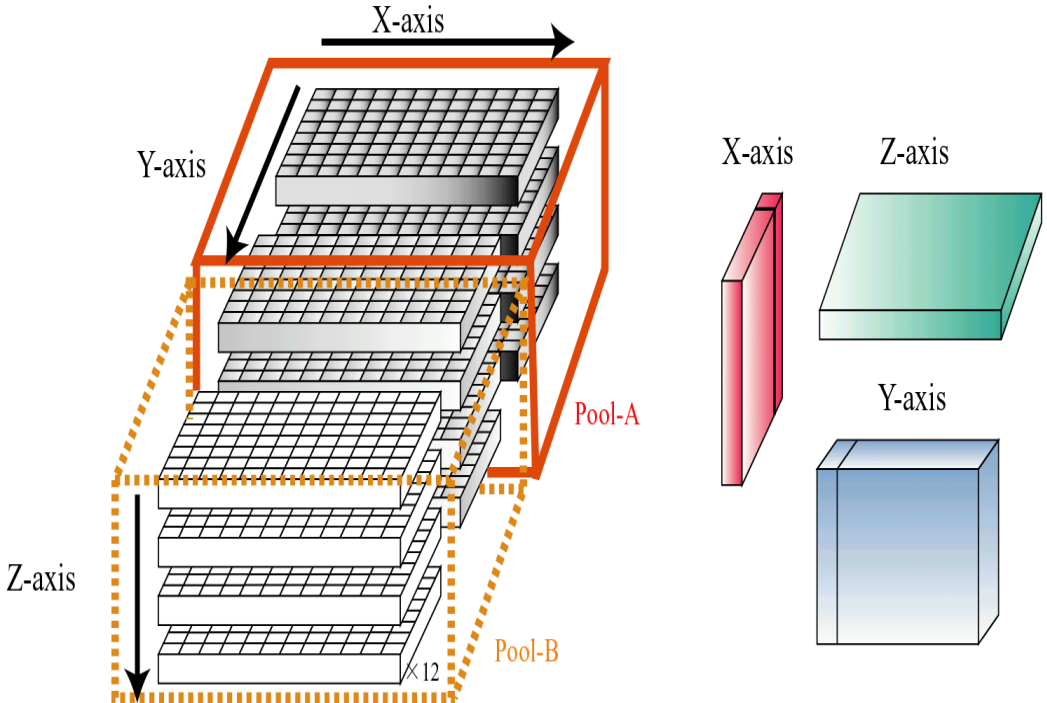
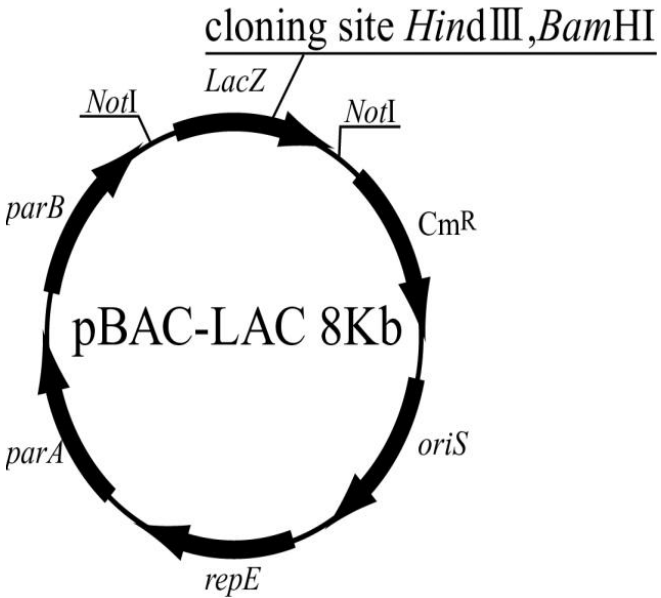


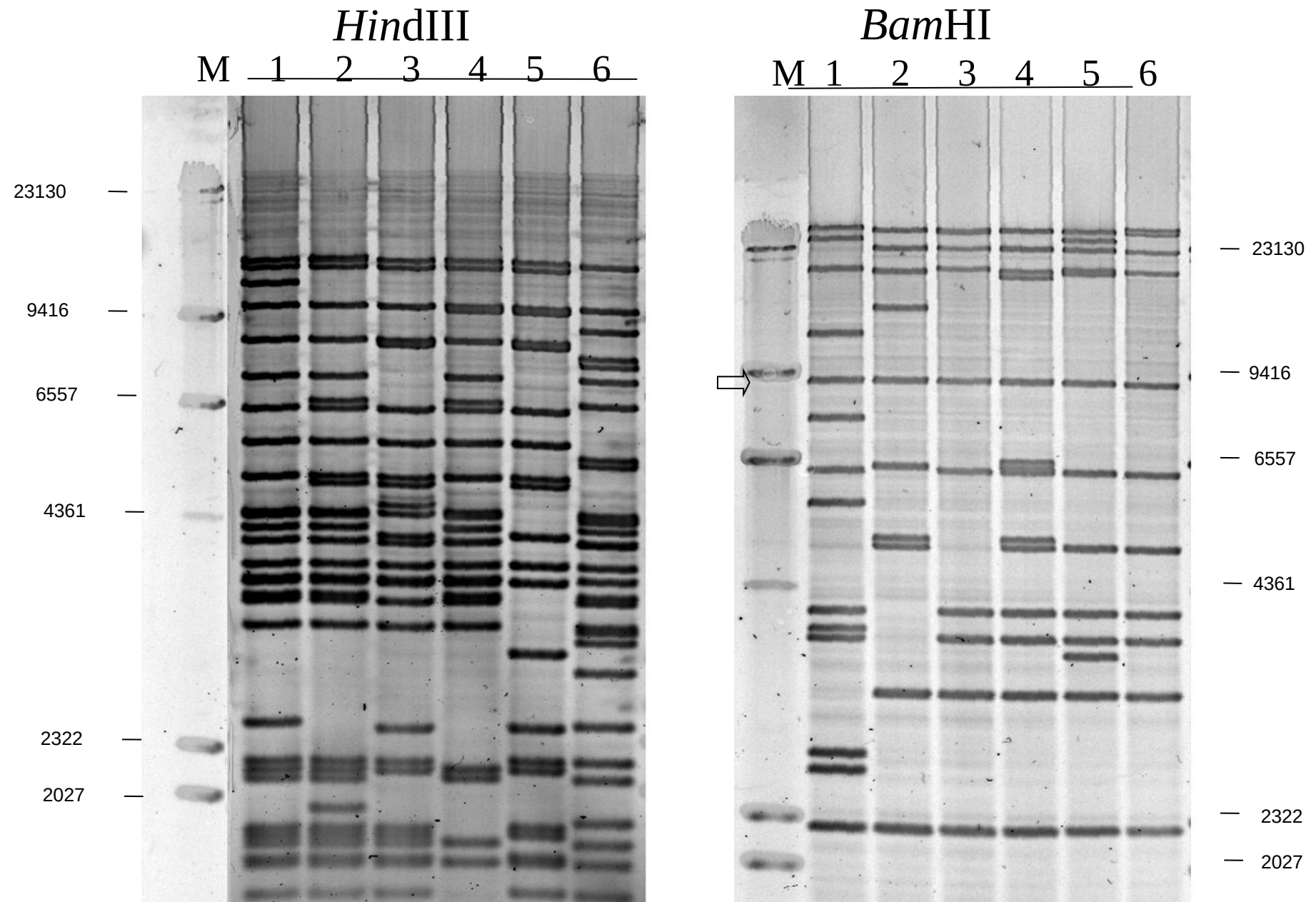
Fig. 1 The schematic drawing for three dimensional pooling



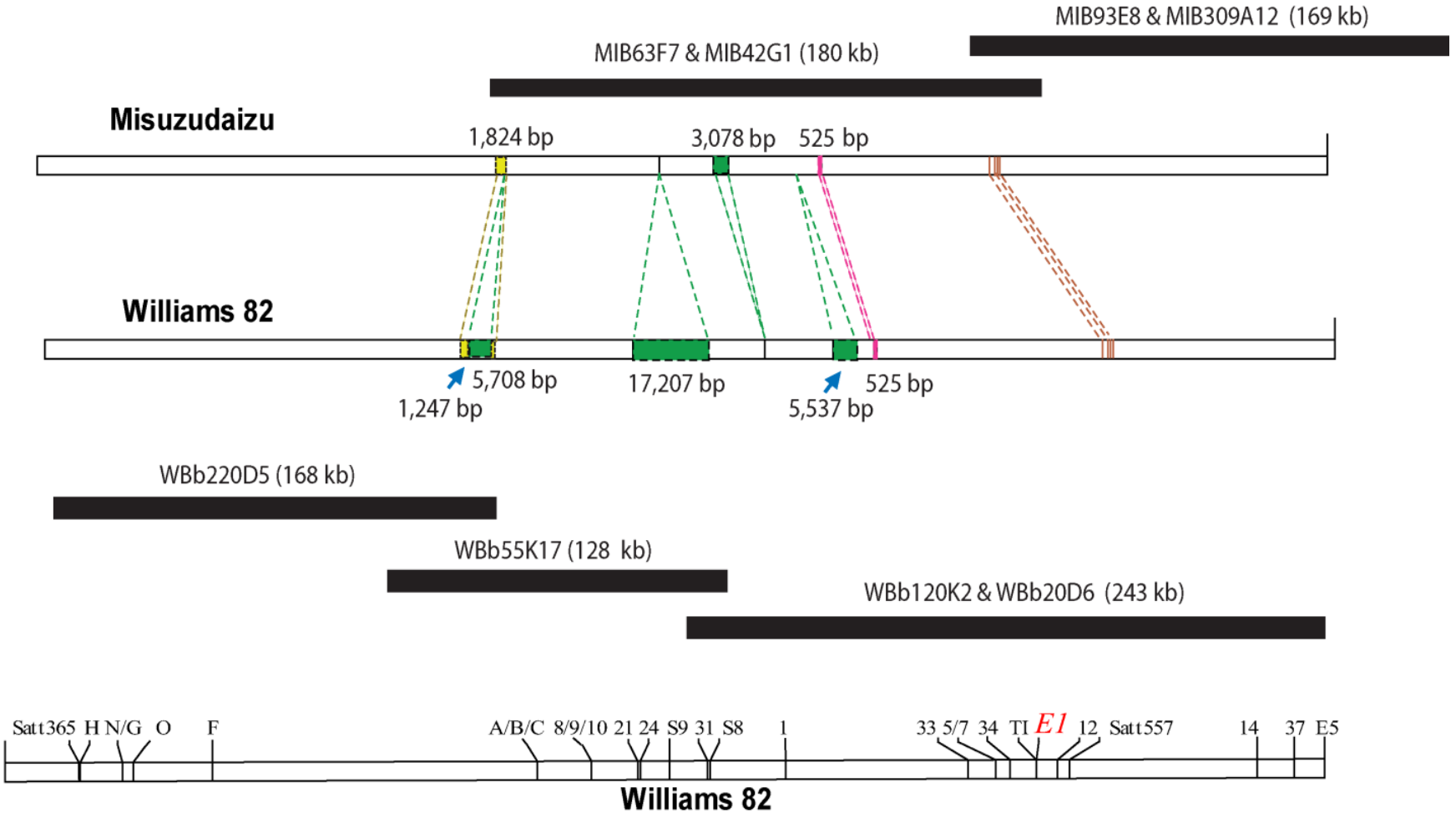




Fingerprinting



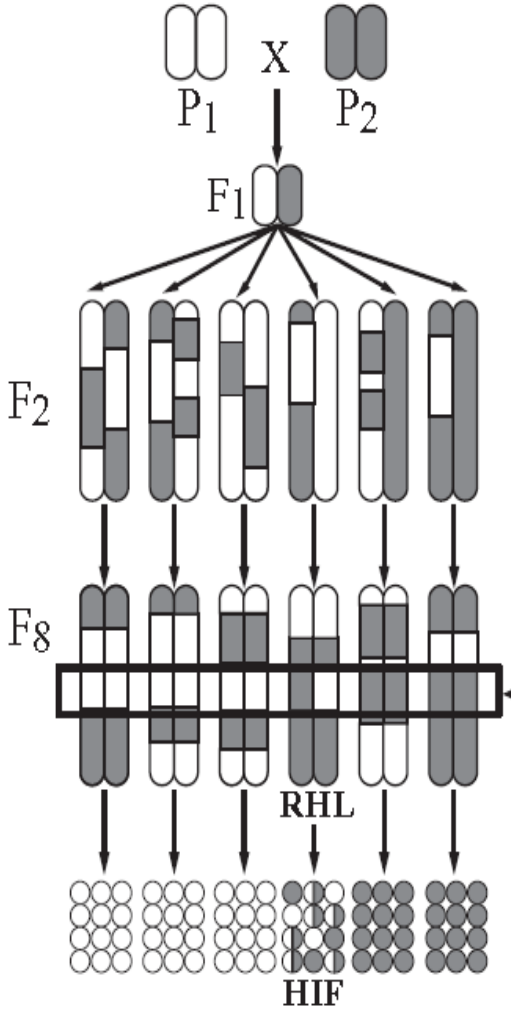
Contig development



RHL Strategy

In the F_2 population, each genetic locus shows a 1:2:1 segregation ratio, theoretically.

Phenotypic scoring of each line



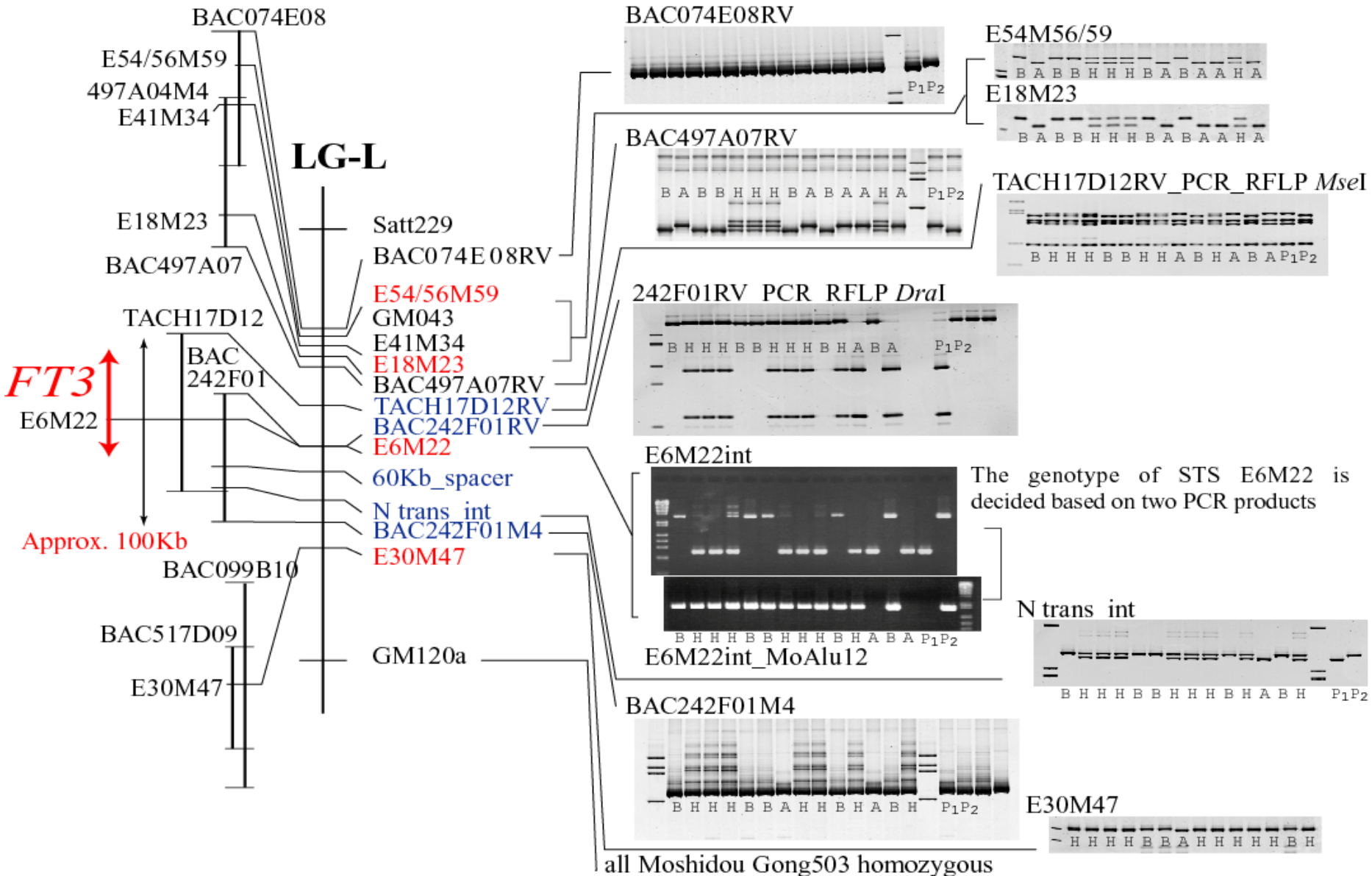
Dissociation of QTLs to single mendelian factor

Residual Heterozygous Line

Many of the loci are fixed in homozygous state, and a few loci are heterozygous in recombinant inbred lines (RILs) derived by single seed descent.

When a QTL is located in a heterozygous region, progeny of the line shows phenotypic segregation.

