Genomic Dissection and Diurnal Expression Analysis Reveal the Essential Roles of the PRR Gene Family in Geographical Adaptation of Soybean

Peiguo Wang 1,2,*, Liwei Wang 2,†, Lixin Zhang 2, Tingting Wu 2, Baiquan Sun 2, Junquan Zhang 2, Enoch Sapey 2,†, Shan Yuan 2, Bingjun Jiang 2, Fulu Chen 2, Cunxiang Wu 2, Wensheng Hou 2, Shi Sun 2,*, Jiangping Bai 1,† and Tianfu Han 2,†

1 Department of Crop Genetics and Breeding, College of Agronomy, Gansu Agricultural University, Lanzhou 730070, China
2 MARA Key Laboratory of Soybean Biology (Beijing), Institute of Crop Sciences, The Chinese Academy of Agricultural Sciences, 12 Zhongguancun South Street, Beijing 100081, China
3 Council for Scientific and Industrial Research (CSIR)-Oil Palm Research Institute, Kade P.O. Box 74, Ghana
* Correspondence: baijp@gsau.edu.cn (J.B.); hantianfu@caas.cn (T.H.)
† These authors contributed equally to this work.

Abstract: Pseudo-response regulator (PRR) family members serve as key components of the core clock of the circadian clock, and play important roles in photoperiodic flowering, stress tolerance, growth, and the development of plants. In this study, 14 soybean PRR genes were identified, and classified into three groups according to phylogenetic analysis and structural characteristics. Real-time quantitative PCR analysis revealed that 13 GmPRRs exhibited obvious rhythmic expression under long-day (LD) and short-day (SD) conditions, and the expression of 12 GmPRRs was higher under LD in leaves. To evaluate the effects of natural variations in GmPRR alleles on soybean adaptation, we examined the sequences of GmPRRs among 207 varieties collected across China and the US, investigated the flowering phenotypes in six environments, and analyzed the geographical distributions of the major haplotypes. The results showed that a majority of non-synonymous mutations in the coding region were associated with flowering time, and we found that the nonsense mutations resulting in deletion of the CCT domain were related to early flowering. Haplotype analysis demonstrated that the haplotypes associated with early flowering were mostly distributed in Northeast China, while the haplotypes associated with late flowering were mostly cultivated in the lower latitudes of China. Our study of PRR family genes in soybean provides not only an important guide for characterizing the circadian clock-controlled flowering pathway but also a theoretical basis and opportunities to breed varieties with adaptation to specific regions and farming systems.

Keywords: soybean; photoperiodic flowering; PRR gene family; haplotype; geographical adaptation

1. Introduction

Soybean (Glycine max) is a typical short-day (SD) plant showing photoperiod sensitivity [1–5]. Photoperiodic regulation of flowering time is an important determinant for the adaptability and productivity of soybean. Various efforts have been made to study the genetic and molecular mechanisms underlying photoperiodic flowering time regulation in soybean [6,7]. The molecular mechanisms of the major genes E1 [8], E2 [9], E3 [10], E4 [11], E6 [12], GmFT1a [13], GmFT2a [14–17], GmFT4 [18], GmFT5a/qDTF-J1 [16,19], J [20–22], qFT12-1/GmPRR37/Tof12/GmPRR3b [23–26], GmPRR3a/Tof11 [25,26] and GmLHYs [27–29] have been identified and characterized. Recent studies have demonstrated that circadian clock genes act upstream of the legume-specific flowering repressor E1 to regulate soybean photoperiodic flowering [24–29]. Therefore, dissecting the genomic basis of the natural variation of circadian clock genes would facilitate elucidating the genetic networks of photoperiodic flowering and adaptation in soybean.
As key components of the circadian clock, pseudo-response regulator (PRR) family genes play important roles in flowering, stress tolerance, and growth in plants [30–32]. In Arabidopsis, PRR family genes consist of five members (APRR1/TOC1, APRR3, APRR5, APRR7 and APRR9), and are subject to a circadian rhythm at the transcriptional level [33,34]. Comprised of the amino acid sequence of Arabidopsis PRRs, which are defined as proteins containing an N-terminal pseudo receiver (PR) domain and a C-terminal CCT (CONSTANS, CO-like, and TOC1) domain, PRR homologues in plants are classified into three groups (PRR1, PRR7/3 and PRR5/9) [31,33].

Arabidopsis PRRs have been shown to regulate flowering via the photoperiod pathway [35]. In contrast, numerous studies have identified PRR3/7 homologues that regulate photoperiod flowering in rice [36–38], wheat [39], barley [40], sorghum [41] and soybean [24–26]. In soybean, natural variants in GmPRR37/GmPRR3b and GmPRR3a affect photoperiodic flowering and have been selected during soybean domestication and facilitated the adaptation of soybean to different cultivated regions [24,25]. To date, there are few studies on other PRR family members in soybean.

