



Allelic Combinations of Soybean Maturity Loci *E1*, *E2*, *E3* and *E4* Result in Diversity of Maturity and Adaptation to Different Latitudes

Bingjun Jiang¹*, Haiyang Nan^{2,3}*, Youfei Gao¹*, Lili Tang², Yanlei Yue¹, Sijia Lu^{2,3}, Liming Ma¹, Dong Cao², Shi Sun¹, Jialin Wang², Cunxiang Wu¹, Xiaohui Yuan², Wensheng Hou¹, Fanjiang Kong^{2*}, Tianfu Han^{1*}, Baohui Liu^{2*}

1 The National Key Facility for Crop Gene Resources and Genetic Improvement and MOA Key Lab of Soybean Biology (Beijing), Institute of Crop Sciences, The Chinese Academy of Agricultural Sciences, Beijing, China, **2** The Key Laboratory of Soybean Molecular Design Breeding, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin, China, **3** University of Chinese Academy of Sciences, Beijing, China

Abstract

Soybean cultivars are extremely diverse in time to flowering and maturation as a result of various photoperiod sensitivities. The underlying molecular genetic mechanism is not fully clear, however, four maturity loci *E1*, *E2*, *E3* and *E4* have been molecularly identified. In this report, cultivars were selected with various photoperiod sensitivities from different ecological zones, which covered almost all maturity groups (MG) from MG 000 to MG VIII and MG X adapted from latitude N 18° to N 53°. They were planted in the field under natural daylength condition (ND) in Beijing, China or in pots under different photoperiod treatments. Maturity-related traits were then investigated. The four *E* maturity loci were genotyped at the molecular level. Our results suggested that these four *E* genes have different impacts on maturity and their allelic variations and combinations determine the diversification of soybean maturity and adaptation to different latitudes. The genetic mechanisms underlying photoperiod sensitivity and adaptation in wild soybean seemed unique from those in cultivated soybean. The allelic combinations and functional molecular markers for the four *E* loci will significantly assist molecular breeding towards high productivity.

Citation: Jiang B, Nan H, Gao Y, Tang L, Yue Y, et al. (2014) Allelic Combinations of Soybean Maturity Loci *E1*, *E2*, *E3* and *E4* Result in Diversity of Maturity and Adaptation to Different Latitudes. PLoS ONE 9(8): e106042. doi:10.1371/journal.pone.0106042

Editor: Fan Chen, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, China

Received: May 30, 2014; **Accepted:** July 27, 2014; **Published:** August 27, 2014

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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported by the National Natural Science Foundation of China (31201232, 31071445, 31171579, 31201222 and 31371643); Open Foundation of Key Laboratory of Soybean Molecular Design Breeding, Chinese Academy of Sciences; "Hundred Talents" Program of Chinese Academy of Sciences; Strategic Action Plan for Science and Technology Innovation of Chinese Academy of Sciences (XDA08030100); Heilongjiang Natural Science Foundation of China (ZD201001, JC201313); China Agriculture Research System (CARS-04) and Chinese Academy of Agricultural Sciences (CAAS) Innovation Project. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: kongfj@iga.ac.cn (FK); hantianfu@caas.cn (TH); liubh@iga.ac.cn (BL)

† These authors contributed equally to this work.

Introduction

Soybean (*Glycine max* (L.) Merrill) is a short-day crop with high protein and oil contents. Many cultivars were bred with different maturity to adapt to various ecological environments. For the convenience of breeding layout, 13 MGs from MG000 to MGX were classified in North America [1–3]. Chinese soybean researchers divided cultivars into different maturity groups as well [4–5]. Soybean production has increased in America in response to recent increases in global demand and maturity is the key factor determining soybean productivity. Therefore, it is rather important to understand the mechanism of soybean maturity diversity and adaptation.

Flowering and maturity were highly controlled by major genes in soybean. Up to now, nine maturity loci have been identified as *E1*–*E8* and *J* [1], [6–12]. These loci have different roles under different photoperiods. Wang et al [13] found that long daylength

condition (LD) might strengthen while short daylength condition (SD) might weaken these maturity loci. More results and progress of maturity genes was reviewed by Xia et al [14]. Furthermore, four loci were identified at molecular level. *E1* gene was identified as a transcription factor which functions as a flowering repressor with a putative nuclear localization signal and a B3-related domain [15]. *E2* is an orthologue of Arabidopsis flowering gene *GIGANTEA* [16]. *E3* and *E4* are phytochrome genes *GmPhyA3* [17] and *GmPhyA2* [18], respectively. In addition, two homologs of soybean *Flowering Locus T* (*FT*) genes, *GmFT2A* and *GmFT5A* were identified and coordinately regulate flowering [19]. Four identified maturity genes *E1*, *E2*, *E3* and *E4* delay flowering and maturity under LD through down regulating *GmFT2A* and *GmFT5A* [15], [16], [19]. As for other loci, more studies should be done before learning their molecular identities.

Although these four known loci *E1*, *E2*, *E3* and *E4* provide an important key to learn the mechanism of flowering and maturity,

Table 1. Cultivars from North America and their respective maturity group.

Code	PI number	Variety	MG	Code	PI number	Variety	MG
CAAE001	P1548594	Maple Presto	000	CAAE021	P1534646	Flyer	IV
CAAE002	P1567787	OAC Vision	000	CAAE022	P1598222	TN4-94	IV
CAAE003	P1548648	Canatto	00	CAAE023	P1564849	Nathan	V
CAAE005	P1592523	Glacier	00	CAAE024	P1572239	Holladay	V
CAAE006	P1629004	MIN0201	0	CAAE025	P1633609	Lomoke	V
CAAE007	P1596541	Trall	0	CAAE026	P1561400	Rhodes	V
CAAE008	P1612764	MIN0901	0	CAAE027	P1633610	Desha	VI
CAAE009	P1599300	Surge	NA	CAAE028	P1592756	Dillon	VI
CAAE010	P1548641	Haroson	I	CAAE029	P1617045	NC-Roy	VI
CAAE011	P1614833	NE1900	I	CAAE030	P1599333	Musen	VI
CAAE012	P1608438	Titan	I	CAAE031	P1531068	Stonewall	VII
CAAE013	P1561858	Holt	II	CAAE032	P1595645	Benning	VII
CAAE014	P1567786	OAC Talbot	II	CAAE033	P1617041	Santee	VII
CAAE015	P1533655	Burlison	II	CAAE034	P1555453	Hagood	VII
CAAE016	P1595926	Athow	III	CAAE035	P1603953	Motte	VIII
CAAE017	P1548634	Zane	III	CAAE036	P1548970	Foster	VIII
CAAE018	P1593258	Macon	III	CAAE037	P1548663	Dowling	VIII
CAAE019	P1578057	Saline	III	CAAE038		Jupiter	X
CAAE020	P1614155	NS93-4118	IV				

NA, not available.
doi:10.1371/journal.pone.0106042.t001

Table 2. Cultivars and accessions from China and far-east Russia and their adaption latitudes.