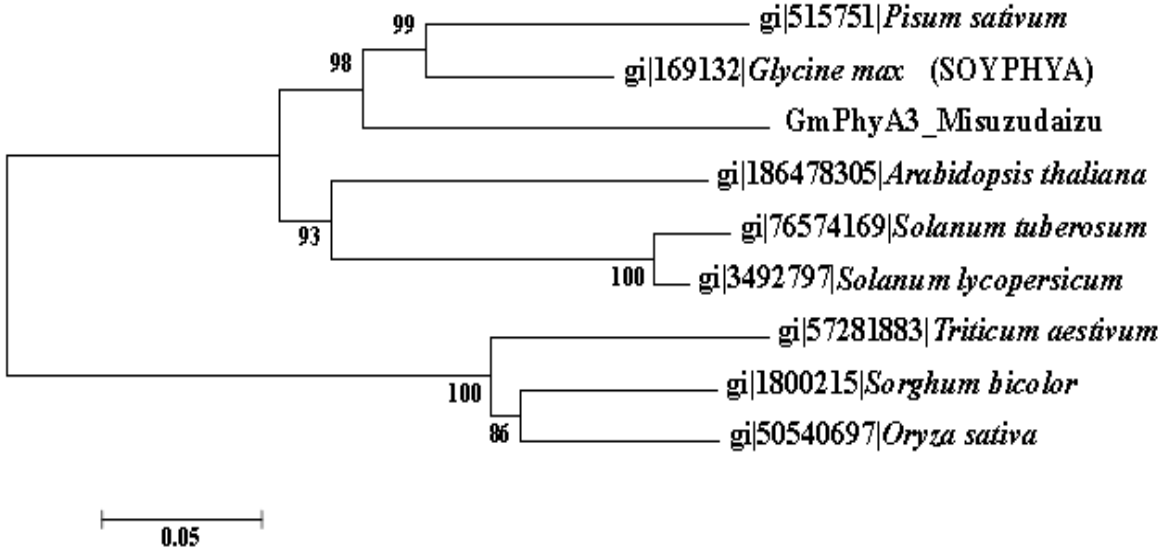
Fine mapping and positional cloning of *E3*



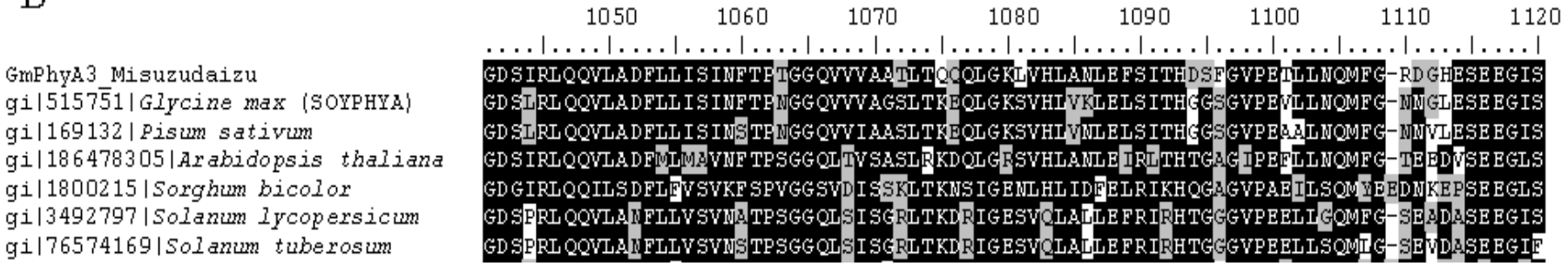
GmPhyA3

Genetics , 2009

A



B

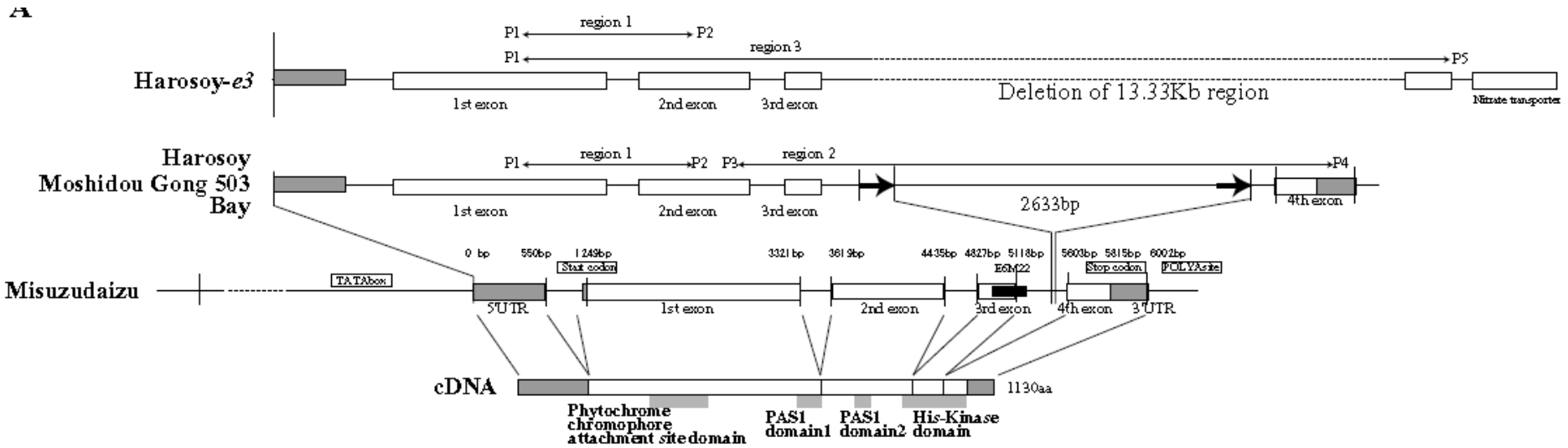


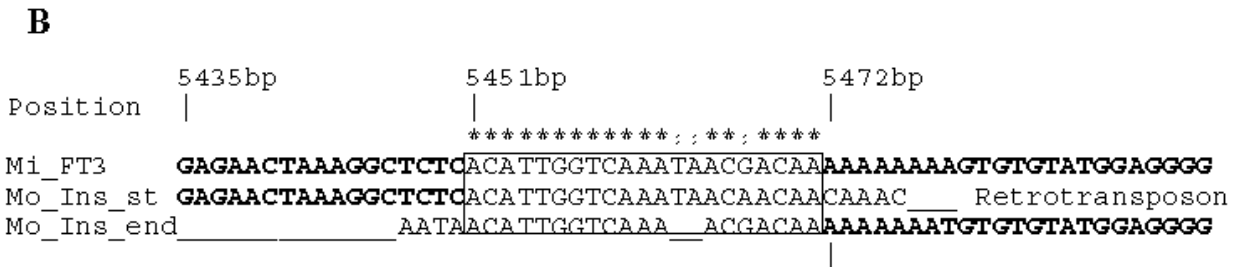
E3

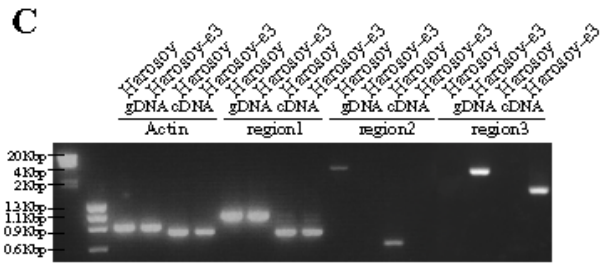
Effects on

Photoperiodic response: medium
Flowering time and maturity: medium

(E3) *GmPhyA3*
- **Light quality**
- **E3 and E4 can control E1**







Positional cloning of *E2* gene

DOI: 10.1534/genetics.110.125062

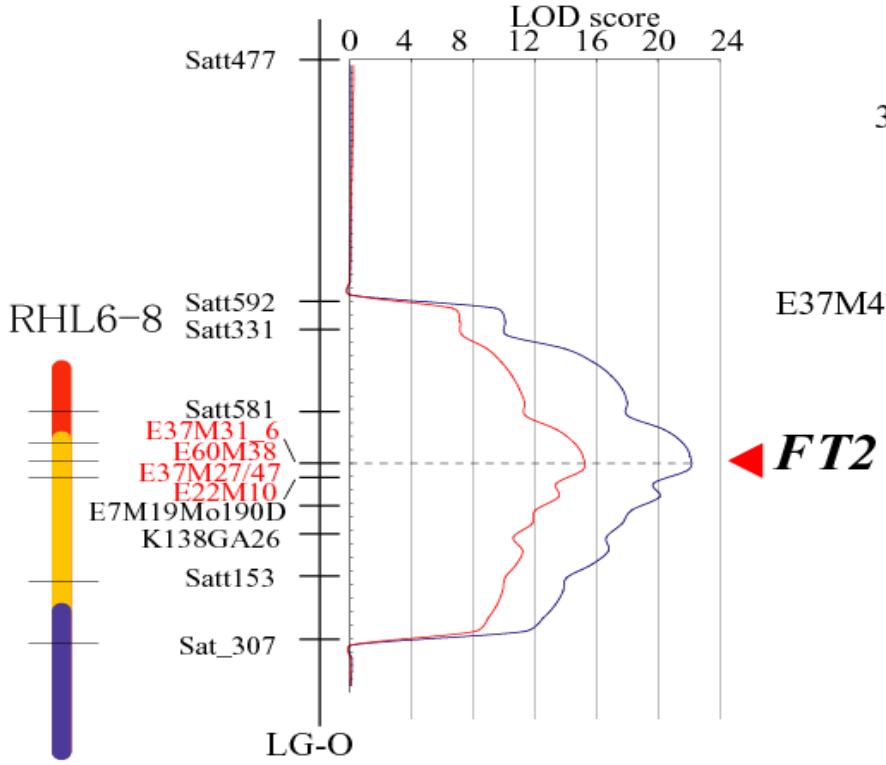
A Map-Based Cloning Strategy Employing a Residual Heterozygous Line Reveals that the *GIGANTEA* Gene Is Involved in Soybean Maturity and Flowering

Satoshi Watanabe,* Zhengjun Xia,*,† Rumiko Hideshima,‡ Yasutaka Tsubokura,* Shusei Sato,§ Naoki Yamanaka,** Ryoji Takahashi,†† Toyoaki Anai,‡ Satoshi Tabata,§ Keisuke Kitamura‡‡ and Kyuya Harada*,1

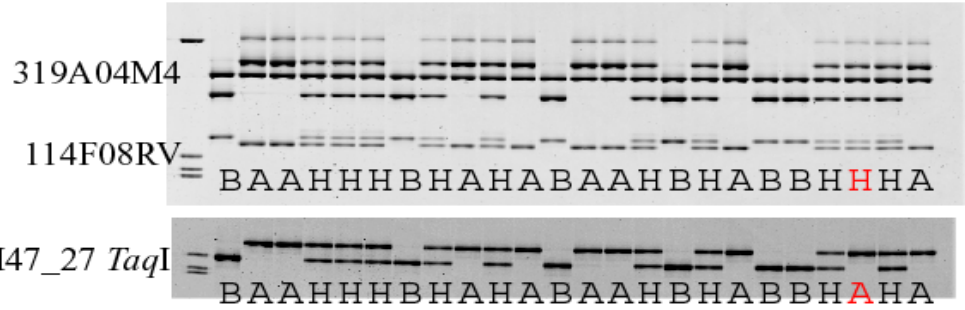
2011. *Genetics* 188(2):395-407.

Segregation in the progeny

QTL analysis for *FT2* locus using RIL population

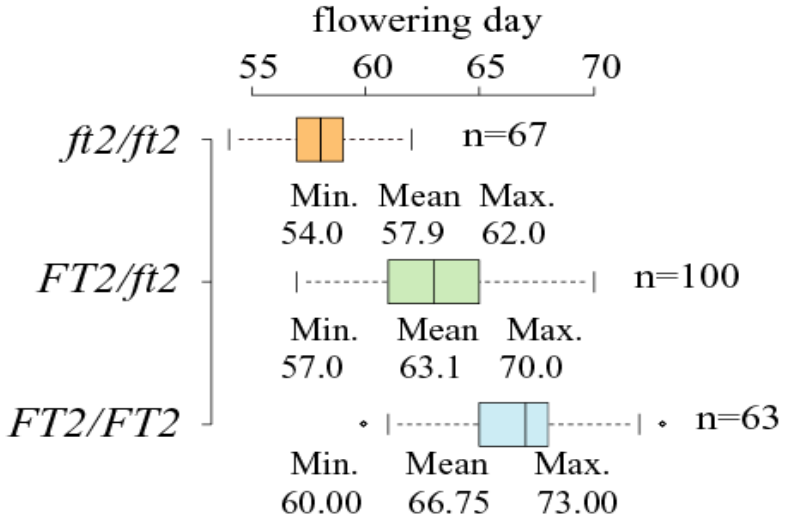


Example of DNA marker linked to *FT2*

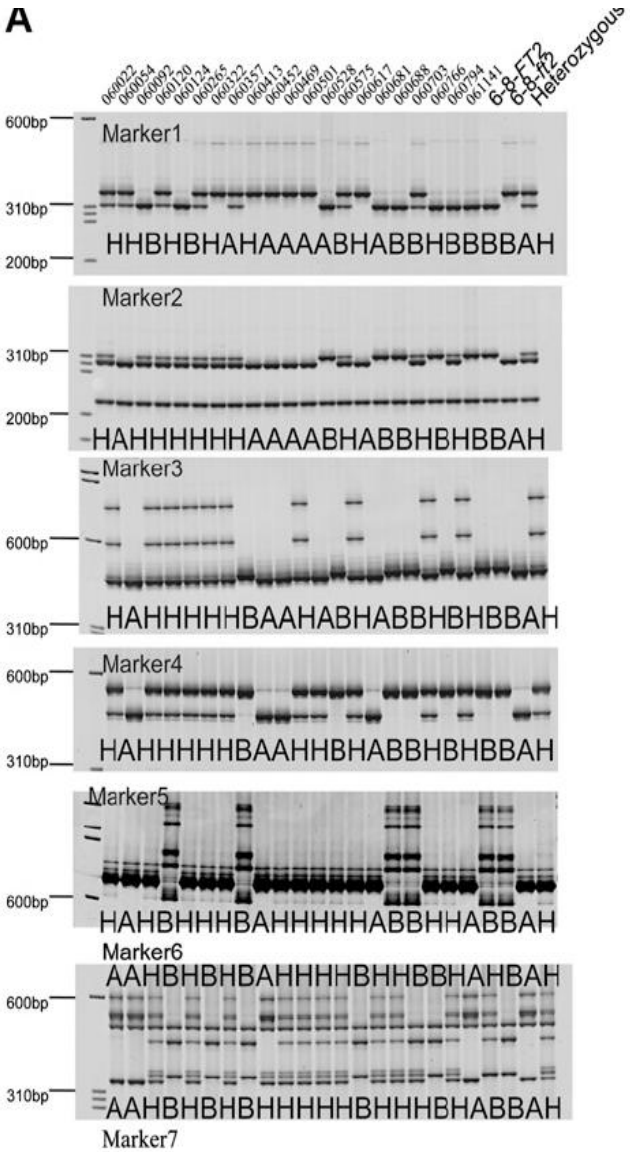


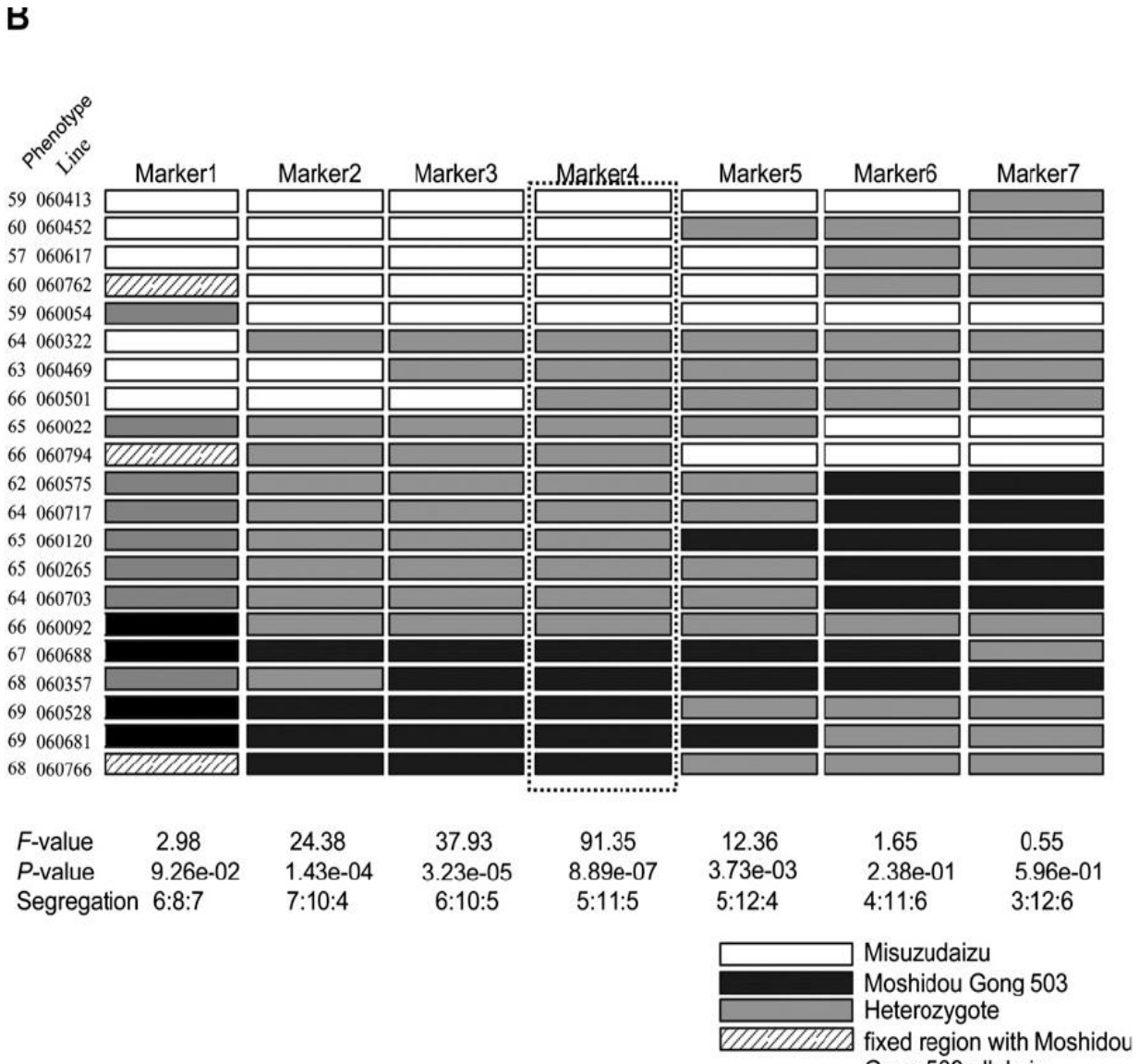
Additive effect	-4.4 days
Dominance effect	0.7 days
Genetic var. / Phenotypic Var.	66%