In the present study, we studied 14 PRR family members of soybean and analyzed their gene structure, evolutionary characteristics, expression pattern under different photoperiodic conditions, and flowering phenotypes across six environments and geographical distributions within the major haplotypes. This study lays a foundation for the further functional characterization of specific genes in the PRR gene family of soybean, which will facilitate the breeding of varieties with better adaptability.

2. Results
2.1. Genome-Wide Identification of the PRR Gene Family in Soybean

In order to identify the PRR gene family in soybean, the known Arabidopsis PRR protein sequences were used as queries in BLASTp searches followed by HMMER profiles. Finally, a total of 14 soybean PRR family genes were confirmed by domain analysis using Pfam and CDD tools. The 14 GmPRR genes were distributed on 11 chromosomes and one scaffold (Supplementary Figure S1). One GmPRR gene was mapped on scaffold-32, Chr04 and Chr06 each contained two GmPRRs, and the other nine chromosomes showed one GmPRR, respectively. They were renamed from GmPRR1 to GmPRR14 according to their chromosomal positions (Supplementary Table S1). Although GmPRR9 and GmPRR10 do not have a CCT domain, we retained them for further analysis, because they have been demonstrated to regulate soybean photoperiodic flowering [25–27]. Further synteny analysis showed fragmental duplication among GmPRRs, suggesting a functional similarity among GmPRR members (Supplementary Figure S1).

Gene characteristics, including the length of the protein sequence, the protein molecular weight, isoelectric point, and the subcellular localization, were analyzed (Supplementary Table S1). The length of the 14 GmPRR proteins ranges from 558 (GmPRR6) to 765 (GmPRR9) amino acids, with molecular weights from 62.2 to 83.2 kDa. The predicted isoelectric points varied from 5.55 (GmPRR4) to 8.01 (GmPRR11). The predicted subcellular localization results showed that all of the GmPRR proteins were located in the nucleus, corresponding to Arabidopsis PRRs encoding transcription factors.

2.2. Evolutionary Analysis of the PRR Gene Family in Soybean

To explore the evolutionary relationships of PRR proteins, a phylogenetic tree was constructed using the whole protein sequences of GmPRRs, APRRs and PRRs of other crops. The results showed that GmPRR homologous genes were clustered into three groups, including the PRR1, PRR3/7 and PRR5/9 groups based on their similarity to the respective Arabidopsis and rice proteins (Figure 1). The PRR1 group contained four GmPRRs (GmPRR2, 4, 6 and 13), the PRR3/7 group consisted of four GmPRRs (GmPRR8, 9, 10 and 11), and the PRR5/9 group contained six GmPRRs (GmPRR1, 3, 5, 7, 12 and 14). In addition, GmPRR2 and GmPRR3, and GmPRR5 and GmPRR6 on the same chromosome
were not divided into the same group, indicating evolutionary divergence and functional diversity of *GmPRRs* on the same chromosome (Figures 1 and S1).

![Phylogenetic tree of PRRs in higher plants](image)

**Figure 1.** Phylogenetic tree of PRRs in higher plants. Different groups are represented by different colors. I, the PRR5/9 group; II, The PRR3/7 group; III, The PRR1 group. The phylogenetic tree was constructed by MEGA 7.0 using the Neighbor-Joining (NJ) method with 1000 bootstrap repeats. The numbers on the branches indicate the bootstrap values. The Arabidopsis CONSTANS (*AtCO*) gene containing a CCT (*CONSTANS, CO-like, and TOC1*) domain was considered as an outgroup. Different subfamilies are represented by different colors.

### 2.3. Gene Structure, Motif Composition and Promoter Characterization of Soybean PRR Gene Family

The architecture of *GmPRR* genes was examined to gain more insight into the evolution of the PRR family in soybean. The results showed that the gene length and structure were diverse among the three groups, but genes in the same group tended to share similar exon and intron structures (Figure 2). The gene length of the second group is longer than the first and third groups due to the longer intron length. Genes in Group I and Group III had a similar gene length. The 14 *GmPRR* genes possessed six to nine exons, and all of the genes in Group I contained eight exons, indicating that different *GmPRR* genes have diverged structurally during evolution. The conserved motifs of the GmPRR proteins were analyzed using Multiple Em for Motif Elcitation (MEME) (Figure 2C). Three motifs (Motif1, 3 and 4) constituted the PR domain, and were conserved in the *GmPRRs*. The Motif6 and Motif10 were the conserved Motifs of the CCT domain except for *GmPRR9* and *GmPRR10*. Among them, the first group is unique to Motif 9 and only existed in the *GmPRRs* of Group I, and Motif 8 was only shared in members of Group III. Taken together, the conserved Motifs and similar gene structures of the GmPRR members in the same group strongly support the reliability of the group classifications. These results suggest an evolutionary divergence among *GmPRR* members, and also the functional similarity of *GmPRRs* in the same group.