Code	Variety	Latitude	Code	Variety	Latitude
CAAE039	Dengke 2	N 47°–53°	CAAE064	Fengchengzaochadou	N 25°–30°
CAAE040	Huaijiang 4	N 48°–50°	CAAE065	Jin 6606	N 41°–43°
CAAE041	Heihe 27	N 46°–48°	CAAE066	Jinzhou 8–14	N 40°–43°
CAAE042	Heihe 3	N 46°–50°	CAAE071	Bahong 1	N 39°–42°
CAAE043	Heihe 43	N 46°–48°	CAAE072	Mianyanghuangwofeng	N 30°
CAAE044	Suinong 14	N 44°–48°	CAAE073	Lüpidou	N 25°–28°
CAAE045	Hefeng 25	N 39°–43°	CAAE074	Liuyueza	N 25°–28°
CAAE047	Jilin 3	N 44°–46°	CAAE075	Ruijinxiaohuangdou	N 24°–27°
CAAE049	Jiunong 21	N 42°–46°	CAAE076	Edou 2	N 30°–35°
CAAE050	Jilin 30	N 41°–43°	CAAE077	Yulindahuangdou	N 23°
CAAE052	Jindou 19	N 35°–40°	CAAE081	Qidou 1	N 25°–30°
CAAE053	Tiefeng 31	N 35°–40°	CAAE082	Jiangledaqingdou	N 27°
CAAE054	Jidou 12	N 35°–38°	CAAE084	Guixia 1	N 22°–26°
CAAE055	Qihuang 28	N 34°–37°	CAAE085	Nandou 12	N 29°–32°
CAAE056	Zhonghuang 13	N 30°–40°	CAAE086	Zigongdongdou	N 29°
CAAE057	Xudou 9	N 32°–35°	CAAE087*	Heiheyesheng	N 50°
CAAE058	Xudou 1	N 32°–35°	CAAE088*	Bayanyesheng	N 46°
CAAE059	Fengshouhuang	N 35°–38°	CAAE089*	Baiyangdianyesheng	N 39°
CAAE061	Dandou 2	N 40°–42°	CAAE090*	Guangxiyesheng	N 23°
CAAE062	Yuejin 4	N 35°–38°	CAAE091	Mohe 1	N 53°
CAAE063	Jinda 814	NA	CAAE092	Ziweicika 4/75	N 50°

CAAE092 is a cultivar from far east Russia; *indicates wild soybeans: CAAE087, CAAE088, CAAE089 and CAAE090.
doi:10.1371/journal.pone.0106042.t002

we mainly got knowledge based on few cultivars but not on a population level. Population-level knowledge will provide another different view of these four loci's role on maturity and adaptation. Therefore, in this study we selected a set of soybean cultivars which cover 12 maturity groups from MG000 to MGVIII and MGX plus some cultivars with wide range of latitude from N 18° to N 53°. These cultivars were subjected to different photoperiod treatments. Traits of beginning bloom (R1), physiological maturity (R7) and full maturity (R8) were investigated [20]. Maturity loci *E1*, *E2*, *E3* and *E4* were genotyped in the population. Further association analysis was done. The results showed that allelic combinations of these four *E* genes significantly determine the ecological-economical adaption of cultivars although they have different impacts on maturity. In addition, the genetic mechanisms underlying photoperiod sensitivity and adaptation in wild soybean seemed unique from those in cultivated soybean.

Materials and Methods

Soybean cultivars were selected from North America (Table 1), China and Russia (Table 2) [21–23]. Four wild soybean accessions were also included for genotyping only (Table 2), which are CAAE087 (Heiheyesheng) collected in Heihe (N 50°22', E 127°53'), Heilongjiang, China; CAAE088 (Bayanyesheng) in Bayan (N 46°08', E 127°39'), Heilongjiang, China; CAAE089 (Baiyangdianyesheng) in Baoding (N 38°51', E 115°30'), Hebei, China; and CAAE090 (Guangxiyesheng) in Nanning (N 22°48', E 108°19'), Guangxi, China. They covered 12 maturity groups from MG 000 to MG VIII and MG X and ranged from N 18° to N 53° indicated in Table 1 and Table 2.

Due to seed availability, 59 cultivars were selected for both field and pot experiments, 12 cultivars only in the field experiment, and 4 cultivars only in the pot experiment. For the field experiment, seeds were sowed on May 14th, 2012 in Beijing (N 39°97', E 116°34') and maturity-related traits of R1, R7 and R8 were recorded regularly [20]. For the pot experiment, seeds were sowed in 10-liter pots on May 16th 2012 and grown under ND in Beijing. After emergence, the seedlings were thinned until each pot contained five uniform plants. These uniform plants were grown until the unifoliate expanded then treated with different photoperiods (LD, 16 h light/8 h dark; SD, 12 h light/12 h dark; and ND). For SD, the plants were transferred to dark room to shorten the daylength. For LD, incandescent bulbs (50 $\mu\text{mol m}^{-2} \text{s}^{-2}$ at the top of plants) with automatic timer controls were used to extend the daylength. Additional details of plant growth and treatments were the same as reported by Wu et al [24]. The days to first flowering of each plant was recorded. Both experiments finished on Oct 15th, 2012. Photoperiod sensitivity (PS) was thus calculated as the following function, where DDF_{LD} is the days to first flowering (R1) from the expansion of the first pair of unifoliate (V1) under LD while DDF_{SD} under SD [25].

$$PS(\%) = (DDF_{LD} - DDF_{SD}) / DDF_{LD} \times 100\%$$

Genomic DNA was isolated from soybean unifoliate leaves using TianGen New Plant Genomic DNA Isolation Kit (DP320). Maturity loci *E1*, *E2*, *E3* and *E4* were genotyped using functional allele specific molecular markers [26].

Table 3. The days from VE to R1, R7 and R8 of soybean Cultivars under ND condition at Beijing.