The effects of *FT2* clearly segregated in the progeny of RHL



E2 and Marker4 is tightly linked





GmGIa (*Gigantea*)

delimited to ~30 kb region

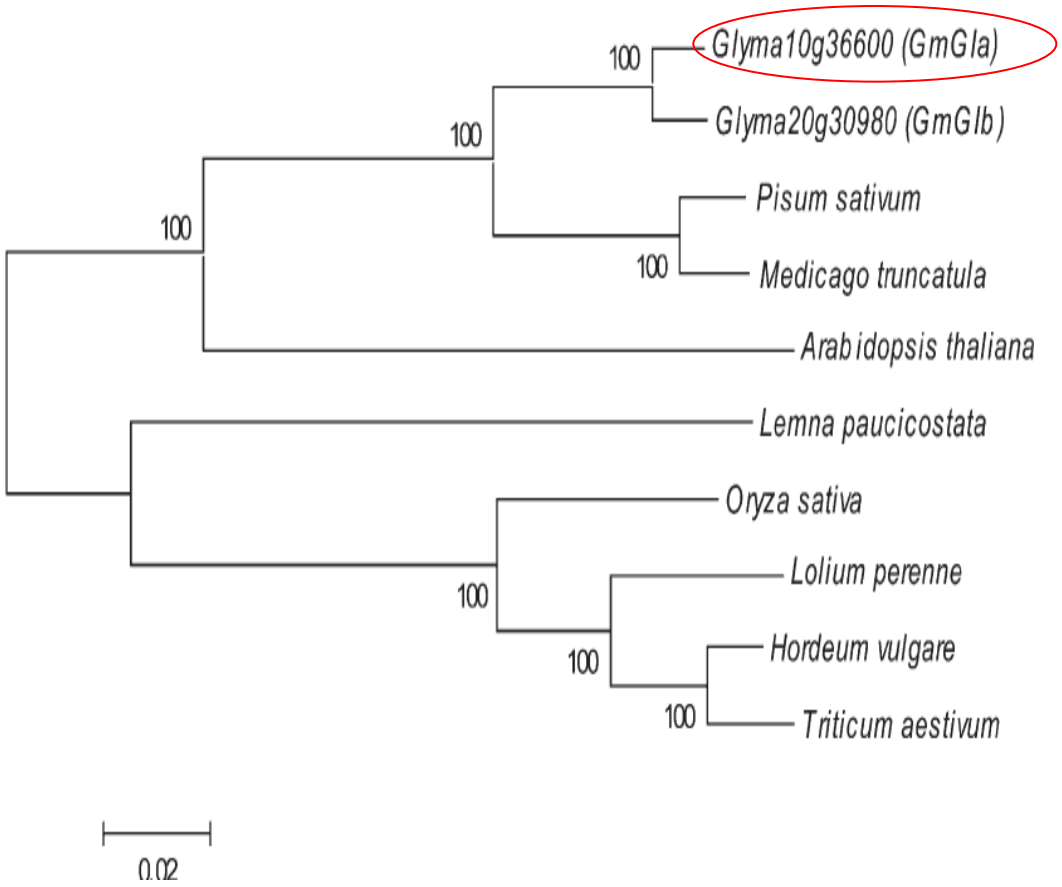
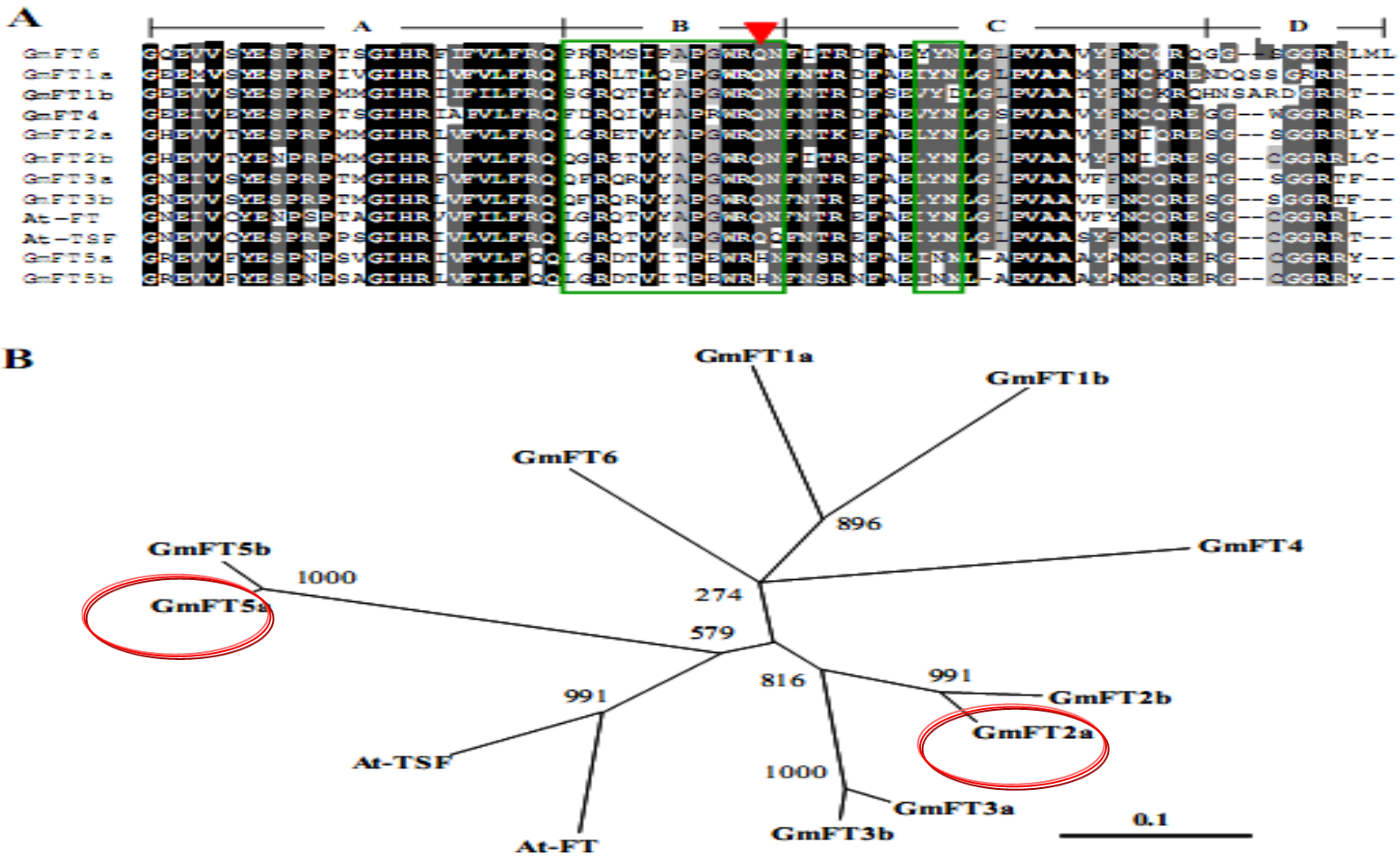


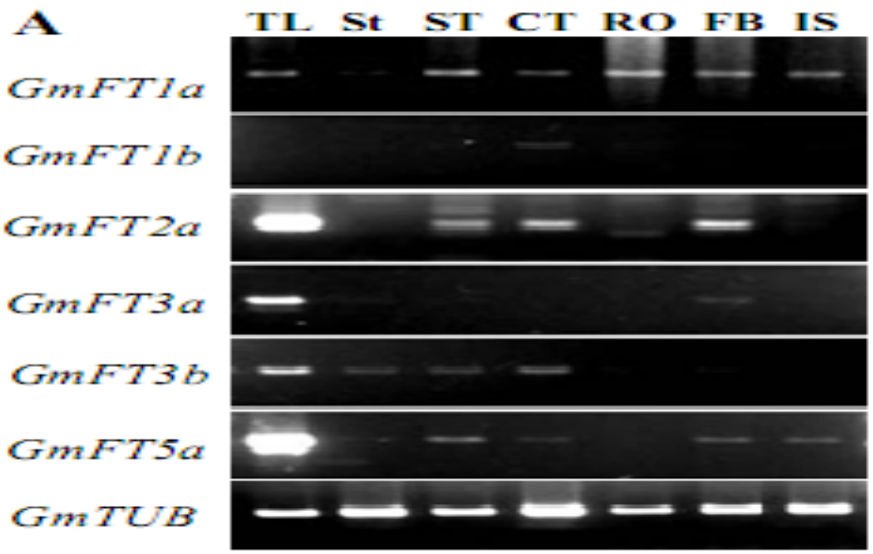
FIGURE 3.—Phylogenic analysis of GIGANTEA proteins. A phylogenic tree of GIGANTEA proteins, constructed by the NJ method with a *p*-distance and a 1000-replication bootstrap confidence value, is shown. Amino acid sequences of *A. thaliana* (AAT80910; FOWLER *et al.* 1999), *P. sativum* (ABP81863; HECHT *et al.* 2007), *Medicago truncatula* (ABE81212), *O. sativa* (NP_001042220; OHYANAGI *et al.* 2006), *H. vulgare* (AAW66946; DUNFORD *et al.* 2005), *T. aestivum* (AAQ11738; ZHAO *et al.* 2005), *Lolium perenne* (ABF83898), and *Lemna paucicostata* (BAD97864) were used.

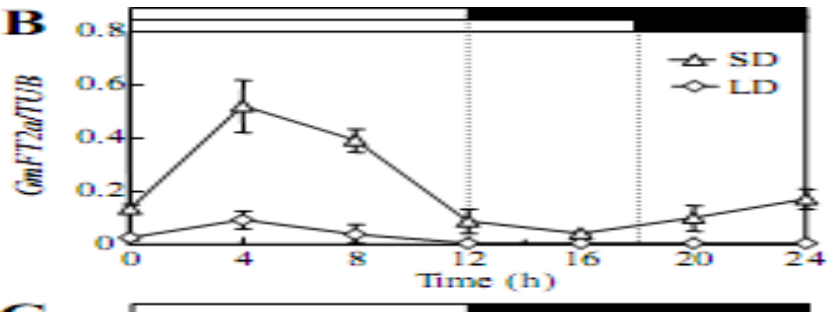
GmFTs

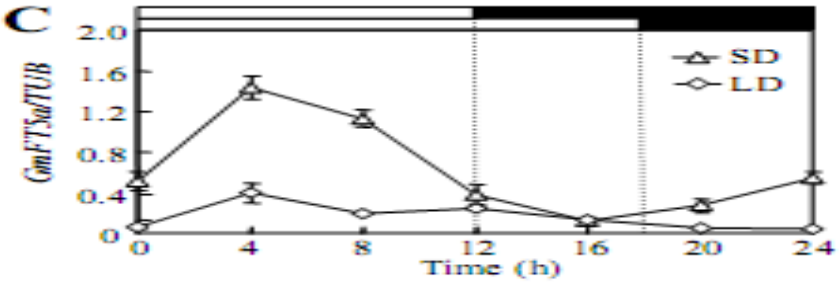


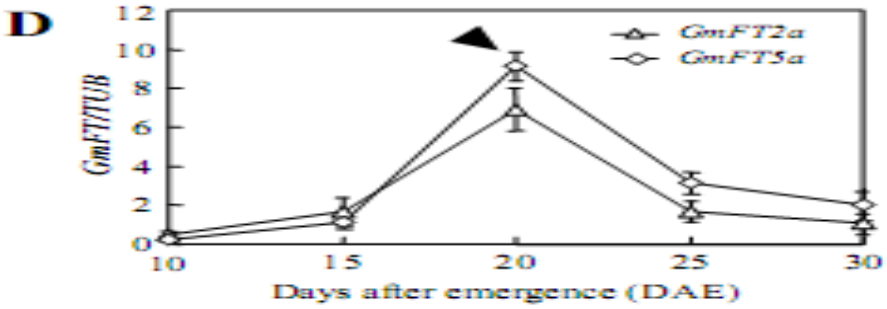
Plant Physiology , 2010

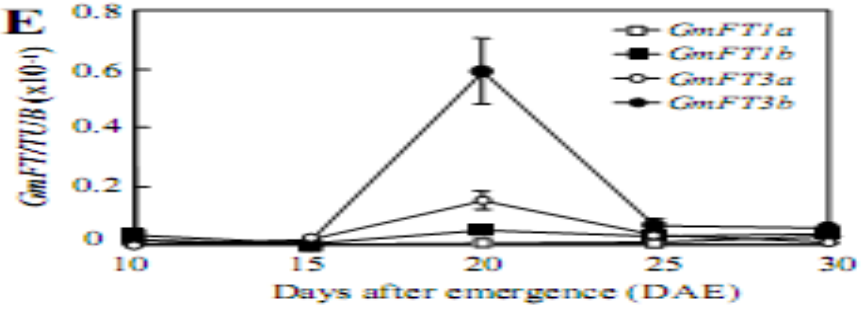
Expression Profiles of *GmFTs*(*GmFT2a/GmFT5a*)