Cis-acting regulatory elements (CAREs) play a vital role in the regulation of gene expression, so a cis-elements analysis was conducted using the 2 kb sequence upstream of the start codon (ATG) of the *GmPRR* genes (Supplementary Figure S2). A large number of cis-elements, including light-responsive elements (I-box, Sp1, GT1-motif, 3-AF1 binding site, AE-box, chs-CMA1a, chs-CMA2a, GA-motif, Box II, LAMP-element, GATT-motif, G-box, ACE, MRE, Box 4), circadian rhythm element (circadian), defense and stress response elements (TC-rich repeats) and hormone response elements (TGA-element, TATC-box, P-box) were identified. These results suggest that *GmPRRs* may play an important role in the photoperiod response and circadian clock in soybean.
Figure 2. The phylogenetic relationships, gene structure, and conserved motifs of the PRR gene family in soybean. (A) Phylogenetic tree of soybean PRR proteins. Different subfamilies are represented by different colors. (B) Genetic structure of GmPRR genes, including introns, UTRs, CDSs and domains specific to the PRR family. (C) The conserved motif of GmPRR proteins and the length of each motif is shown proportionally.

2.4. The Expression Pattern of GmPRR Genes

For investigating the expression patterns of GmPRR genes, a qRT-PCR analysis was carried out. GmPRRs were expressed widely in the trifoliolate leaf, unifoliolate leaf, shoot apex, stem, hypocotyl, and root of Zigongdongdou (ZGDD) and Heihe 27 (HH27) (Supplementary Figure S3). GmPRR2 and GmPRR6 were expressed in all tissues, with higher expression in the root and lower expression in the trifoliolate leaf and unifoliolate leaf. GmPRR4 and GmPRR13 were more highly expressed in the trifoliolate leaf and less highly expressed in the root. The transcript levels of GmPRR3 and GmPRR5 were highest in the unifoliolate leaf and trifoliolate leaf, and lower in the stem and hypocotyl. GmPRR8, GmPRR10 and GmPRR11 were highly expressed in the unifoliolate leaf and the expression of GmPRR9 was higher in unifoliolate and trifoliolate leaves. The expression of GmPRR1, GmPRR7, GmPRR12 and GmPRR14 was higher in the unifoliolate and trifoliolate leaves but lower in the root and shoot apex.

The leaf is the major organ for sensing the photoperiod. To evaluate the circadian clock properties of GmPRRs, we examined the expression of GmPRRs in trifoliolate leaves of ZGDD, a photoperiod-sensitive soybean variety under long-day (LD) and short-day (SD) treatments. All the GmPRRs showed obvious rhythmic expression except for GmPRR12 under LD and SD conditions (Figure 3). The transcripts of GmPRR1, GmPRR7, GmPRR9, GmPRR10 and GmPRR14 peaked at 8 h after exposure to light. GmPRR2, GmPRR4, GmPRR6, GmPRR8, GmPRR11 and GmPRR13 peaked at 12 h after exposure to light. GmPRR3 and GmPRR5 transcripts peaked at 8 h under SD conditions and 12 h under LD conditions. We also found that GmPRRs within the same group exhibit similar rhythmic expression pattern. Except for GmPRR3 and GmPRR5, GmPRR genes showed a higher expression level under LD conditions, suggesting that LD induced their expression in leaves. These results indicate that GmPRRs may regulate soybean circadian rhythm and photoperiodic flowering.
LD conditions. We also found that GmPRRs within the same group exhibit similar rhythmic expression pattern. Except for GmPRR3 and GmPRR5, GmPRR genes showed a higher expression level under LD conditions, suggesting that LD induced their expression in leaves. These results indicate that GmPRRs may regulate soybean circadian rhythm and photoperiodic flowering.

Figure 3. Expression levels of GmPRRs throughout a 48 h period in the unifoliolate leaves of soybean variety ZGDD (Zigongdongdou) on days 10 and 11 of long-day (LD, 16:8, light: dark) or short-day (SD, 12:12, light: dark) treatment. Relative transcript levels of GmPRRs were normalized to GmActin. The data are given as the means ± SE of three biological replicates.