Code	Phenotype			Code	Phenotype		
	VE-R1	VE-R7	VE-R8		VE-R1	VE-R7	VE-R8
CAAE001	20.7±1.7	61.0±3.8	70.9±1.8	CAAE038	122.3±1.0	NA	NA
CAAE002	20.0±1.8	63.9±3.4	71.5±1.9	CAAE039	20.4±0.8	61.5±4.8	71.7±2.0
CAAE003	20.1±0.4	57.2±1.0	68.0±0.0	CAAE040	19.0±0.0	67.0±6.7	76.7±3.4
CAAE005	20.3±1.8	68.4±1.6	73.7±1.0	CAAE041	20.1±0.4	69.7±0.5	76.7±1.3
CAAE006	19.9±1.3	69.3±3.0	77.0±2.2	CAAE042	20.4±1.5	71.3±1.8	78.3±2.1
CAAE007	20.3±2.0	70.1±2.2	77±2.2	CAAE043	19.1±0.5	71.0±2.2	81.3±6.5
CAAE008	22.4±1.6	93.4±10.6	108.5±15	CAAE044	21.1±0.7	71.3±2.4	80.7±6.3
CAAE009	21.9±1.2	86.9±2.1	100.7±3.6	CAAE045	20.2±0.4	71.3±1.8	78.3±2.1
CAAE010	22.5±1.4	85.3±2.0	90.9±1.6	CAAE047	23.1±2.3	83.9±1.3	94.4±4.2
CAAE011	25.8±1.9	81.1±11.1	103.9±5.9	CAAE049	23.4±1.2	83.9±9.9	101.4±7.0
CAAE012	21.9±1.4	89.7±1.6	101.3±2.6	CAAE050	24.9±1.1	98.6±5.2	109.9±7.3
CAAE013	22.7±1.4	94.2±1.8	109.9±4.7	CAAE052	24.5±0.8	99.9±2.8	114.2±2.4
CAAE014	21.6±0.5	92.5±3.0	100.3±3.1	CAAE053	23.1±0.3	113.6±3.0	121.7±2.2
CAAE015	25.2±1.9	102.1±7.3	113.9±5.3	CAAE054	37.4±0.6	119.8±6.6	127.3±7.0
CAAE016	24.9±1.0	106.3±7.0	118.5±3.5	CAAE055	42.7±2.3	127.0±8.0	136.8±5.1
CAAE017	26.9±1.8	117.1±2.3	125.5±1.5	CAAE056	36.4±2.0	113.7±6.3	121.1±2.7
CAAE018	24.7±0.6	117.9±1.8	124.9±3.3	CAAE057	31.3±1.0	111.4±2.5	119.9±1.4
CAAE019	28.6±1.2	123.4±2.7	131.6±3.3	CAAE058	24.2±1.0	95.9±2.8	106.2±5.2
CAAE020	24.2±1.2	116.4±2.8	125.7±1.9	CAAE059	25.5±0.5	111.2±3.1	118.7±2.6
CAAE021	26.3±1.3	123.4±3.3	133.4±2.9	CAAE063	41.9±2.1	116.0±2.0	122.0±0.0
CAAE022	32.5±4.4	125.1±4.1	134.6±1.4	CAAE064	41.3±1.0	101.9±3.7	111.3±4.7
CAAE023	56.1±3.9	131.9±7.8	139.3±2.4	CAAE065	27.1±1.2	103.9±9.1	115.7±5.1
CAAE024	50.6±2.6	132.9±6.1	141.0±3.9	CAAE066	42.5±1.5	116.0±3.2	126.8±6.3
CAAE025	53.7±1.1	140.6±4.5	142±0.0 [†]	CAAE071	49.1±4.0	132.6±6.4	140.0±5.6
CAAE026	54.9±1.9	141.5±4.4	143.2±1.8	CAAE072	91.3±2.7	141.0±1.4	NA
CAAE027	61.8±4.6	142.5±4.9	142.0±0.0 [†]	CAAE073	54.2±1.1	140.0±4.7	144.0±2.8 [†]
CAAE028	63.3±5.5	141.8±4.3	144.0±1.7 [†]	CAAE074	69.0±0.0	125.4±4.6	133.5±4.6
CAAE029	59.0±5.2	142.0±3.1	145.0±0.0 [†]	CAAE075	94.0±2.1	NA	NA
CAAE030	75.1±2.0	141.9±3.0	145.0±0.0 [†]	CAAE076	65.5±5.5	143.8±1.6	NA
CAAE031	77.7±3.3	142.8±1.1	NA	CAAE077	71.8±9.8	NA	NA
CAAE032	70.8±3.2	NA	NA	CAAE081	NA	NA	NA
CAAE033	70.4±3.1	145.0±0.0	NA	CAAE082	96.8±1.8	NA	NA
CAAE034	77.7±1.6	NA	NA	CAAE084	98.3±2.9	NA	NA
CAAE035	75.9±4.4	145.0±0.0	NA	CAAE085	95.1±2.6	NA	NA

Table 3. Cont.

Code	Phenotype			Code	Phenotype		
	VE-R1	VE-R7	VE-R8		VE-R1	VE-R7	VE-R8
CAAE036	74.3±4.5	NA	NA	CAAE086	NA	NA	NA
CAAE037	69.6±4.6	146.1±0.9	NA				

^apartial plants (<50%) matured. NA, not available.
doi:10.1371/journal.pone.0106042.t003

Ethics Statement

No specific permissions were required for domestic research of the collections of wild soybean accessions in Heihe (N 50°22', E 127°53'), Bayan (N 46°08', E 127°39'), Baoding (N 38°51', E 115°30') and Nanning (N 22°48', E 108°19'), China. All the field studies did not involve endangered or protected species.

Results

Soybean cultivars have diverse flowering and maturity dates

Seventy-one cultivars were planted under ND in the field at Beijing. These cultivars showed rich diversity in maturity (Table 3). Two cultivars CAAE081 and CAAE086 failed to flower until the experiment ended. Nine cultivars CAAE032, CAAE034, CAAE036, CAAE038, CAAE075, CAAE077, CAAE082, CAAE084 and CAAE085, flowered but did not reach R7. Six cultivars CAAE031, CAAE033, CAAE035, CAAE037, CAAE072 and CAAE076 did not reach R8 although they flowered and podded. For other cultivars that regularly flowered and matured, the days to R1 from emergence (VE) ranged from 19.0 (CAAE040) to 75.1 (CAAE030), the days to R7 ranged from 57.2 (CAAE003) to 142.5 (CAAE027), and the days to R8 ranged from 68.0 (CAAE003) to 145.0 (CAAE029 and CAAE030). Thus, the range of time to R1, R7 and R8 showed the maturity diversity of these cultivars. The cultivars that reached R1 less than 50 days after emergence could mature before the frost in Beijing, those that reached R1 in greater than 50 days but less than 70 days could mature partially, and the ones that reached R1 after 70 days could hardly mature (Table 3). In Figure 1 and Figure 2, maturity-related traits of R1, R7 and R8 generally increased from early MG to late MG and from high latitude to low latitude.