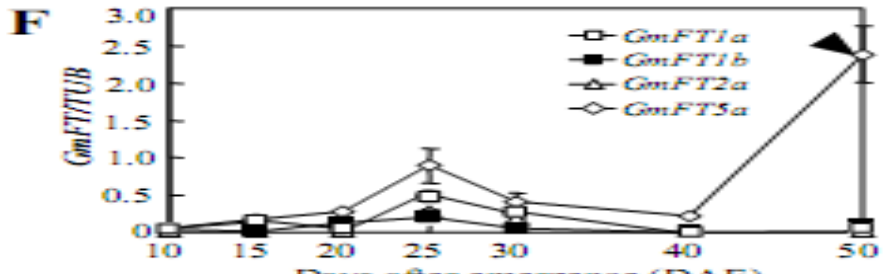


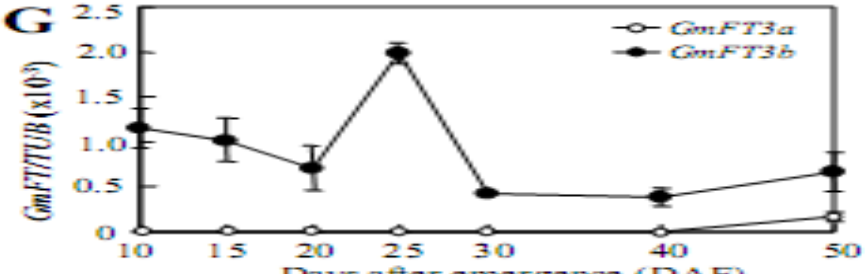










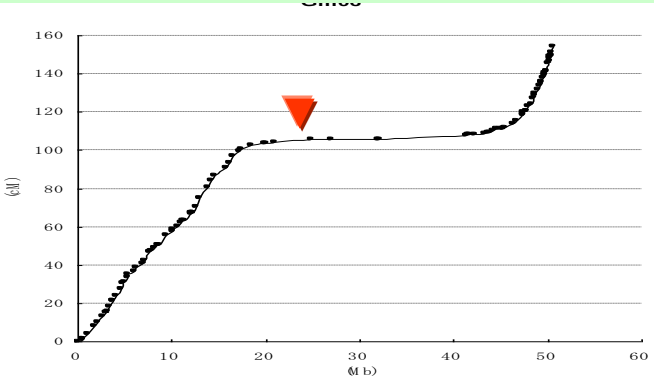


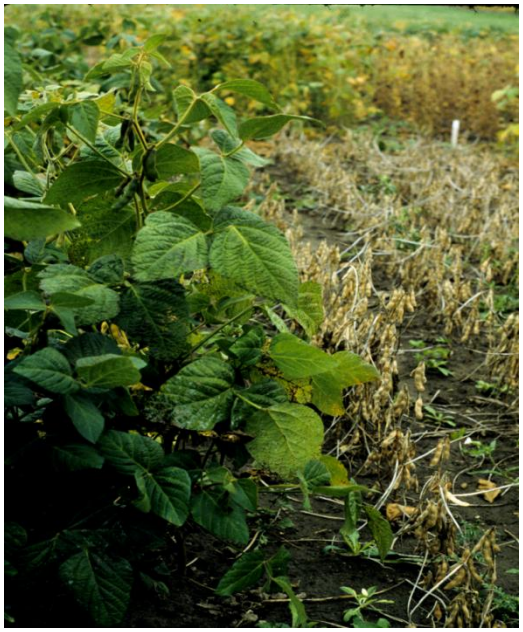
Positional cloning of *E1*

1920 Garner and Allard Photoperiodism

1923 Woodworth *S / s* ?
1927 Owen *E / e*
1971 Bernard *E1 / e1*

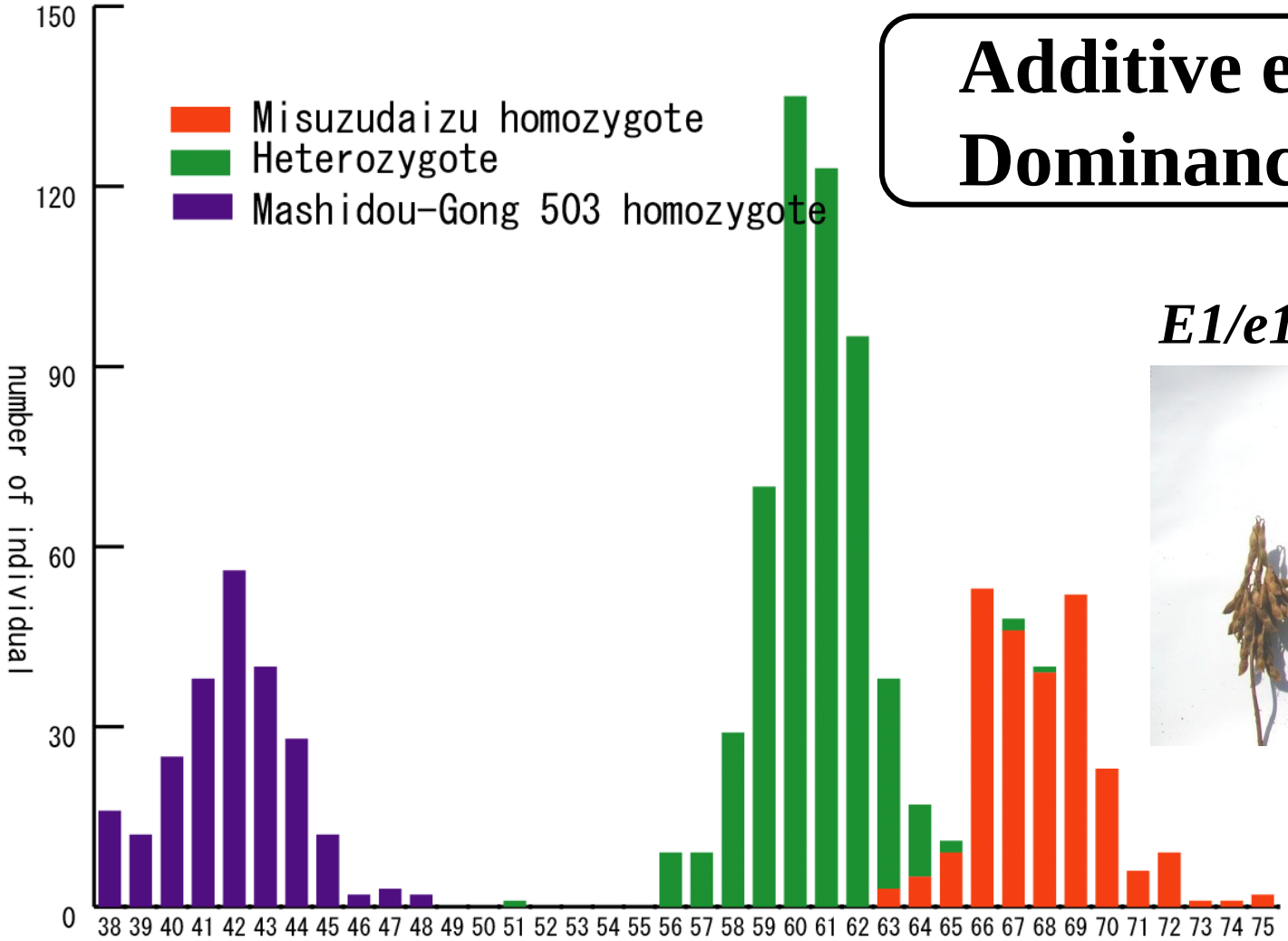
- **Major locus (gene)**
- **Difficult to clone due to low combination rate**





Canada
(E Cober)

RHL 1-156



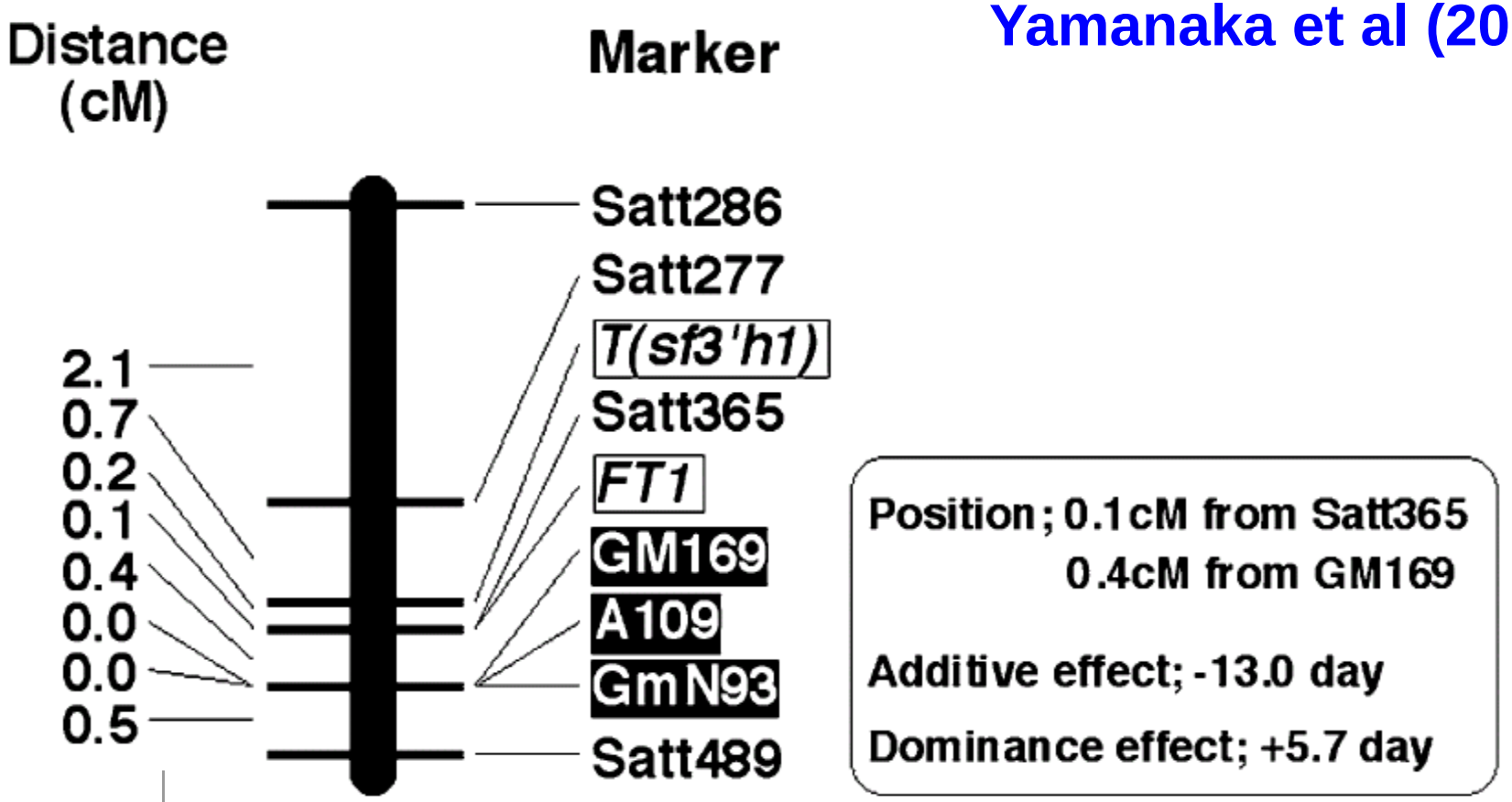
Additive effect 9.7
Dominance effect 4.0

E1/e1 ***E1/E1***

1-156

0.1 cM is too large

Yamanaka et al (2005)



Position and genetic effects of *FT1* determined using 1,006 plants

Plant Material

Harosoy *E1*
×
Harosoy (*e1*)