2.5. Haplotype Analysis of the 14 Soybean PRR Family Genes in Soybean Germplasm with Diverse Geographical Origins

In an attempt to evaluate the effect of natural variations in GmPRR alleles on soybean adaptation, the genotypes of GmPRRs were detected among 207 resequencing soybean varieties (Supplementary Figure S4), which have been traditionally planted in China and
the USA (Supplementary Table S2). We also investigated the flowering time across six environments (Sanya 2016, Xiangtan 2017, Xinxiang 2016, Beijing 2016, Changchun 2017 and Heihe 2017) and the geographical distribution of varieties with major haplotypes (Figures 4 and 5). By comparing the flowering times of different GmPRR haplotypes in the six environments of China, we found that natural variations in all of the GmPRRs were associated with soybean flowering time and the phenotypic difference tendency becomes more pronounced with increasing latitude in China (Figures 4 and 5).

Figure 4. Association analysis of GmPRR haplotypes with flowering time in soybean germplasm. (A–N) Flowering time of the soybean varieties with major haplotypes of GmPRR1-GmPRR14. The number within each box indicates the number of varieties that did not flower. The data are means ± standard deviations, and a, b and c indicate ranking by Duncan’s test at $p < 0.05$. SY2016: Sanya2016; XT2017: Xiangtan 2017; XX2016: Xinxiang2016; BJ2016: Beijing2016; CC2017: Changchun2017; and HH2017: Heihe2017.
Figure 5. Geographical distribution of soybean varieties harboring different alleles of GmPRR genes. (A–N) The geographic distribution of GmPRR1-GmPRR14 haplotypes. NE: Northeast China; HHH: Huang-Huai-Hai; SC: South China.

GmPRR1 sequence comparisons identified nine polymorphic loci, including seven SNPs and two indels, and defined five haplotypes (Supplementary Figure S4A). GmPRR1H3 carried a missense mutation resulting in an amino acid substitution (Ser/Pro) outside of the PR and CCT domains (Supplementary Figure S4A). GmPRR1H3 and GmPRR1H4 were widely distributed across China and the US with similar flowering times (Figures 4A and 5A). GmPRR1H1 showed significantly earlier flowering (Figure 4A) and was distributed in Northeast China (NE) (Figure 5A). GmPRR1H5 showed significantly later flowering (Figure 4A) and was all distributed in the US germplasm (Figure 5A). Further characterization of these two unique haplotypes (GmPRR1H1 and GmPRR1H5) might facilitate soybean genetic improvement in China and the US. Seven SNPs and six haplotypes were identified for GmPRR2 (Supplementary Figure S4B). SNP-Chr04:41757081 was located in the PR domain, resulting in amino acid substitution (Gln/His) (Supplementary Figure S4B), which may lead to late flowering for GmPRR2H6 (Figure 4B). Except for Sanya2016, GmPRR2H1 resulted in significantly earlier flowering, whereas GmPRR2H5 and GmPRR2H6 displayed later flowering (Figure 4B). GmPRR2H4 was the most abundant. With the increase in latitude of China,
the proportion of GmPRR2\textsuperscript{H1} increased, whereas the proportion of GmPRR2\textsuperscript{H3}, GmPRR2\textsuperscript{H4} and GmPRR2\textsuperscript{H5} decreased (Figure 5B).

For GmPRR3, seven SNPs and five haplotypes were defined, and SNP-Chr04:49760646, was a missense mutation (Cys/Ser) (Supplementary Figure S4C). GmPRR3\textsuperscript{H3} and GmPRR3\textsuperscript{H4} flowered significantly later than GmPRR3\textsuperscript{H1} (Figure 4C). The frequency of GmPRR3\textsuperscript{H3} increased with increasing latitude in China (Figure 5C). GmPRR3\textsuperscript{H4} only existed in South China (SC), indicating that GmPRR3\textsuperscript{H4} was strongly selected in low-latitude regions.

Eight SNPs were found, and four haplotypes were defined in GmPRR4 and SNP-Chr05:21778871, a missense mutation resulting in amino acid substitutions (Leu/Ser) (Supplementary Figure S4D). Compared with GmPRR4\textsuperscript{H1}, GmPRR4\textsuperscript{H2} flowered significantly later (Figure 4D). GmPRR4\textsuperscript{H1} was mainly distributed in NE, while GmPRR4\textsuperscript{H2} was mainly distributed in Huang-Huai-Hai (HHH) and SC (Figure 5D).

Among the 16 SNPs in GmPRR5, two led to amino acid substitution sites, and SNP-Chr06:11184185(Glu/Asp) was in the PR domain (Supplementary Figure S4E). GmPRR5\textsuperscript{H2} and GmPRR5\textsuperscript{H4} flowered significantly later in Beijing and Changchun (Figure 4E) and were mostly found in HHH and SC (Figure 5E). GmPRR5\textsuperscript{H1} flowered significantly earlier in all environments and the frequency of GmPRR5\textsuperscript{H1} increased with increasing latitude in China, indicating that GmPRR5\textsuperscript{H1} was strongly selected at high latitudes during soybean improvement, especially in NE (Figure 5E).