Soybean cultivars react variously to photoperiod treatments

Sixty-three cultivars were planted in pots. They were treated with different photoperiods after V1. They began flowering in 18.4 to 32.8 days after emergence under SD, in 20.0 to 122.3 days under ND, and in 20.5 to 113.7 under LD, while under LD three cultivars CAAE031 (PI531068, MGVII), CAAE084 (Qjudou 1), and CAAE086 (Zigongdongdou) did not flower (Table 4). The PS was calculated according to the equation [25], which ranged between 10% and 80%. For the three cultivars that did not flower, it could be set at 100%. Thus, CAAE035 (PI603953, MGVIII), CAAE072 (Mianyanghuangwofeng), CAAE085 (Nandou 12), CAAE075 (Ruijinxiaohuangdou) and the former three cultivars are most sensitive to photoperiod (PS>75%). CAAE001 (PI548594, MG000), CAAE002 (PI567787, MG000), CAAE005 (PI592523, MG00), CAAE007 (PI596541, MG0), CAAE042 (Heihe 3) and CAAE091 (Mohe 1) could be classified as photoperiod insensitive because their photoperiod sensitivities are lower than 10%. These data suggested that these soybean cultivars diversify significantly in photoperiod sensitivity. Moreover, as shown in Figure 2, the photoperiod sensitivity of these cultivars generally increased from early MG to late MG and from high to low latitude.

Genotyping soybean cultivars of maturity loci *E1*, *E2*, *E3* and *E4*

Eighty-five cultivars were genotyped at four maturity loci *E1*, *E2*, *E3* and *E4* (Table 4). Thirty-eight cultivars are from North America, which cover 12 maturity groups from MG000 to MGVIII and MGX. Other cultivars are from China except

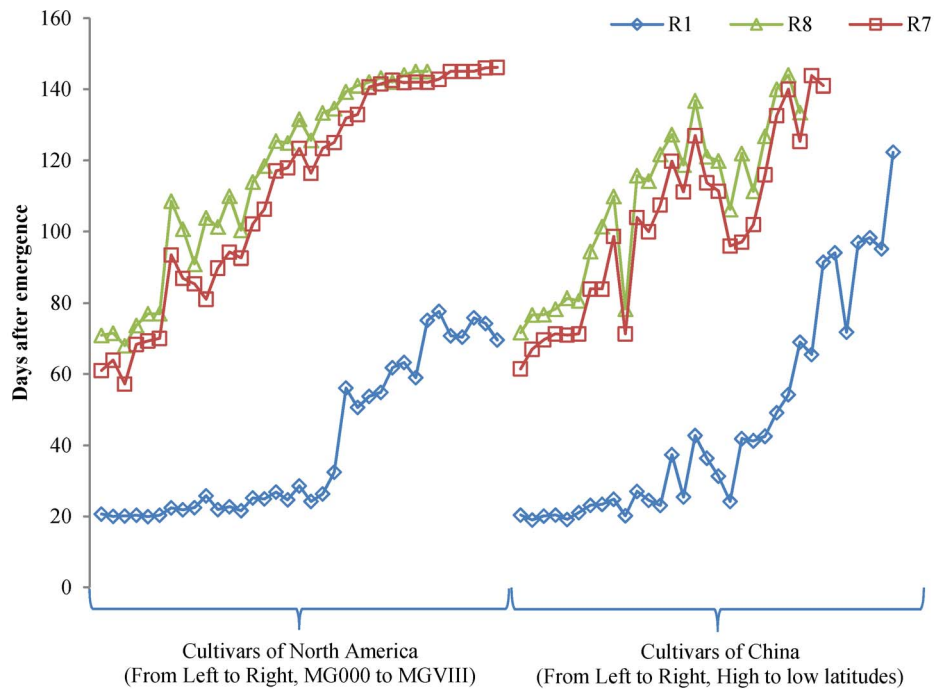


Figure 1. Maturity-related traits of soybean R1, R7 and R8 in the field at Beijing. Left, cultivars of North America, which are approximately sorted by maturity group. Right, cultivars of China, which are sorted roughly by adaption latitude.
doi:10.1371/journal.pone.0106042.g001

CAAE092, which is from Russia (Tables 1 and 2). There are ten genotypes in total in this population (Table 5). The genotypes of *E1/E2/E3/E4* and *E1/e2/E3/E4* are the majority types, which were identified in 28 and 19 cultivars respectively (Table 5). Three genotypes of *e1-as/E2/e3/E4*, *e1-as/e2/e3/e4* and *e1/e2/e3/E4* were

identified only in one variety each (Table 5). For the *E1* locus, allele *e1* and *e1-as* are always detected in early-maturing cultivars from MG000 to MGIV or from high latitudes adapted cultivars. Moreover, all of the four wild soybeans were *E1/E2/E3/E4* type.

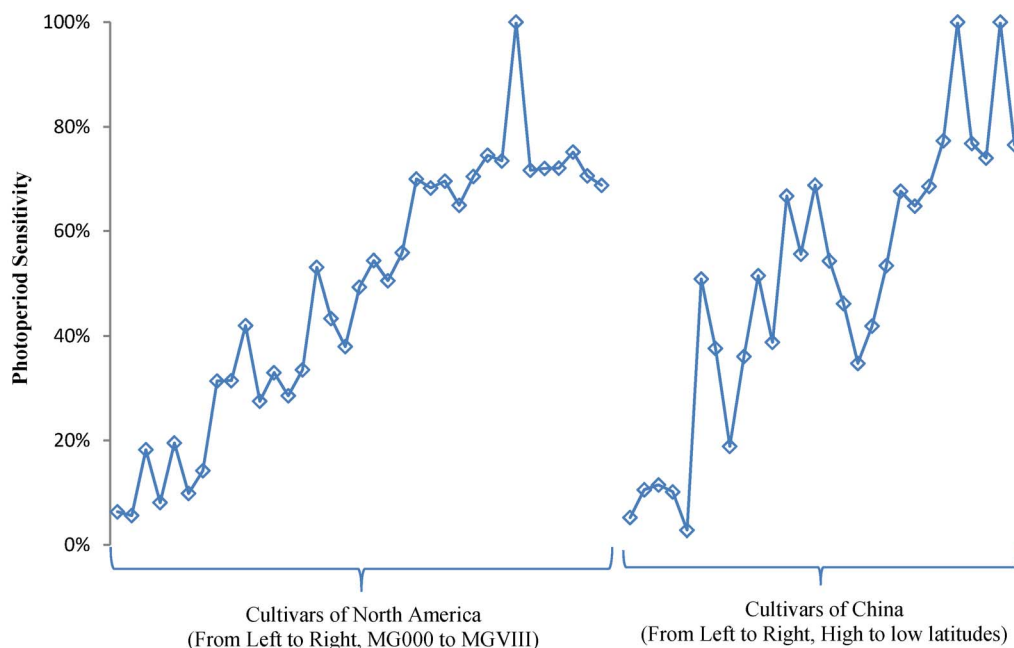


Figure 2. Photoperiod sensitivity of soybean cultivars. Left, cultivars of North America, which are approximately sorted by maturity group. Right: cultivars of China, which are sorted roughly by adaption latitude.
doi:10.1371/journal.pone.0106042.g002

Table 4. The days to R1 from V1 under different photoperiod treatments and the resultant PS at Beijing.