2005 : F2: 105 individuals

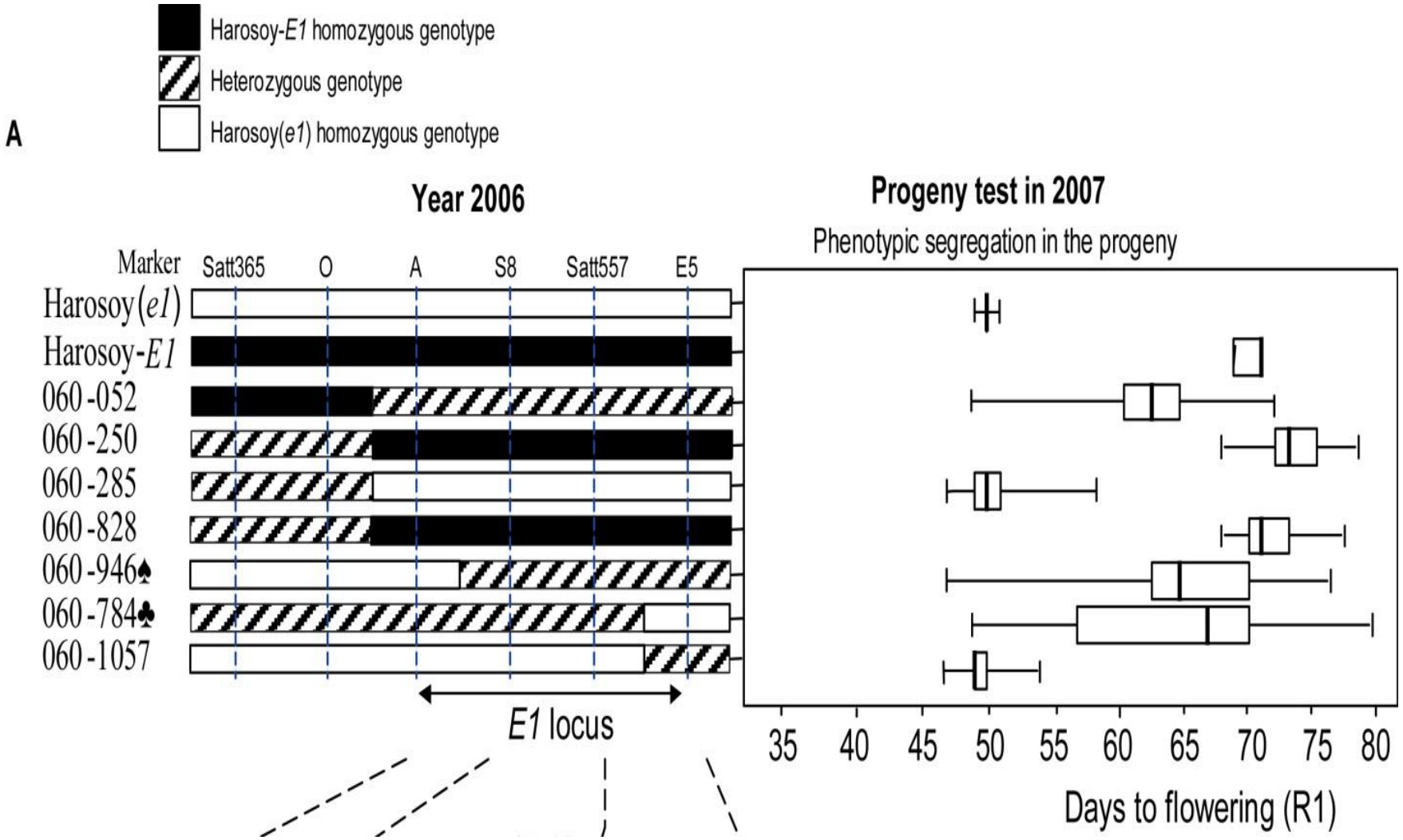
2006: F3: 1,442 individuals 289 kb

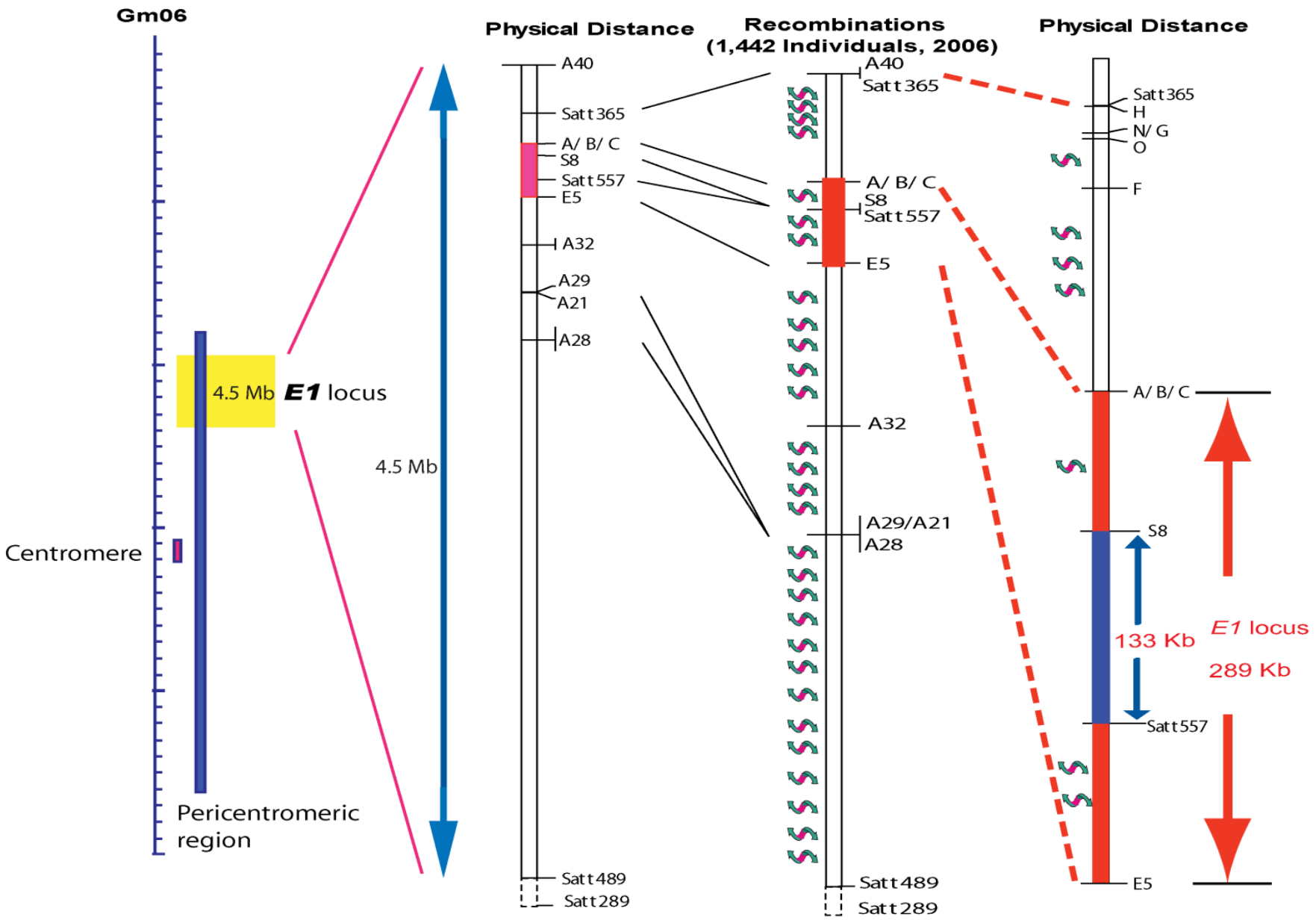
2007: F4: 1446 individuals

2008: F5: 1,3762 individuals 17.4 kb

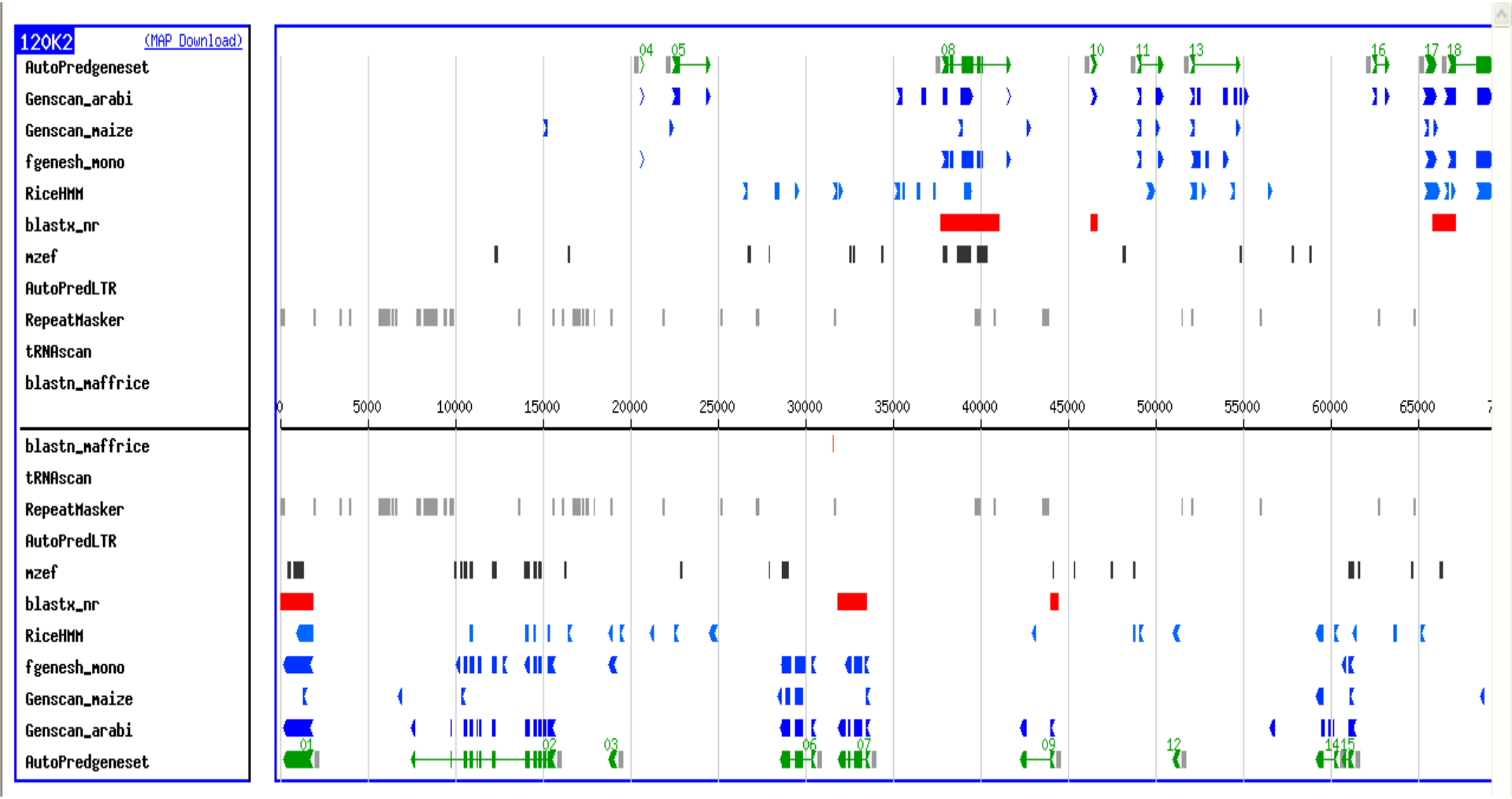

Harosoy *E1E1*
Harosoy *e1e1*

Mapping with 1,442 plants



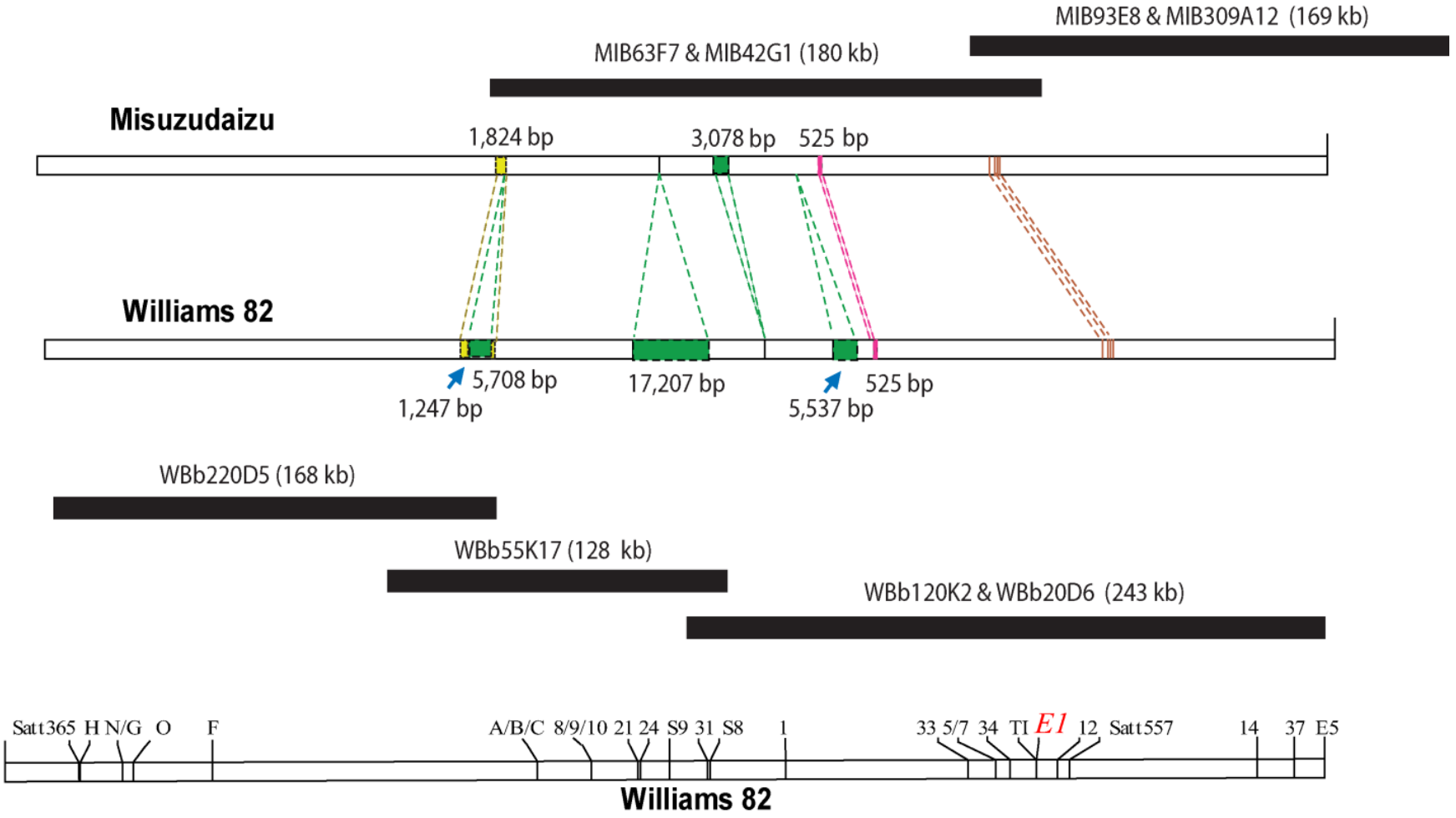

Gm06
4.5 Mb E1 locus
4.5 Mb
Centromere
Pericentromeric region
Physical Distance
A40
Satt365
A/ B/ C
S8
Satt557
E5
A32
A29
A21
A28
Satt489
Satt289
Recombinations
(1,442 Individuals, 2006)
A40
Satt365
A/ B/ C
S8
Satt557
E5
A32
A29/A21
A28
Satt489
Satt289
Physical Distance
Satt365
H
N/ G
O
F
A/ B/ C
S8
133 Kb
E1 locus
289 Kb
Satt557
E5

More than 40 genes were identified within 289 kb region

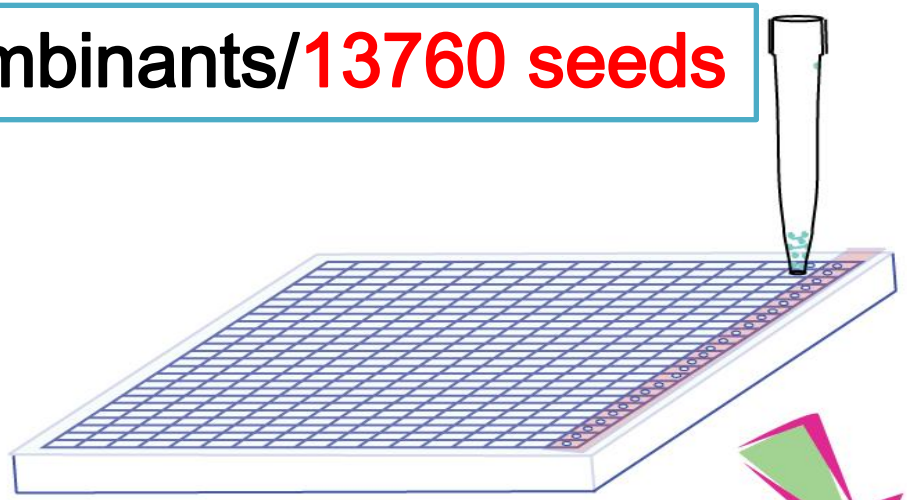


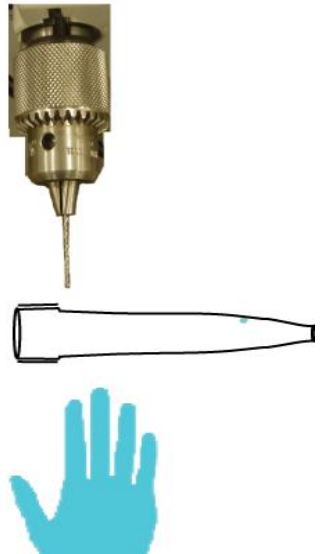
Annotation using RiceGAAS

Contig and marker development



10 recombinants/13760 seeds





Air flow

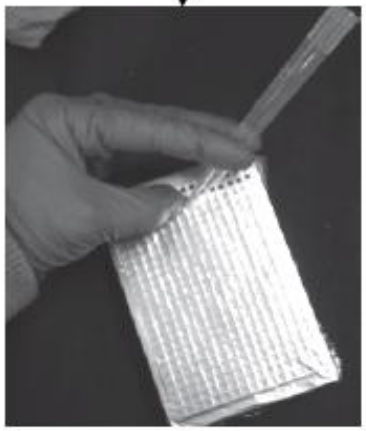
Drilling

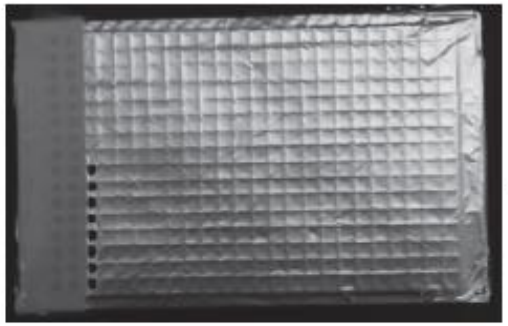
Cleaning with air flow

Seed powder Collection

Cleaning with air flow

Transfer





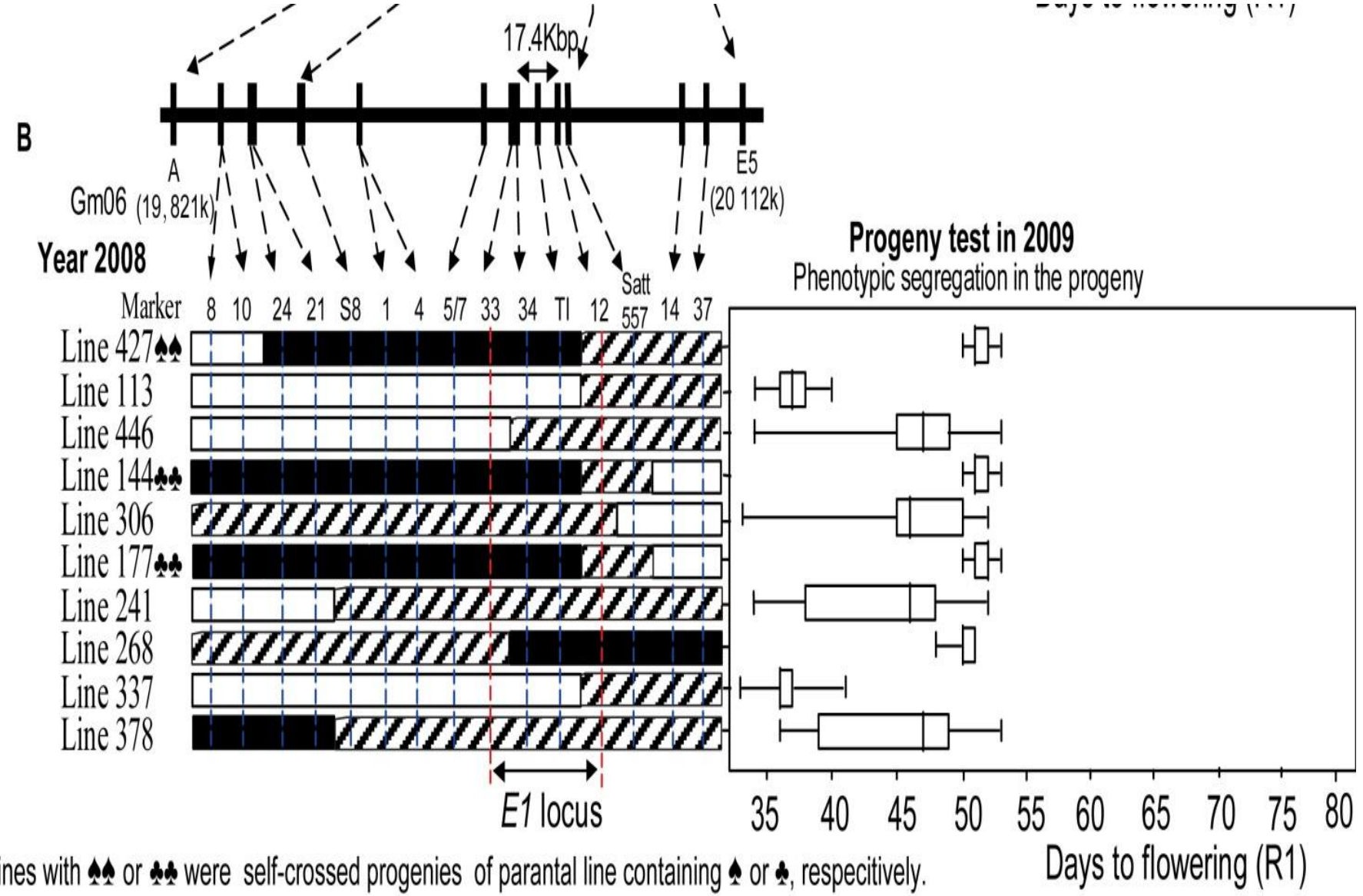
DNA extraction

PCR and Genotyping

Seeds

Selection of recombinant plants

10 recombinants identified



Lines with ♠♠ or ♣♣ were self-crossed progenies of parantal line containing ♠ or ♣, respecitively.