One indel and 15 SNPs were identified corresponding to five haplotypes for GmPRR6. SNP-Chr06:17610272 was a missense mutation (Leu/Ser) located in the PR domain (Supplementary Figure S4F). More than half of the varieties in the NE belong to GmPRR6\textsuperscript{H1} with significantly earlier flowering (Figures 4F and 5F). GmPRR6\textsuperscript{H2} was mostly distributed in middle and high latitudes (Figure 5F). GmPRR6\textsuperscript{H3} and GmPRR6\textsuperscript{H4} were all associated with later flowering, the frequencies of which increased with decreasing latitude (Figures 4F and 5F). These results revealed that diverse GmPRR6 haplotypes with varied flowering times have adapted to target regions during soybean breeding.

For GmPRR7, one indel and 15 SNPs were detected, and five caused a missense mutation (Supplementary Figure S4G). Among the four haplotypes, GmPRR7\textsuperscript{H3} was the most widely distributed across China and the US (Figure 5G), while GmPRR7\textsuperscript{H4}, associated with significantly later flowering, only existed in HHH and SC (Figures 4G and 5G).

Based on 17 SNPs, GmPRR8 was divided into three haplotypes (Supplementary Figure S4H). The percentage of GmPRR8\textsuperscript{H1} was high in NE, and GmPRR8\textsuperscript{H2} and GmPRR8\textsuperscript{H3} showed later flowering and were mainly found in HHH and SC (Figures 4H and 5H).

Four haplotypes were defined for GmPRR9 according to one indel and 16 SNPs (Supplementary Figure S4I). GmPRR9\textsuperscript{H1} carried a frameshift mutation that resulted in the loss of the CCT domain in the encoded protein (Supplementary Figure S4I). Compared with GmPRR9\textsuperscript{H2}, GmPRR9\textsuperscript{H3} and GmPRR9\textsuperscript{H4}, GmPRR9\textsuperscript{H1} flowered significantly earlier (Figure 4I), and was the most widely distributed across China and the US (Figure 5I). The frequency of GmPRR9\textsuperscript{H1} increased with increasing latitude in China, whereas the percentages of GmPRR9\textsuperscript{H2}, GmPRR9\textsuperscript{H3} and GmPRR9\textsuperscript{H4} decreased (Figure 5I). These results suggested that GmPRR9 might function as a flower repressor in soybean, and the large effect mutation causing the deletion of the CCT domain, might result in early flowering.

We found one indel and five SNPs in GmPRR10 and defined three haplotypes (Supplementary Figure S4K). GmPRR10\textsuperscript{H1} carried a nonsense mutation that causing deletion of the CCT domain (Supplementary Figure S4K). GmPRR10\textsuperscript{H1} was significantly associated with earlier flowering than GmPRR10\textsuperscript{H2} and GmPRR10\textsuperscript{H3} (Figure 4I). GmPRR10\textsuperscript{H1} was the most widely distributed, and the frequency of GmPRR10\textsuperscript{H1} strongly increased with increasing latitude in China, whereas the frequencies of GmPRR10\textsuperscript{H2} and GmPRR10\textsuperscript{H3} decreased (Figure 5I). Taken together, GmPRR9 and GmPRR10 both contained large effect mutations causing the loss of the CCT domain, which may disrupt their function in the regulation of flowering time in soybean.

For GmPRR11, 17 SNPs were found and SNP-Chr13:24842201, SNP-Chr13:24842656 and SNP-Chr13:24844364 were missense mutations resulting in amino acid substitutions
were identified on 12 chromosomes (Supplementary Figure S1). Phylogenetic analysis revealed extensive genetic plasticity that may contribute to soybean cultivated across diverse latitudes. Further, we investigated haplotype combinations of GmPRRs with early flowering time tended to distribute in higher latitude regions, while varieties harboring more haplotypes of GmPRRs with later flowering time were mostly cultivated in the lower latitudes of China (Figure 5M).

Among the six SNPs, one indel was found in GmPRR13 (Supplementary Figure S4I), and SNP-Chr17:8022010 was a missense mutation resulting in amino acid substitutions (Ile/Thr). Four haplotypes were identified, and GmPRR13H1 was the most widely distributed. GmPRR13H1 had significantly earlier flowering in Changchun and Heihe (Figure 4M) and varieties with GmPRR13H1 were distributed at higher latitudes in China (Figure 5M).

Fifteen SNPs and one indel were found for GmPRR14, and SNP-Chr19:50366034 was a missense mutation (Phe/Ser) in the PR domain (Supplementary Figure S4N). Based on the allele polymorphism, a total of five haplotypes were identified, of which GmPRR14H2 was the most widely distributed. GmPRR14H2 flowered significantly earlier in Changchun and Heihe (Figure 4N) and appeared only in the NE varieties (Figure 5N).