Cultivar	Days from V1 to R1				Cultivar	PS (%)	Days from V1 to R1				PS (%)
	SD	ND	LD	LD			SD	ND	LD	LD	
CAAE001	21.4±1.1	20.4±0.7	22.9±2.4	22.9±2.4	CAAE035	6.3	26.9±1.3	83.2±1.1	108.2±8.9	75.2	
CAAE002	22.6±1.1	20.9±1.4	23.9±2.2	23.9±2.2	CAAE036	5.6	26.7±2.4	83.9±1.4	90.6±0.5	70.6	
CAAE003	21.4±1.5	24.7±1.7	26.2±3.3	26.2±3.3	CAAE037	18.2	27.6±0.7	73.9±3.1	88.4±0.5	68.8	
CAAE005	21.4±2.0	20.0±0.6	23.2±0.9	23.2±0.9	CAAE050	8.1	23.1±0.9	30.2±1.4	37.1±5.0	37.6	
CAAE006	21.7±2.1	22.0±2.5	27.0±5.4	27.0±5.4	CAAE053	19.5	23.1±1.7	28.9±2.2	37.8±6.5	38.8	
CAAE007	20.6±1.3	20.1±0.6	22.9±1.7	22.9±1.7	CAAE052	9.8	22.9±0.8	31.9±1.9	47.2±3.8	51.5	
CAAE008	24.1±3.2	25.5±0.9	28.1±4.1	28.1±4.1	CAAE054	14.2	24.1±1.1	44.1±1.8	54.3±3.0	55.6	
CAAE009	22.6±2.7	29.8±1.9	32.9±3.4	32.9±3.4	CAAE057	31.4	27.0±0.8	45.0±2.7	50.2±1.9	46.2	
CAAE010	22.8±1.7	28.5±2.1	33.2±1.6	33.2±1.6	CAAE056	31.4	28.0±1.7	49.5±1.4	61.3±3.2	54.3	
CAAE011	24.8±1.6	32.5±1.5	42.7±3.4	42.7±3.4	CAAE061	42.0	26.7±3.1	50.3±4.6	54.3±7.1	50.9	
CAAE012	25.1±3.0	27.9±2.3	34.6±3.1	34.6±3.1	CAAE076	27.5	27.3±0.8	73.3±1.5	86.9±0.9	68.5	
CAAE013	22.2±2.3	28.6±2.1	33.1±2.5	33.1±2.5	CAAE064	33.0	32.8±2.0	45.9±2.4	56.5±2.8	41.9	
CAAE014	24.8±1.9	28.7±2.6	34.7±4.7	34.7±4.7	CAAE059	28.5	24.1±1.4	44.8±1.6	77.4±2.0	68.8	
CAAE015	26.7±2.1	31.2±2.6	40.1±3.7	40.1±3.7	CAAE084	33.5	30.8±1.9	106.4±0.7	NA	100.0	
CAAE016	22.0±1.9	31.2±3.2	46.9±5.2	46.9±5.2	CAAE045	53.1	24.3±2.1	27.6±1.5	30.0±2.6	18.9	
CAAE017	26.2±2.1	35.6±6.0	46.3±0.5	46.3±0.5	CAAE041	43.4	21.2±1.7	22.8±0.9	23.6±1.3	10.2	
CAAE018	25.1±0.4	31.5±0.8	40.5±1.6	40.5±1.6	CAAE042	38.0	23.0±1.7	24.3±1.4	23.7±1.7	2.8	
CAAE019	25.3±2.1	39.4±1.0	49.9±3.1	49.9±3.1	CAAE040	49.3	20.2±1.5	20.4±0.5	22.8±1.6	11.5	
CAAE020	21.3±1.6	34.1±6.0	46.7±4.2	46.7±4.2	CAAE065	54.4	26.4±1.4	34.1±2.9	41.3±2.7	36.0	
CAAE021	23.7±2.7	36.7±3.1	47.9±3.6	47.9±3.6	CAAE066	50.5	26.5±0.5	48.6±1.3	56.9±1.9	53.4	
CAAE022	23.2±2.2	45.2±2.5	52.5±5.0	52.5±5.0	CAAE074	55.9	32.2±1.0	62.9±0.3	91.5±0.9	64.8	
CAAE024	25.0±0.8	60.2±1.2	83.3±0.6	83.3±0.6	CAAE073	70.0	26.6±1.2	63.8±5.1	82.0±0.7	67.6	
CAAE025	27.9±1.4	67.1±1.4	87.9±1.1	87.9±1.1	CAAE072	68.3	28.2±1.8	92.7±3.3	124.4±0.8	77.3	
CAAE026	26.4±1.3	67.2±0.8	86.9±0.8	86.9±0.8	CAAE091	69.6	20.4±2.8	22.9±0.7	21.6±1.2	5.2	
CAAE027	29.5±1.0	67.0±0.5	84.0±0.0	84.0±0.0	CAAE085	64.9	29.8±1.7	98.6±1.3	126.8±1.7	76.5	
CAAE028	26.3±2.0	68.1±1.3	89.0±2.3	89.0±2.3	CAAE075	70.5	28.9±1.4	98.3±1.4	124.5±1.2	76.8	
CAAE029	24.1±0.8	82.1±3.3	94.7±0.5	94.7±0.5	CAAE058	74.5	23.9±1.4	30.9±1.9	36.6±7.0	34.7	
CAAE030	25.0±0.8	83.8±1.4	94.1±0.6	94.1±0.6	CAAE077	73.4	29.6±2.8	96.4±0.5	113.7±1.0	74.0	
CAAE031	24.7±0.8	83.6±0.5	NA	NA	CAAE062	100.0	27.6±0.9	55.3±2.3	83.0±1.4	66.7	
CAAE032	25.1±0.7	72.6±1.3	88.6±1.1	88.6±1.1	CAAE092	71.7	18.4±2.0	20.9±1.6	20.5±1.8	10.6	
CAAE033	24.9±0.4	73.9±2.2	88.9±1.9	88.9±1.9	CAAE086	72.0	32.8±0.8	122.3±0.8	NA	100.0	
CAAE034	24.9±0.6	84.6±1.1	89.0±1.7	89.0±1.7		72.0					

NA, not available.
doi:10.1371/journal.pone.0106042.t004

Table 5. Genotype of soybean cultivars at four maturity loci *E1*, *E2*, *E3* and *E4*.