Progeny test

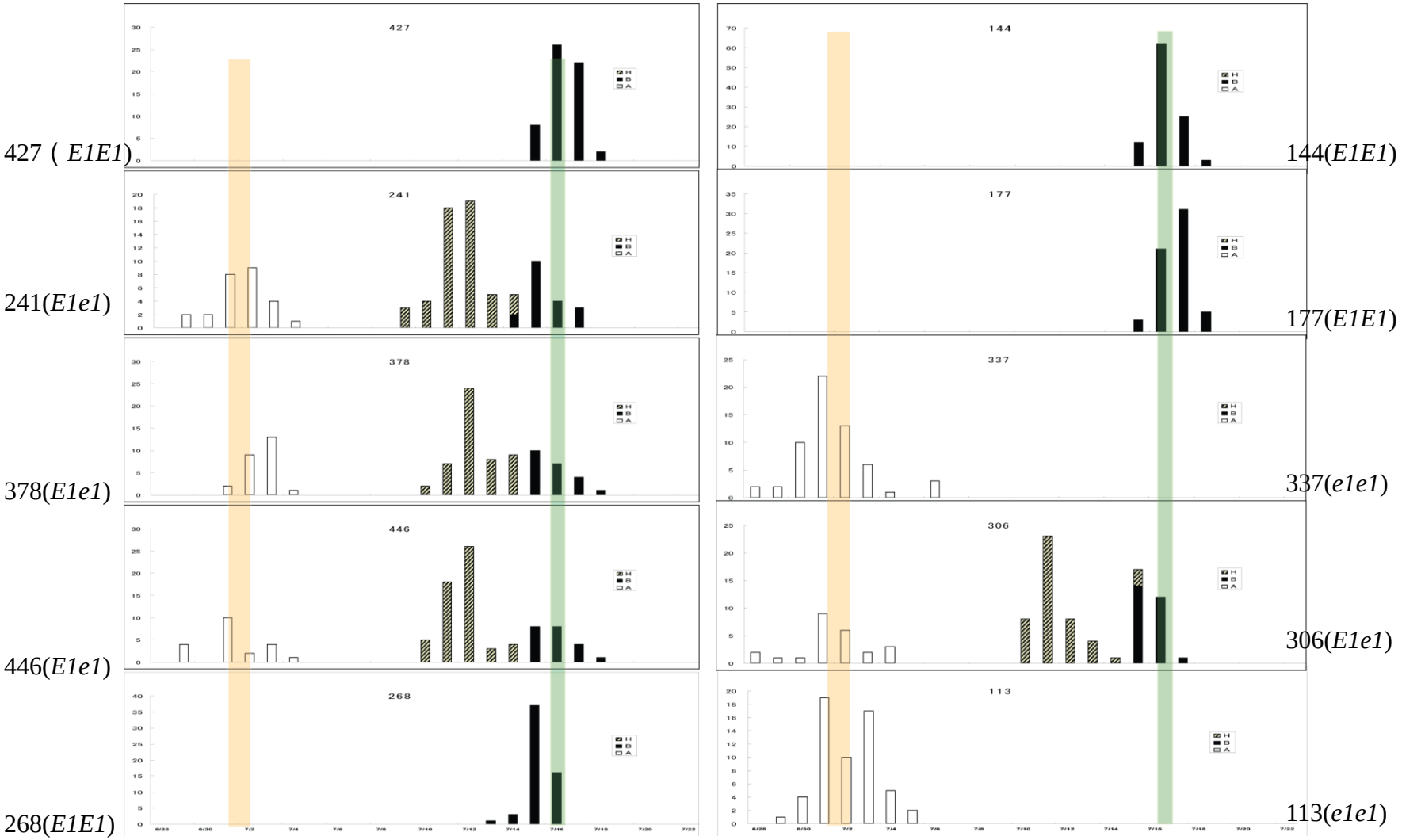
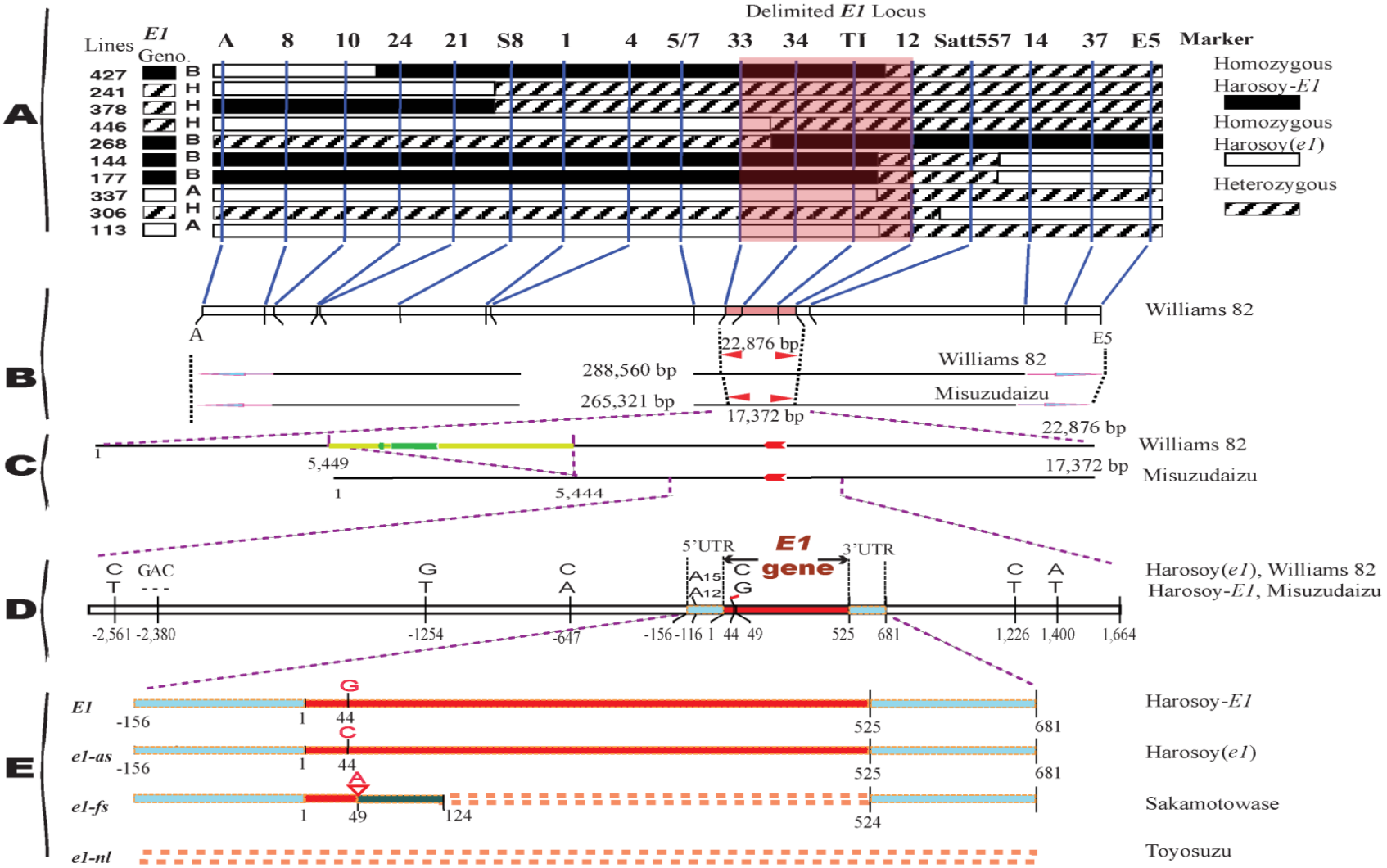
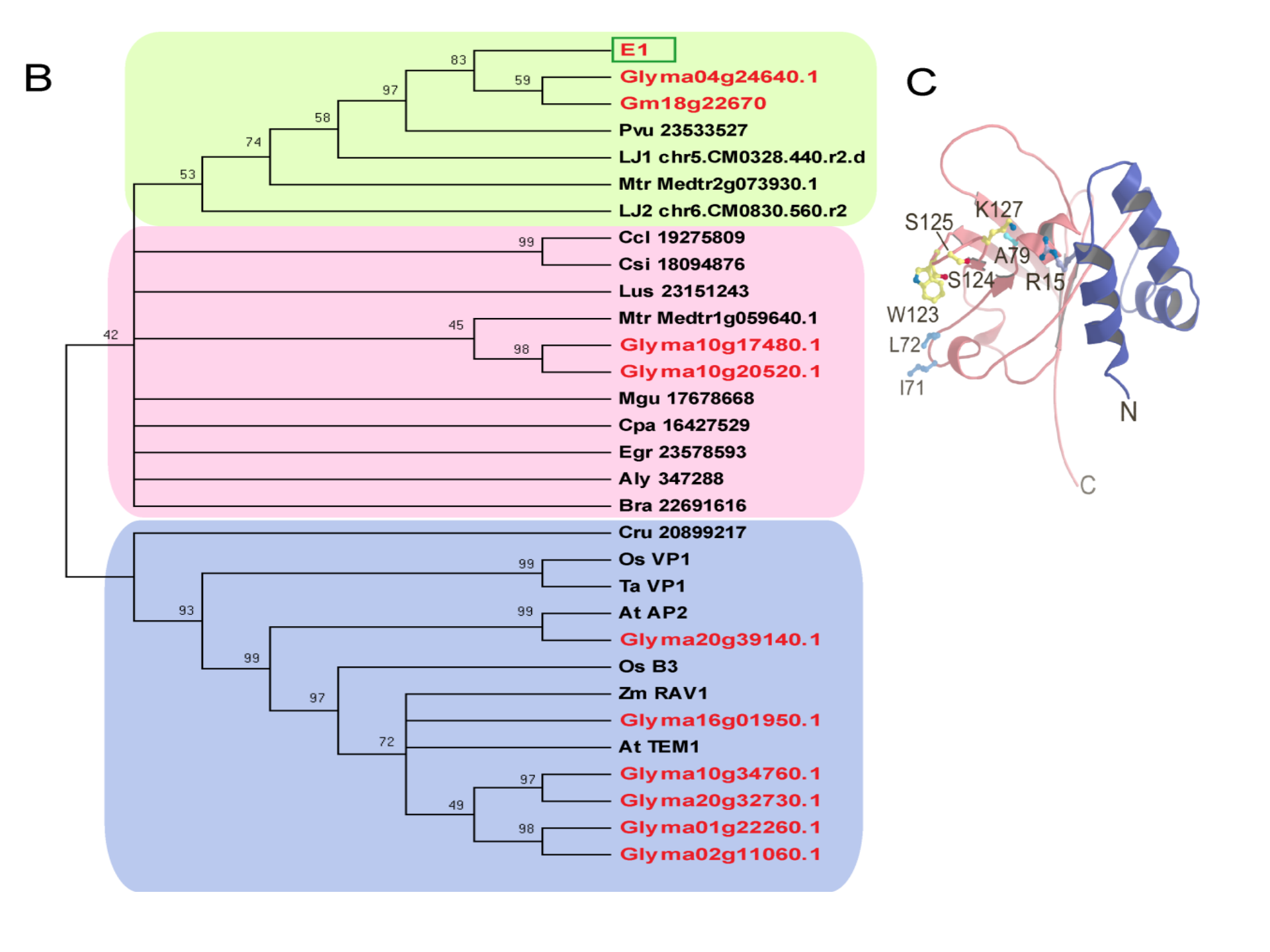


Figure S4. The segregation patterns of recombinants for flowering time in progeny. The flowering time for both parent lines ,Harosoy and Harosoy E1 were indicated in yellow and green color. The column with different with white, black and hatched background represented the genotypes of *e1-as*, *E1* and heterozygote using dCAPS marker TI, respectively. The phenotype for flowering time corresponded to the genotypes of maker TI, indicating genetic factor underlying the segregation is located in *E1* region.

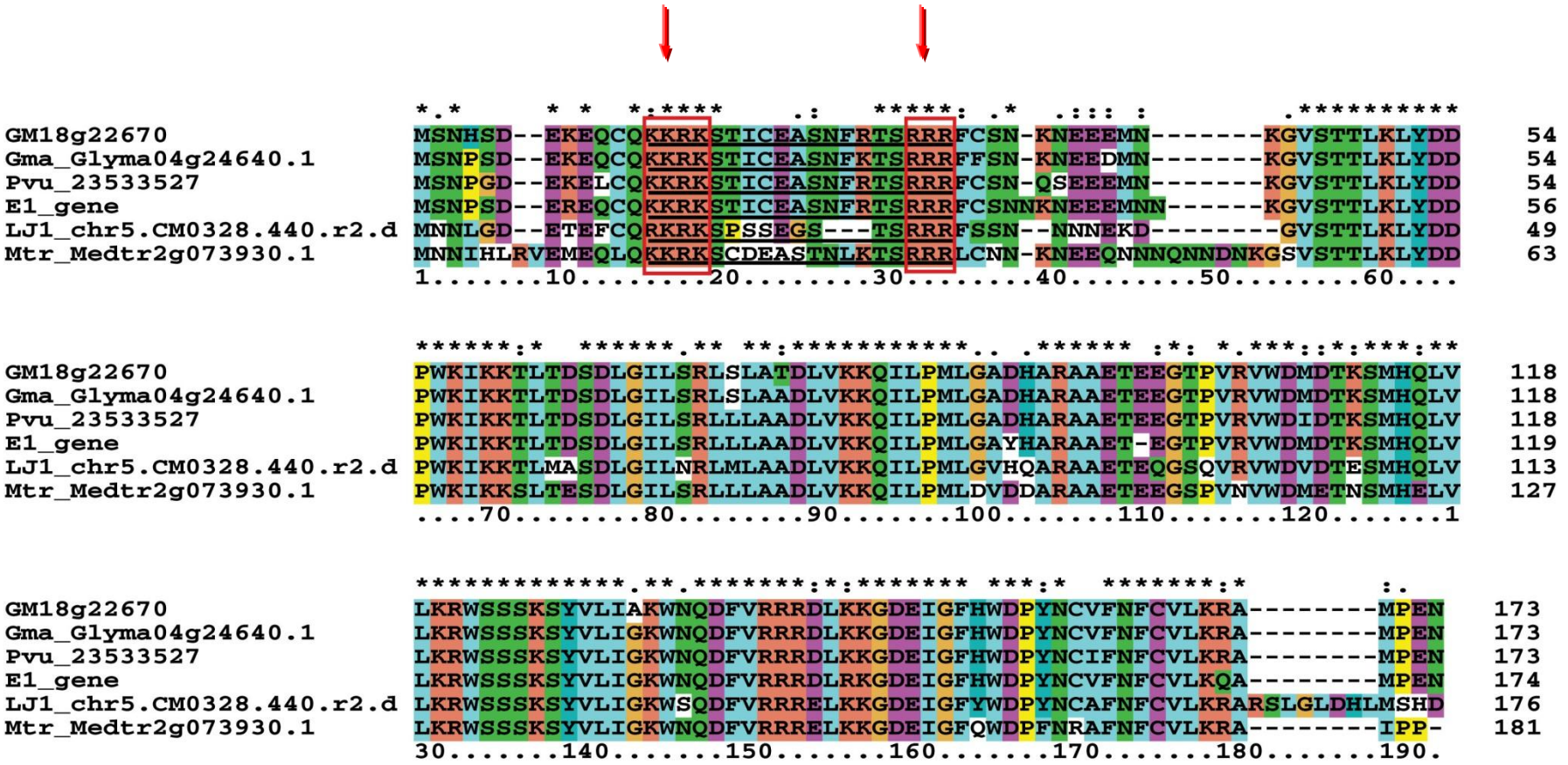
Delimit E1 locus to a 17.4 kb region




B
E1
Glyma04g24640.1
Gm18g22670
Pvu 23533527
LJ1 chr5.CM0328.440.r2.d
Mtr Medtr2g073930.1
LJ2 chr6.CM0830.560.r2
Ccl 19275809
Csi 18094876
Lus 23151243
Mtr Medtr1g059640.1
Glyma10g17480.1
Glyma10g20520.1
Mgu 17678668
Cpa 16427529
Egr 23578593
Aly 347288
Bra 22691616
Cru 20899217
Os VP1
Ta VP1
At AP2
Glyma20g39140.1
Os B3
Zm RAV1
Glyma16g01950.1
At TEM1
Glyma10g34760.1
Glyma20g32730.1
Glyma01g22260.1
Glyma02g11060.1
83
59
97
58
74
53
99
45
98
42
99
93
99
99
97
72
97
49
98
C
S125
K127
A79
S124
R15
W123
L72
I71
N
C

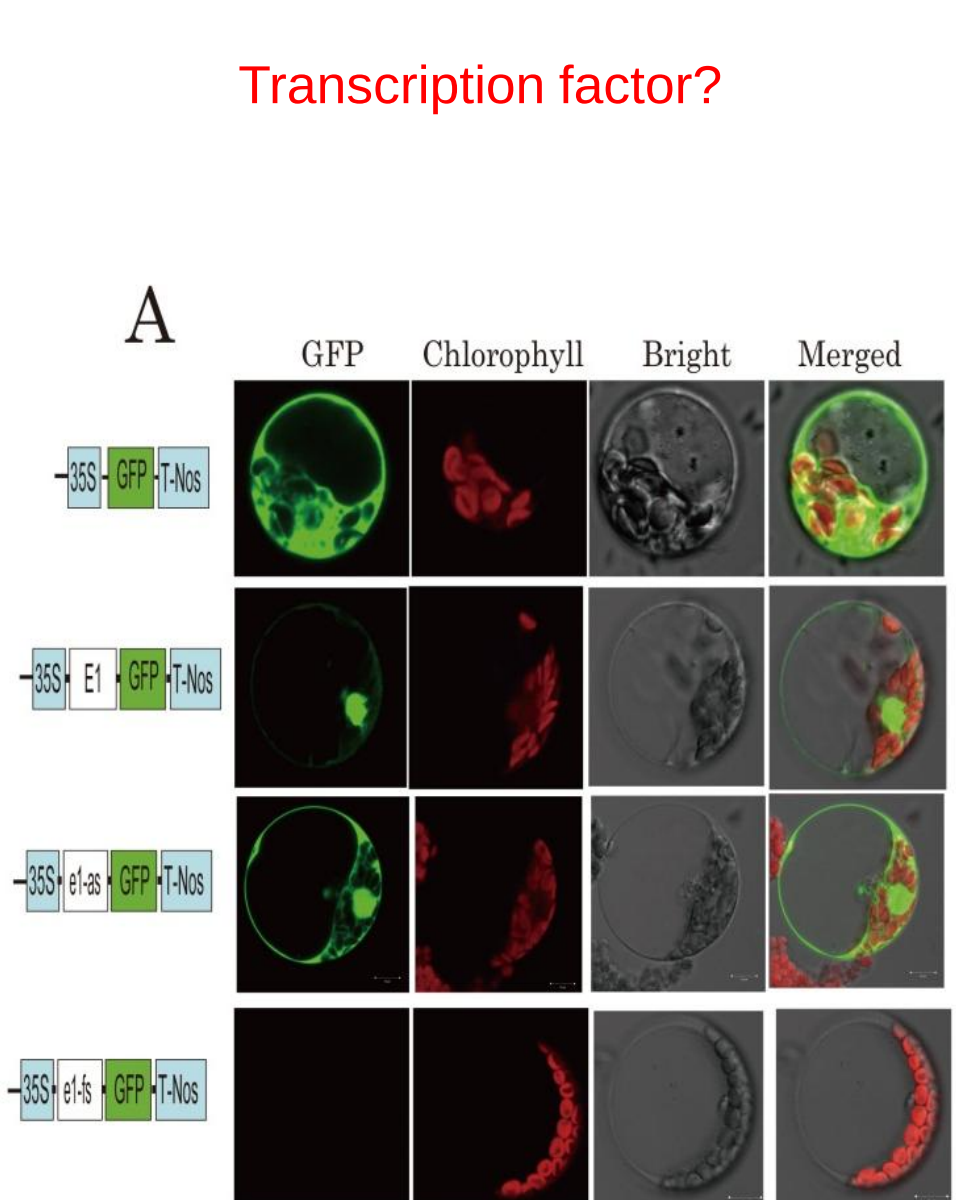
E1 Contains a bipartite nuclear localization signal