2.6. Haplotype Combinations of GmPRRs in 207 Soybean Varieties

To evaluate the combinatorial effects of GmPRRs on the local fitness of soybean, we analyzed the haplotype combinations of GmPRRs among the 207 soybean varieties. A total of 150 haplotype combinations were confirmed, which suggested that GmPRR family members were highly diversified in cultivated soybean (Supplementary Table S3). Soybean varieties harboring more haplotypes of GmPRRs with early flowering time tended to distribute in higher latitude regions, while varieties harboring more haplotypes of GmPRRs with later flowering time were mostly cultivated in the lower latitudes of China (Figure 5, Supplementary Table S2). These findings substantially coincided with haplotype analysis for individual GmPRR family genes (Figures 4 and 5). Thus, we speculate that this diverse allelic combination of GmPRRs contributed to the regional adaptability of soybean. Further, we investigated haplotype combinations of GmPRRs in a widely-grown soybean variety of Zhonghuang 13, which ranked No.1 in planting area in the first two decades of the 21st century in China [42–44]. The result showed Zhonghuang 13 harbors GmPRR1H4, GmPRR2H4, GmPRR3H3, GmPRR4H2, GmPRR5H4, GmPRR6H4, GmPRR7H3, GmPRR8H2, GmPRR9H1, GmPRR10H1, GmPRR11H1, GmPRR12H4, GmPRR13H2 and GmPRR14H2 of GmPRR genes, and other varieties carrying these haplotypes, except GmPRR5H4 and GmPRR12H4, are distributed in all three regions (NE, SC and HHH) in China (Figure 5, Supplementary Figure S4). An association analysis revealed that GmPRR7H3, GmPRR9H1 and GmPRR10H1 in Zhonghuang 13 were associated with earlier flowering; GmPRR3H3, GmPRR4H2, GmPRR5H4, GmPRR6H4 and GmPRR12H4 were related to later flowering; and the other haplotypes (GmPRR1H4, GmPRR2H4, GmPRR13H2 and GmPRR14H2) were associated with a medium flowering time (Figure 4). These findings might partly explain the wide adaptability of Zhonghuang 13. Taken together, various combinations of mutations at GmPRRs provided extensive genetic plasticity that may contribute to soybean cultivated across diverse latitudes.

3. Discussion

PRR family genes play an essential role in maintaining circadian clock stability and affect plant growth and developmental processes, such as flowering time, photosynthesis response, heat shock response, oxidative stress response, stomatal conductance, mitochondrial metabolism, and cold stress [45–47]. In this study, a total of 14 soybean PRR genes were identified on 12 chromosomes (Supplementary Figure S1). Phylogenetic analysis classified the 14 GmPRR genes into three main groups (PRR1/TOC1, PRR3/7, PRR5/9)
(Figure 1), which was in accordance with PRR proteins in model plants, such as Arabidopsis and rice [31,33,48]. The number of PRR genes in soybean exceeds that in Arabidopsis and rice, so we speculated that soybean PRR genes may have a higher duplication rate or a lower gene loss rate after duplication (Supplementary Figure S1) [49].

GmPRRs have both the N-terminal response-regulator receiver domains and the C-terminal CCT domain, except for GmPRR9 and GmPRR10, whose proteins from reference genome W82 carry large effect mutations causing the loss of the CCT domain. This is consistent with previous studies [24–26]. qRT-PCR analysis (Figure 3) revealed that the transcripts of 13 GmPRRs exhibit diurnal patterns in leaves that peaked 8 h, 12 h, and 14 h after the lights were turned on, indicating that GmPRR expression is modulated by the circadian clock. These findings will facilitate the characterization of the circadian clock in soybean.

In Arabidopsis, the PR and CCT domain of APRRs confer repression activity and DNA-binding activity, respectively [50,51]. In rice, missense mutations occurring in the conserved CCT domain, are predicted to affect OsPRR37 function in the regulation of photoperiod sensitivity, leading to an early heading date [36,38]. In this study, GmPRR12H4 carried a missense mutation in the CCT domain and was significantly late flowering (Supplementary Figure S4M, Figure 4L). Additionally, GmPRR3a and GmPRR3b, corresponding to GmPRR9 and GmPRR10 in this study [25] (Supplementary Figure S1, Supplementary Table S1), respectively, carry mutations that delete the CCT domain leading to early flowering in soybean [24–26]. Thus, it is worth clarifying the function of GmPRR12 in the photoperiodic flowering pathway. However, non-synonymous variants in the PR domain appeared in GmPRR2, GmPRR5, GmPRR6, GmPRR7, and GmPRR14, and only the variation in GmPRR2 was clearly associated with flowering time (Figure 4). Further studies could verify the effect of the PR domain on the regulation of soybean photoperiod flowering. For GmPRR1, GmPRR4, GmPRR8, GmPRR11 and GmPRR13, there were some missense variants occurring in the coding regions outside the CCT and PR domains, which were associated with flowering time. These variations might weakly affect gene function and finely tune the developmental rate. Developing Kompetitive Allele Specific PCR (KASP) markers for these variations could facilitate soybean improvement with the desired flowering time for planting in target areas.