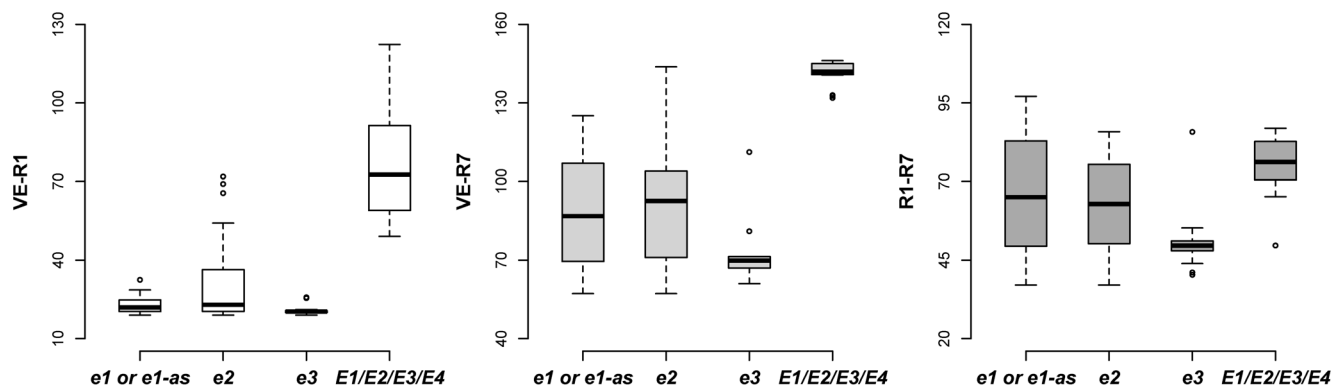
Genotype				Num	Variety
<i>E1</i>	<i>E2</i>	<i>E3</i>	<i>E4</i>		
<i>E1</i>	<i>E2</i>	<i>E3</i>	<i>E4</i>	28	CAAE023 (MGV), CAAE024 (MGV), CAAE025 (MGV), CAAE026 (MGV), CAAE027 (MGVI), CAAE028 (MGVI), CAAE029 (MGVI), CAAE030 (MGVI), CAAE031 (MGVII), CAAE032 (MGVII), CAAE033 (MGVII), CAAE034 (MGVII), CAAE035 (MG VIII), CAAE036 (MG VIII), CAAE037 (MG VIII), CAAE038 (MGX), CAAE071, CAAE072, CAAE075, CAAE081, CAAE082, CAAE084, CAAE085, CAAE086, CAAE087, CAAE088, CAAE089, CAAE090
<i>E1</i>	<i>e2</i>	<i>E3</i>	<i>E4</i>	19	CAAE047, CAAE049, CAAE050, CAAE052, CAAE054, CAAE055, CAAE056, CAAE057, CAAE058, CAAE061, CAAE062, CAAE063, CAAE064, CAAE065, CAAE066, CAAE073, CAAE074, CAAE076, CAAE077
<i>E1</i>	<i>e2</i>	<i>e3</i>	<i>E4</i>	3	CAAE044, CAAE045, CAAE059
<i>e1-as</i>	<i>E2</i>	<i>E3</i>	<i>E4</i>	8	CAAE016 (MGIII), CAAE017 (MGIII), CAAE018 (MGIII), CAAE019 (MGIII), CAAE020 (MGIV), CAAE021 (MGIV), CAAE022 (MGIV), CAAE053
<i>e1-as</i>	<i>E2</i>	<i>e3</i>	<i>E4</i>	1	CAAE011 (MGI)
<i>e1-as</i>	<i>e2</i>	<i>E3</i>	<i>E4</i>	8	CAAE003 (MG00), CAAE008 (MG0), CAAE009, CAAE010 (MGI), CAAE012 (MGI), CAAE013 (MGII), CAAE014 (MGII), CAAE015 (MGII)
<i>e1-as</i>	<i>e2</i>	<i>e3</i>	<i>E4</i>	7	CAAE005 (MG00), CAAE006 (MG0), CAAE007 (MG0), CAAE040, CAAE041, CAAE042, CAAE043
<i>e1-as</i>	<i>e2</i>	<i>e3</i>	<i>e4</i>	1	CAAE039
<i>e1</i>	<i>e2</i>	<i>e3</i>	<i>E4</i>	1	CAAE092
<i>e1</i>	<i>e2</i>	<i>e3</i>	<i>e4</i>	3	CAAE001 (MG000), CAAE002 (MG000), CAAE091

doi:10.1371/journal.pone.0106042.t005

Maturity loci *E1*, *E2*, *E3* and *E4* have different impacts on maturity and photoperiod response

In general, recessive alleles *e1*, *e1-as*, *e2*, *e3* and *e4* promoted flowering and maturity but with different impacts (Figure 3). The allele *e4* was detected in only four cultivars (CAAE039 with the genotype *e1-as/e2/e3/e4*, and CAAE001, CAAE002 and CAAE091 with the genotype of *e1/e2/e3/e4*) in the population. The four cultivars with recessive *e4* alleles were adapted to high latitude and showed photoperiod insensitivity suggesting the importance of the *e4* allele for high latitude adaptation. The

cultivars with the allele *e1*, *e1-as* or *e2* exhibited a narrower range of the days from VE to R1 (VE-R1) than that of the days from VE to R7 (VE-R7) and that of the days from R1 to R7 (R1-R7) (Figure 3). In contrast, the cultivars with the allele *e3* or *e4* showed a consistently narrow range although some outliers existed (Figure 3). Moreover, these four recessive alleles promoted flowering under different photoperiod conditions (Figure 4), and the cultivars with more recessive alleles of *e1*, *e1-as*, *e2*, *e3* and *e4* had a lower PS during photoperiod treatments (Figure 5).