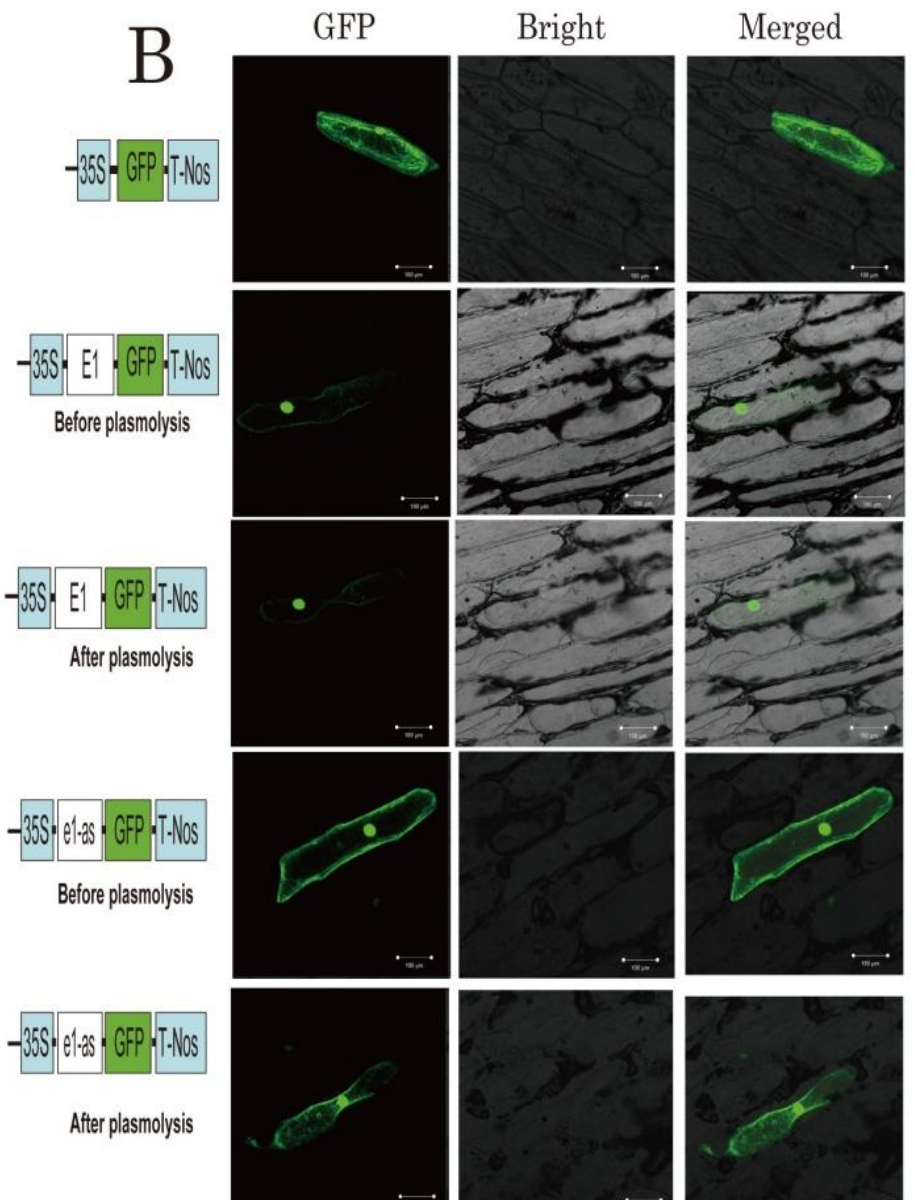
A



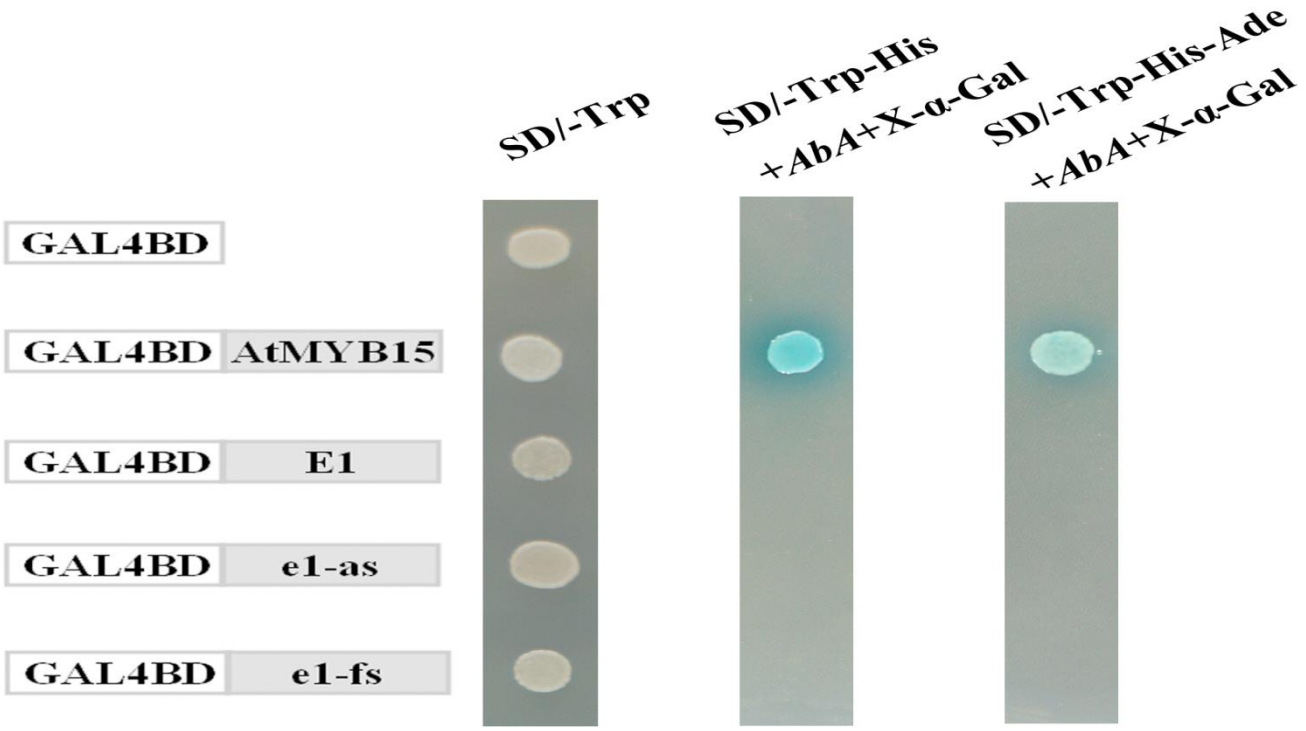
Subcellular localization

Transcription factor?

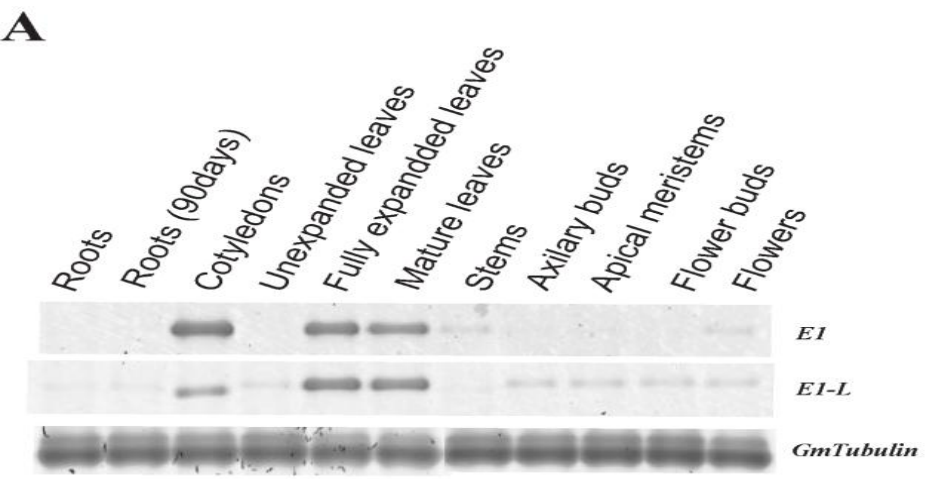


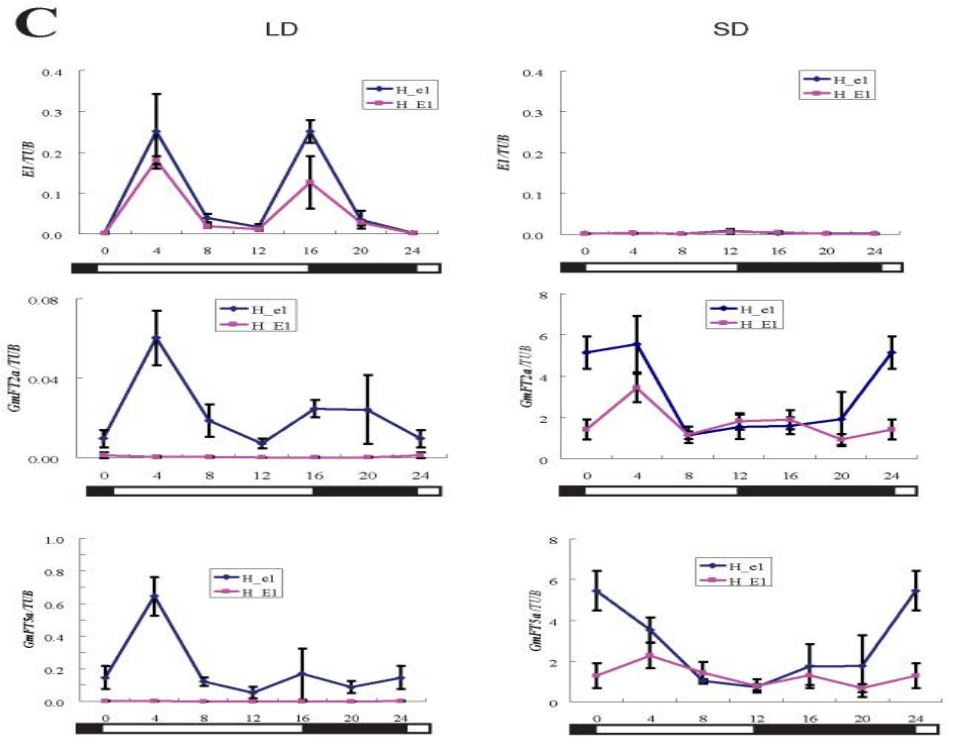


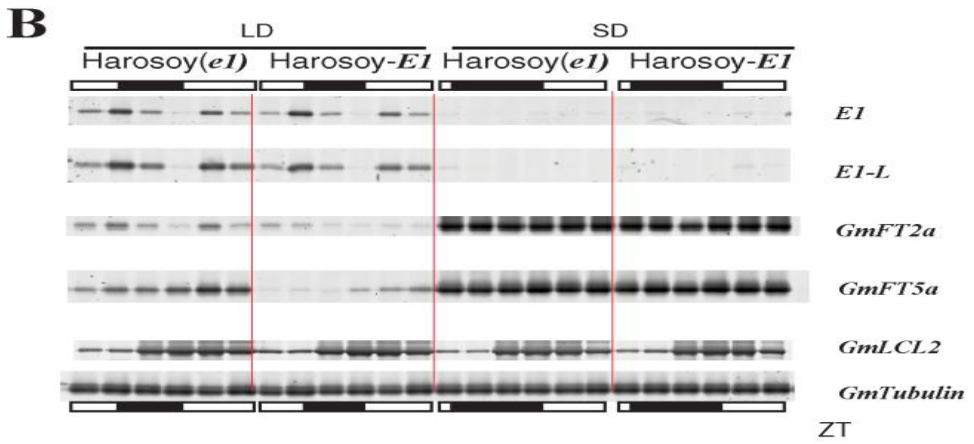
E1 showing transcriptional repression activity

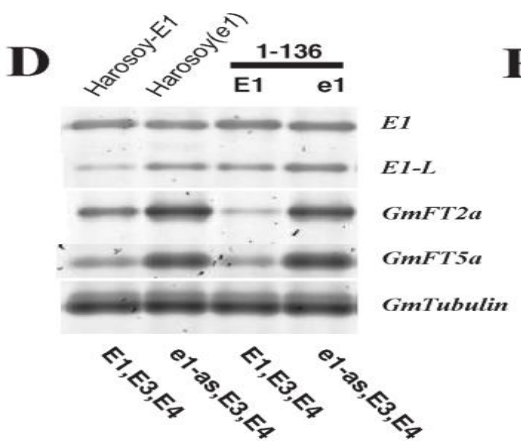


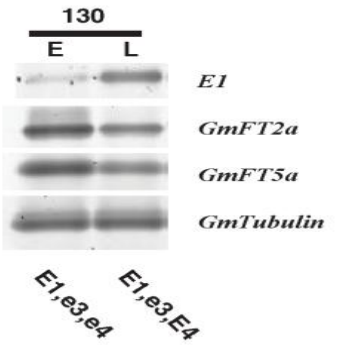
Expression


A
Roots
Roots (90days)
Cotyledons
Unexpanded leaves
Fully expandded leaves
Mature leaves
Stems
Axilary buds
Apical meristems
Flower buds
Flowers
E1
E1-L
GmTubulin


C
LD
SD
H_e1
H_E1
E1/TUB
GmFT2a/TUB
GmFT5a/TUB

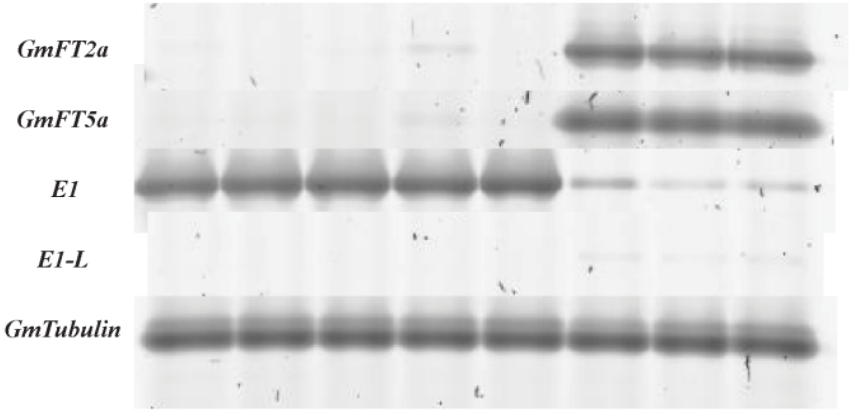

B
LD
SD
Harosoy(e1)
Harosoy-E1
Harosoy(e1)
Harosoy-E1
E1
E1-L
GmFT2a
GmFT5a
GmLCL2
GmTubulin
ZT

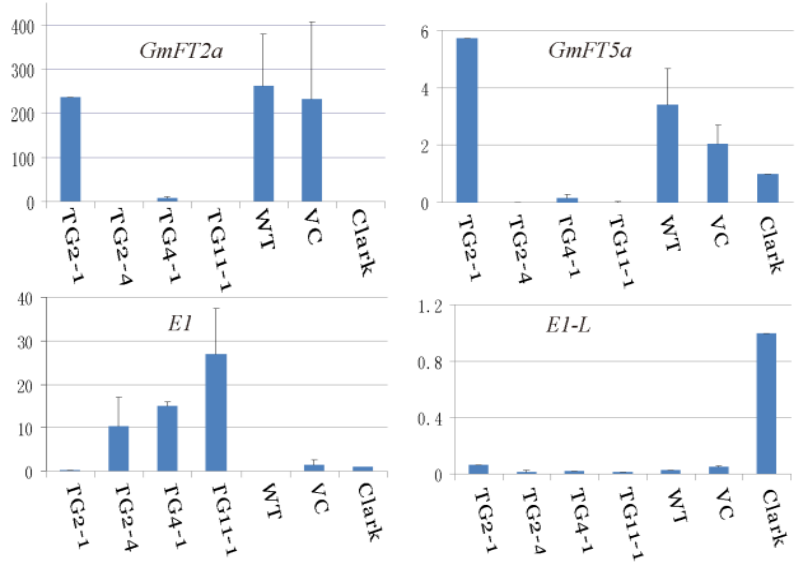

D
Harosoy-E1
Harosoy(e1)
1-136
E1
e1
E1
E1-L
GmFT2a
GmFT5a
GmTubulin
E1,E3,E4
e1-as,E3,E4
E1,E3,E4
e1-as,E3,E4


E
130
E
L
E1
GmFT2a
GmFT5a
GmTubulin
E1,e3,e4
E1,e3,E4

Transformation

	TG2-3	TG2-4	TG2-5	TG4-1	TG11-1	TG2-1	VC	WT
Copy no.	1	2	1	3	1	8	0	0
R1 (days)	53	59	64	37	>70	24	29	29





The high *E1* expression in transgenic lines led to a later flowering pheotype

T_0 Line	T_1 Plant	Copy No.	*E1* 15d	*E1* 30d	*GmFTs* 15d	*GmFTs* 30d	Flowering time
TG2	2	7~8	-	-	+++	+++	24.5 ± 0.5
	4	1~2	+++	+++	-	-	>53days
TG11	2	1~2	+++	+++	-	-	>70days
TG4	1	3	+++	+	±	++	37
WT	6	0	-	-	+++	+++	29.1 ± 0.69
NC	6	0	-	-	+++	+++	29.4 ± 0.90