The circadian clock coordinates the internal biological processes with the external environmental factors, and thus provides an adaptive advantage. The occurrence of circadian timekeeping is of great importance to living beings. Studies have shown that naturally occurring variations in clock parameters (period, phase, and amplitude) are necessary for the circadian clock to contribute to the fitness of organisms over a wide range of latitudes [52–57]. In Arabidopsis thaliana, latitude-specific selection effects have been found on circadian properties by analyzing natural variation in the period, phase, and amplitude of 150 accessions, and Pseudo-response regulator (PRR) family members are key candidates for clock quantitative trait loci [52]. Crops are commonly cultivated over a broader geographical range compared with their ancestors and increasing evidence has shown that breeders have been indirectly selecting for circadian parameters, which might contribute to improved performance in distinct latitudes. The allelic variation of EID1 responsible for the phase delay was selected in cultivated tomato during domestication due to the enhanced performance under long-day photoperiods [53]. Similarly, latitudinal clines in circadian period were found in elite soybean cultivars from six maturity groups [54]. Thus, further analysis of the association between the genetic diversity and circadian period of GmPRRs will facilitate elucidating the adaptation of soybean to different cultivated regions.

Unlike other traits that generally experience breeding selection in one direction, soybean flowering time becomes more diverse during breeding for different environments with variable day lengths. It has been a major goal for breeders to decipher the genetic mechanisms of soybean flowering time and regional adaptability. In this study, we analyzed the distribution of major GmPRR haplotypes among 180 varieties traditionally cultivated in China. Soybean varieties with GmPRR1H1, GmPRR2H1, GmPRR3H1, GmPRR4H1,
GmPRR5H1, GmPRR6H1, GmPRR7H2, GmPRR7H3, GmPRR8H1, GmPRR9H1, GmPRR10H1, GmPRR11H1, GmPRR12H1, GmPRR12H2, GmPRR12H3, GmPRR13H1, and GmPRR14H1 were associated with earlier flowering and tend to be distributed in higher latitudes in China (Figures 4 and 5). However, varieties of GmPRR1H5, GmPRR2H5, GmPRR2H6, GmPRR3H3, GmPRR3H4, GmPRR4H2, GmPRR5H2, GmPRR5H4, GmPRR6H3, GmPRR6H4, GmPRR7H4, GmPRR8H2, GmPRR9H2, GmPRR9H3, GmPRR9H4, GmPRR10H2, GmPRR10H3, GmPRR11H2, GmPRR11H4, GmPRR12H3, and GmPRR12H4, which were related to later flowering, are mainly found in Huang-Huai-Hai (HHH) and South China (SC) (Figures 4 and 5). We found high diversity of haplotype combinations of GmPRRs in the 207 accessions, and the combinational effects of GmPRRs may contribute to the wide adaptation of Zhonghuang 13 (Figure 5, Supplementary Table S3). Furthermore, these rich natural variations and combinations of GmPRRs may be useful for precise prediction of flowering time.

Soybean flowering time is a quantitative feature regulated by various genes [8–29]. Recent studies have shown that clock genes play important roles in soybean flowering and ecological adaptation. Natural mutants of GmELF3/J [20–22] and LHY1a [27] improve soybean adaptation to the tropics, and natural variations in GmPRR37/GmPRR3b and GmPRR3a are associated with soybean adaptation to high latitudes [24–26]. GmLUX1 and GmLUX2 both interact with GmELF3/J to form an evening complex and play essential roles in soybean flowering and adaptation [22]. Thus, we also investigated the number of homologs for clock genes including CCA1/LHY, LNK family, ELF3, ELF4, and LUX, and found that the number of these genes (CCA1/LHY-4, LNK-4, ELF3-5, ELF4-2, LUX-2 homologs) are much fewer than PRR genes in soybean. Further analysis of the allelic combinations of GmPRRs with other genes controlling flowering would allow us to precisely manipulate flowering time, and to breed soybean varieties best adapted to diverse environments.

4. Materials and Methods
4.1. Plant Materials, Photoperiod Treatments and Multiple-Site Experiments

For the analysis of the expression patterns of GmPRRs, the photoperiod sensitive soybean variety Zigongdongdou (ZGDD) and the photoperiod insensitive soybean variety Heihe27 (HH27) were grown in a controlled culture room at 26 °C under short-day (SD) (12 h: 12 h, light: dark) and long-day (LD) (16 h: 8 h, light: dark) conditions. Different organs of the plant, including the trifoliolate leaf, unifoliolate leaf, shoot apex, stem, hypocotyl, and root were sampled after 4 h exposure to light at 10 days after emergence (DAE). The trifoliolate leaves of soybean variety ZGDD were sampled at 4 h intervals throughout a 48 h period on days 10 and 11 of LD or SD treatment. Each sample consisted of material collected from three plants.