**Figure 3.** Quartile box plots showing the days between the stages of VE, R1 and R7. Circles show outliers.

doi:10.1371/journal.pone.0106042.g003

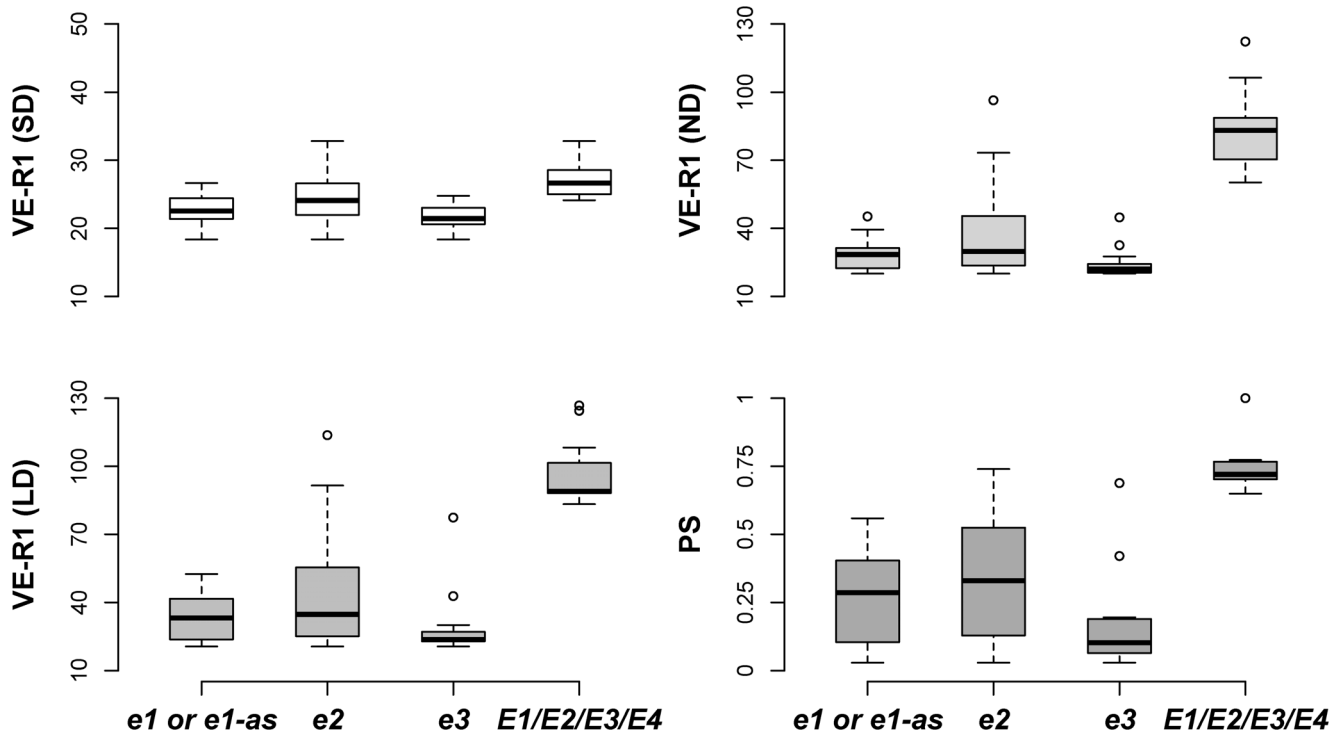


Figure 4. Quartile box plots showing days between the stages of VE and R1 under different photoperiod conditions and PS. Circles show outliers.

doi:10.1371/journal.pone.0106042.g004

Discussion

Soybean cultivars from different maturity groups show diversity in flowering, maturity and photoperiod sensitivity

The tested soybean cultivars were selected from North America, China and Russia. Some of them covered from MG000 to MGVIII and MGX, almost all of the total 13 MG [2] and the others were collected from N 18° to N 53° to cover the wide range of latitude, which represents the main soybean producing area in China. In addition, some wild soybean accessions were also included. Thus, the population of lines used here should exhibit the diversity of maturity not only in phenotype but also in genotype. The field experiment under ND provided strong evidence. Some cultivars failed to flower (R1), some could not reach pod yellowing (R7) and some could not reach full maturity (R8) (Table 3). Even for those cultivars that flowered and matured, the days to first flowering, physiological maturity and full maturity varied significantly (Table 3). What's more, in the experiment of photoperiod treatments, the population also showed diversity of photoperiod sensitivity as expected. The days to first flowering varied between different photoperiod treatments and between different cultivars. The photoperiod sensitivity also varied from 10% to 80% (Table 4). Six cultivars were classified as photoperiod insensitive: CAAE001 (PI548594, MG000, *e1/e2/e3/e4*), CAAE002 (PI567787, MG000, *e1/e2/e3/e4*), CAAE005 (PI592523, MG00, *e1-as/e2/e3/E4*), CAAE007 (PI596541, MG0, *e1-as/e2/e3/E4*), CAAE042 (Heihe 3, *e1-as/e2/e3/E4*) and CAAE091 (Mohe 1, *e1/e2/e3/e4*). These results were consistent with previous report that soybean photoperiod insensitivity was at least conditioned by three genetic mechanisms according to allelic

combinations of *E1*, *E2* and *E4*: *e3/e4*; *e1/e3* or *e1/e4* and *e1-as/e3/E4*. In the genetic mechanism of *e1-as/e3/E4*, novel unidentified gene/genes participated in photoperiod insensitivity [26], [27].

E genes have different impacts on flowering and maturation

Soybean, as a short-day crop, has many cultivars with diversified maturity structure. *E1*, *E2* and *E3* are involved with different impacts. In the field experiment under ND, the recessive allele *e1*, *e1-as* and *e2* significantly narrowed the variation of VE-R1 more than that of VE-R7 and R1-R7 (Figure 3), suggesting that *E1* and *E2* genes have significant impact on pre-flowering development other than post-flowering responses. However, the loci *E3* and *E4* might function not only in pre-flowering development but also in post-flowering development, indicated by the narrow variation in VE-R1, VE-R7 and R1-R7 of cultivars with *e3* and *e4* alleles (Figure 3). This result is consistent with Xu et al [26], that *E3* and *E4* respond not only to pre-flowering but also to post-flowering by increasing pod filling duration, number of nodes and pod numbers by up-regulating the expression of growth habit gene *D11*. This result implies the significance of *E3* and *E4* loci for molecular genetic breeding to increase soybean productivity. Moreover, the outliers suggested that the disfunction of *E3* and *E4* might be interrupted by other genes. Similarly, the loci *E1* and *E3* are related with photoperiod sensitivity (Figure 4).

For the genotype of *E1/E2/E3/E4*, the photoperiod sensitivity was mostly above 70%. Compared with genotypes *E1/E2/E3/E4*, *e1-as/E2/E3/E4*, *e1-as/e2/E3/E4* and *e1-as/e2/e3/E4* (Figure 5), the photoperiod sensitivity decreased with the numbers of recessive