Three EMS derived *E1* mutants

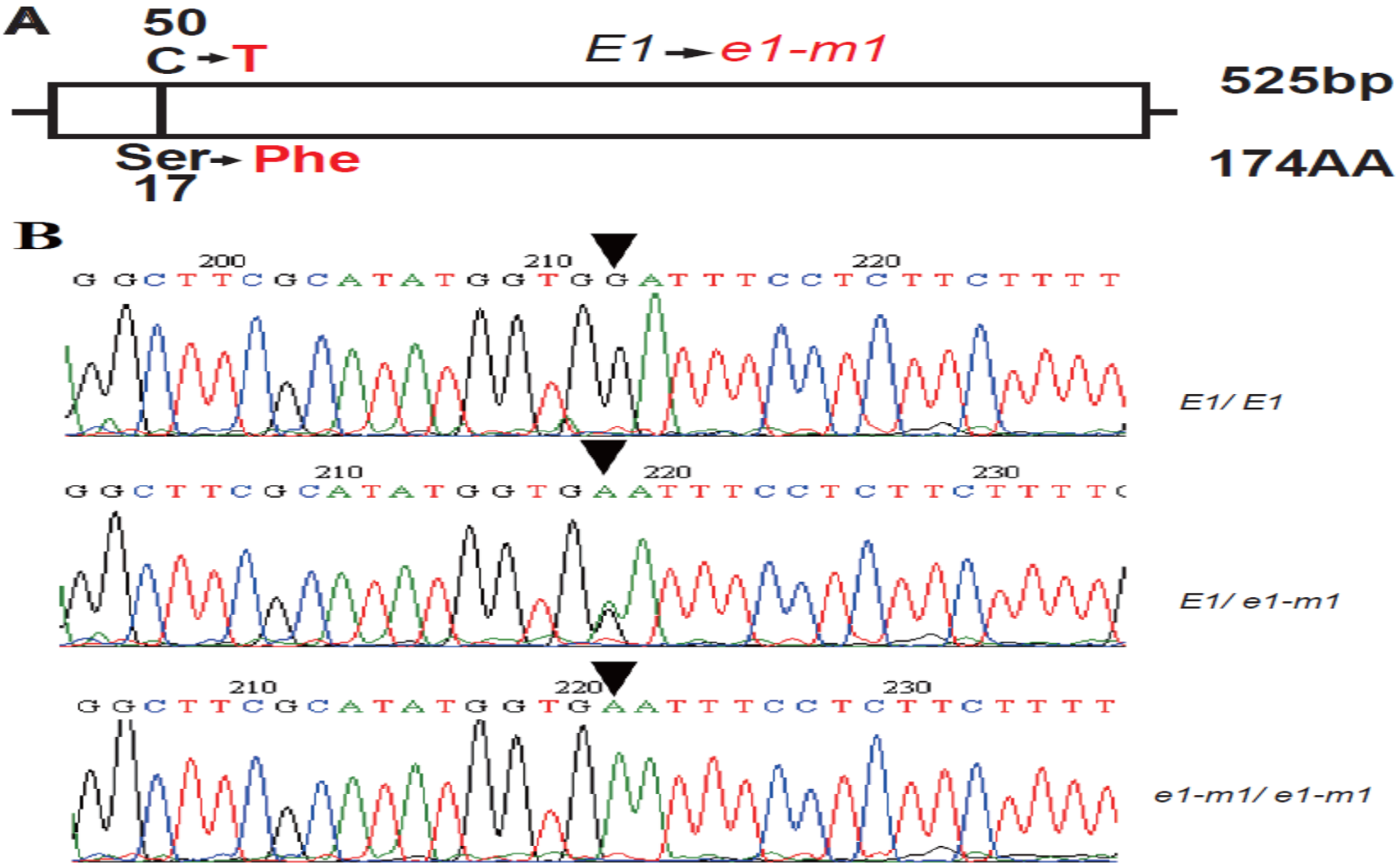


Table 1. Phenotypic analysis of flowering time in the wild-type (WT) and the progenies of *E1* mutants (*mut*) obtained using EMS mutagenesis.

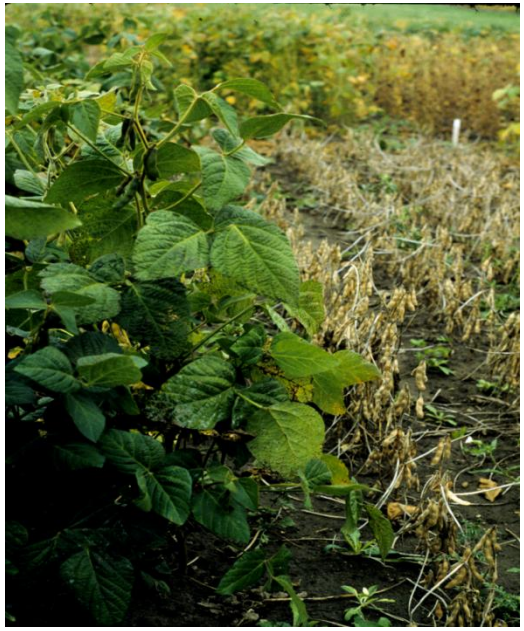
			Flowering time (days after emergence)						
			WT/WT		*WT/mut*		*mut/mut*		
Mutant line	Mutation in E1 protein[3]	Population	Average	S.D.	Average	S.D.	Average	S.D.	Significance[*4]
E1-m1 [*1]	Ser17Phe	Homozygous *mut* × wild- type, F_2	69.70 (*n* = 12)	±1.37	66.80 (*n* = 12)	±1.82	63.80 (*n* = 12)	±1.07	*P<0.001*
E1-m2 [*2]	Arg15Lys	Heterozygous *mut*, self-crossed	47.67 (*n* = 9)	±1.25	45.38 (*n* = 16)	±2.15	38.71 (*n* = 7)	±1.03	*P<0.001*
	Arg15Lys	Homozygous *mut*					38.11 (*n* = 9)	±1.73	
E1-m3 [*2]	Thy65Ile	Heterozygous *mut*, self-crossed	46.75 (*n* = 4)	±1.92	43.75 (*n* = 8)	±1.64	37.33 (*n* = 3)	±2.05	*0.01<P<0.05*
	Thy65Ile	Homozygous *mut*					35.20 (*n* = 5)	±1.72	
Wild-type		WT, Fukuyutaka	48.57 (*n* = 7)	±0.90					

E1 genotype

Cultivar or NIL	Accession number	Genotype	Flowering time (R1, days after emergence)			
			Growth Chamber[1]			Field[2] (NIAS, Japan)
			12L/12D	14.5L/9.5D	16L/8D	
		E1				
Aokimame	JP 28298	*E1,E2,E3,E4*	31.0	n.d.	106.0	89.6
Peking	JP 28432	*E1,E2,E3,E4*	n.d.	65.0	98.0	55.2
Bay	PI 553043	*E1,E2,E3,E4*	29.5	n.d.	75.0	60.0
Harosoy-*E1*	PI 547676	*E1,e2,E3,E4*	31.0	51.0	73.0	46.4
1-136(*E1*)[a]	-	*E1,E2,e3,E4*	n.d.[c]	66.0	n.d.	n.d.
Enrei	JP 28862	*E1,e2,e3,E4*	n.d.	51.0	71.0	49.0
9L[b]	-	*E1,e2,e3,E4*	29.0	n.d.	58.5	n.d.
Kariyutaka	JP 86520	*E1,E2,e3,e4*	n.d.	32.0	36.0	36.6
130L[b]	-	*E1E4*	n.d.	39.0	n.d.	n.d.
130E[b]	-	*E1e4*	n.d.	33.0	n.d.	n.d.
		e1-as				
Clark	PI 617268	*e1-as,E2,E3,E4*	n.d.	40.0	58.0	42.4
Williams 82	PI 518671	*e1-as,E2,E3,E4*	29.0	n.d.	54.0	41.8
Harosoy-*E2*	PI 547768	*e1-as,E2,E3,e4*	30.0	n.d.	57.0	36.9
1-136(*e1*)[a]	-	*e1-as,E2,e3,E4*	n.d.	40.0	n.d.	n.d.
Harosoy	PI 547707	*e1-as,e2,E3,E4*	29.0	33.5	49.0	35.4
Harosoy-*e4*	PI 591435	*e1-as,e2,E3,e4*	29.0	n.d.	44.0	33.2
Harosoy-*e3*	PI 547716	*e1-as,e2,e3,E4*	29.5	n.d.	43.0	32.3
		e1-fs				
Sakamotowase	JP 27450	*e1-fs,e2,e3,E4*	29.0	31.0	30.0	29.7
9E[b]	-	*e1-fs,e2,e3,E4*	29.0	n.d.	32.0	n.d.
		e1-nl				
Yukihomare	-	*e1-nl,e2,E3,e4*	n.d.	34.0	36.0	34.9
Fiskeby V	JP 30465	*e1-nl,e2,e3,E4*	29.0	n.d.	30.0	32.0

E1 effect

Harbin



Canada
(E Cober)

Transgenic plants

Positional cloning and characterization reveal the molecular basis for soybean maturity locus *E1* that regulates photoperiodic flowering

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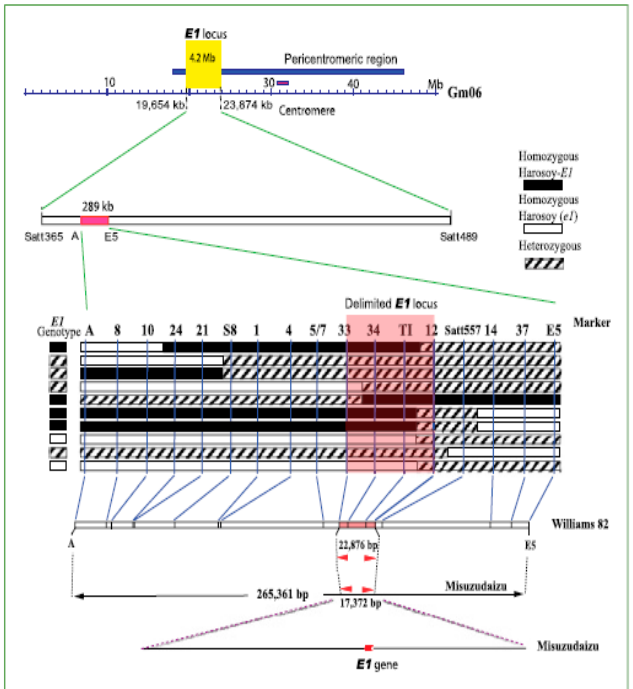
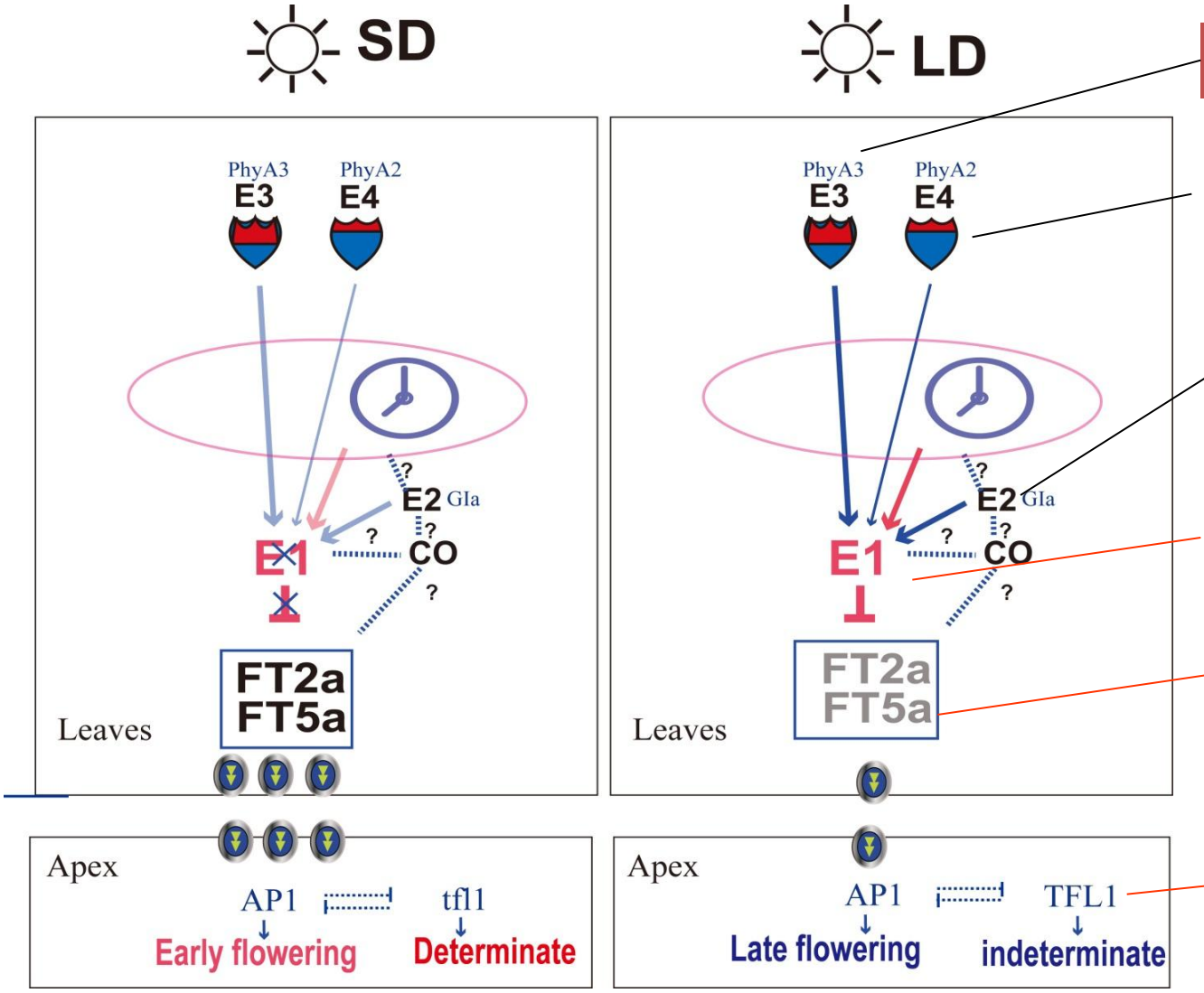
AUTHOR SUMMARY

Various external cues, such as photoperiod and temperature, are known to initiate plant flowering under the appropriate seasonal conditions. Nearly a century ago, photoperiodism, the general concept of photoperiodic induction of flowering, was established for the first time based on evidence that many plant species flower in response to changes in day length. In *Arabidopsis thaliana* and rice (*Oryza sativa*), a conserved pathway that confers day-length responses through the regulation of transcription of the *FLOWERING LOCUS T* (*FT*) by the transcription factor *CONSTANS* (*CO*) has been well characterized. However, the regulatory



The *E1* locus resides in the pericentromeric region of chromosome 6 in soybean (http://www.phytozome.net), a region that is characterized by a high ratio of physical to genetic distance; such regions make it difficult to identify genes that underlie important agronomic traits through positional cloning. To overcome this difficulty, we developed a mapping population by crossing two near-isogenic lines, Harosoy-*E1* and Harosoy (*e1*), which have identical genomes except at the *E1* locus. Among 13,761 seeds produced by crosses between these two lines, we successfully identified 10 recombinants in the *E1* region

Putative photoperiod response pathway



Genetics, 2009

Genetics, 2008

Genetics, 2011

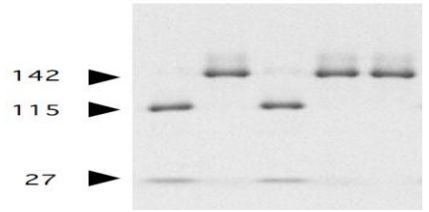
PNAS, 2012

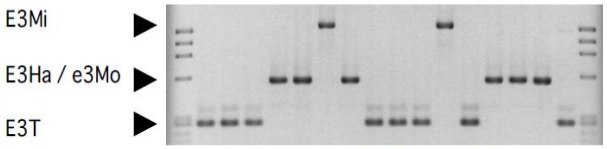
Plant Physiology, 2010

Plant Physiology, 2010

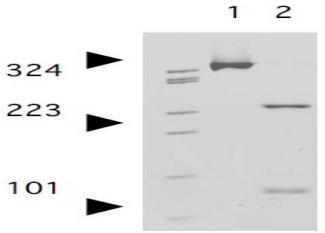
Genotyping

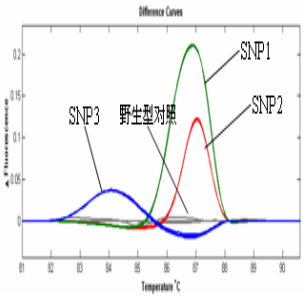
E1	*E3*	*E4*	*E2*
e1-as	*e3-mi*	*e4a*	*e2*
e1-nl	*e3-ha*	*e4b*	
e1-fs	*e3-mo*	*e4-c*	…
…	*e3-t*	*e4-d*	…

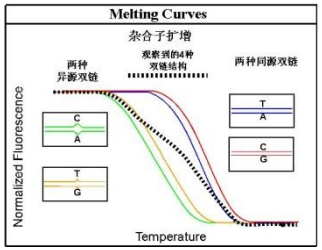




2% Agarose100V 45 min







Association with photoperiod response and flowering / maturity time

Conclusion and future prospective

- **With the use of the large populations, we successfully cloned the *E1* gene**
- **The E1 gene is legume specific, implying soybean has a specific photoperiod pathway deferent from Arabidopsis and rice**
- **E1 contains a bipartite nuclear signal and a domain distantly related to AP2**
- ***E1* genotype and expression level control flowering time**
- **The functional mechanism (ongoing)**
  - **how the E1 expression is regulated?**
  - **how *E1* controls *GmFTs*?**

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CAS, China

Thank you!

Map-based cloning in soybean

Genome wide association study