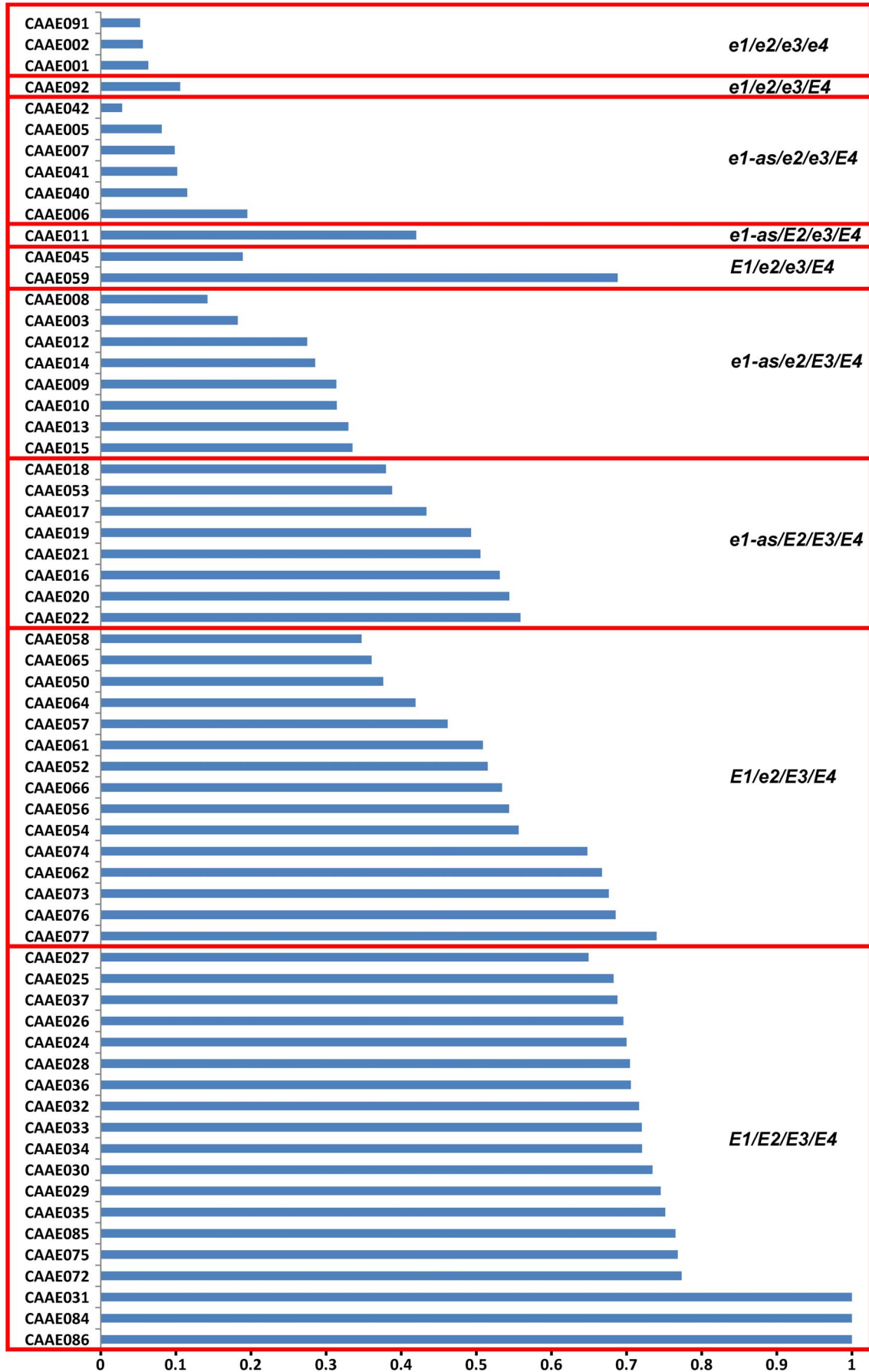


Figure 5. Photoperiod sensitivity grouped by *E* genotypes.
doi:10.1371/journal.pone.0106042.g005

alleles. While comparing *E1/e2/E3/E4* and *e1-as/E2/E3/E4* with *E1E2E3E4*, it was suggested that *E1* plays a more important role than *E2* because *e1* decreased the photoperiod sensitivity more significantly and narrowed its range. These results further proved that soybean photoperiod insensitivity was involved by four maturity loci *E1*, *E3*, *E4* and *E7* while *E2* locus was not involved [28], [29]. In these tested cultivars of China, *E1/e2/E3/E4* is much more abundant. It ranged from N 18° -N 42° while *E1/E2/E3/E4* was distributed south of N 39° except for the four wild soybeans (CAAE087, Heiheyesheng; CAAE088, Bayanyesheng; CAAE089, Baiyangdianyesheng; and CAAE090, Guangxiyesheng). For *e1* or *e1-as* alleles, the associated cultivars were mostly located above N 45° except for CAAE053 (Tiefeng 31, N 35°-40°). Thus, to improve the ecological adaptability of cultivars, the *E1* gene must function less because of its most strong impact on delaying maturity while other *E* genes might become important in adaptation. Unlike cultivated soybean, two wild soybean accessions, CAAE087 (Heiheyesheng) and CAAE088 (Bayanyesheng) adapted north of N 46° in China where cultivars generally had less photoperiod sensitivity where genotyped as *E1/E2/E3/E4*. It is greatly important for wild soybean to adapt and survive during season alteration. The genetic mechanisms underlying photoperiod insensitivity and adaptation in wild soybean were therefore unique from those in cultivated soybean.

Allelic combinations of *E* genes determine maturity groups

In the population analyzed here, MG_V to MG_X have the same genotype at *E1/E2/E3/E4*. It suggests that in these maturity groups, other unknown maturity genes should be involved in the determination of Mature Group. MG_{III} to MG_{IV} are mainly genotyped as *e1-as/E2/E3/E4*, and MG_{II} is *e1-as/e2/E3/E4*. For MG_{II} to MG_X, each group has one genotype. MG_I has the genotypes of *e1-as/e2/E3/E4* and *e1-as/E2/e3/E4*; MG₀ and

MG₀₀ both have the genotypes of *e1-as/e2/E3/E4* and *e1-as/e2/e3/E4*; and MG₀₀₀ has *e1/e2/e3/e4*. From MG₀₀₀ to MG_I, each maturity group has two genotypes, which means that photoperiod is the first key factor in these region to determine maturity group. However, the more recessive alleles at *E* genes, the earlier cultivars mature. Due to the limited number of cultivars used in each MG, the maturity genotypes for each group may be underestimated and additional genotypes for each MG may be identified with a larger sample of cultivars. Although it is not possible to enumerate all genotypes of a given maturity group, this limited sample of cultivars showed that allelic combinations of *E* genes determine maturity groups in general.

Conclusions

The *E* genes (*E1*, *E2*, *E3* and *E4*) have different roles in maturity and photoperiod sensitivity and their allelic combinations determine maturity group and adaptation to different latitude.

Acknowledgments

We thank Randall L. Nelson (Soybean/Maize Germplasm, Pathology and Genetics Research Unit, USDA and Department of Crop Sciences, University of Illinois, Urbana, Illinois, USA), Lijuan Qiu and Zhangxiong Liu (Institute of Crop Sciences, Chinese Agricultural Academy of Sciences, China) for providing some soybean germplasm. We also appreciate Dr. Elroy R. Cober (Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Canada) and Dr. Jun Abe (Research Faculty of Agriculture, Hokkaido University) for proofreading of the manuscript.

Author Contributions

Conceived and designed the experiments: BJ FK TH BL. Performed the experiments: BJ HN YG LT YY SL LM JW SS DC. Analyzed the data: BJ HN YG CW XY WH. Contributed reagents/materials/analysis tools: FK TH BL. Contributed to the writing of the manuscript: BJ FK.

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