A 207-accession panel including 180 soybean varieties from China (97 from Northeast China (NE), 46 from Huang-Huai-Hai (HHH), 37 from South China (SC)) and 27 from the US were used for haplotype analysis (Supplementary Table S2) [58]. The panel was planted in Sanya (18°18′ N, 112°39′ E), Xinxian (35°08′ N, 113°45′ E)) and Beijing (40°13′ N, 116°33′ E) in 2016, and in Xiantan (27°40′ N, 112°39′ E), Changchun (43°50′ N, 124°82′ E) and Heihe (50°24′ N, 127°49′ E) in 2017 [59]. These six environments were named SY2016, BJ2016, XX2016, XT2017, CC2017 and HH2017, respectively. All of the 207 soybean varieties were grown in rows 1.5 m long by 0.5 m row space with 0.1 m between the plants. All materials were arranged in randomized complete blocks with two replications.

4.2. Identification, Phylogenetic and Bioinformatic Analysis of GmPRRs

The potential PRR family members in soybean were identified based on Arabidopsis PRR sequences using both BLASTp and Hidden Markov Model (HMM). The protein sequences of Arabidopsis PRRs were downloaded from the TAIR database (https://www.arabidopsis.org/) (accessed on 8 February 2020). Soybean genome annotation was downloaded from the Phytozome 13.0 database (https://phytozome-next.jgi.doe.gov/) (accessed on 8 February 2020). Soybean PRR members resulting from both searches
with an E-value threshold of \( <e^{-10} \) were pooled, and all redundant putative PRR sequences were removed. The remaining PRR sequences were further confirmed by the Pfam (http://pfam.xfam.org/) (accessed on 12 February 2020) and NCBI Conserved Domains Database (https://www.ncbi.nlm.nih.gov/cdd/) (accessed on 12 February 2020) for the existence of a PR domain and CCT domain.

The Multiple Collinearity Scan toolkit (MCScanX) was used to analyze the GmPRRs duplications with the default parameters (MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity). The syntenic analysis map was constructed using TBtools [60]. Physiochemical parameters of soybean PRR proteins, such as polypeptide length, the protein molecular weight, isoelectric point was investigated using ExPASy (http://web.expasy.org/protparam/) (accessed on 15 September 2021). The subcellular localization of GmPRR proteins was predicted by PSORT II (https://psort.hgc.jp/form2.html) (accessed on 15 September 2021).

To reveal the evolutionary relationships among PRR genes in different plant species, potential PRR genes from Arabidopsis, rice and other crops were selected for phylogenetic analysis. The phylogenetic tree was constructed using the Neighbor-Joining (NJ) method of MEGA 7.0 software (https://www.megasoftware.net/home) (accessed on 8 February 2020) with a bootstrap test of 1000 replicates [61].

4.3. Gene Structure, Conserved Motif and Promoter Sequence Analyses

For the analysis of the exon–intron patterns, the genomic DNA sequence and CDS of each GmPRR gene were retrieved from the soybean genome database. Conserved motifs of GmPRRs were analyzed by the Multiple Em for Motif Elicitation (MEME, https://meme-suite.org/meme/tools/meme) (accessed on 26 May 2021), and the maximum number of motifs was set to 10.

To identify the potential photoperiod and circadian related cis-elements, the 2 kb genomic sequences upstream of the start codon (ATG) of GmPRR genes were extracted from the soybean genome database. The cis-acting elements were confirmed using Plant Cis-Acting Regulatory Element (PlantCARE, http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) (accessed on 26 May 2021). TBtools software (https://github.com/CJ-Chen/TBtools) (accessed on 30 May 2021) was used for visualization of these analysis results [60].

4.4. RNA Extraction and Quantitative Real-Time PCR

Total RNA was extracted using the Easy Fast Plant Tissue Kit (TianGen, Beijing, China), and cDNA was synthesized with the FastKing RT Kit (With gDNase) (TianGen, Beijing, China). Primers were designed using the NCBI (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) (accessed on 13 May 2021) online program, and the primers (Supplementary Table S4) were synthesized by Tsingke Biotechnology Co., Ltd. (Beijing, China). Quantitative RT-PCR (qRT-PCR) was performed using an ABI QuantStudio™ 7 flex (Applied Biosystems, San Francisco, CA, USA) with Taq Pro Universal SYBR qPCR Master Mix (Vazyme, Beijing, China). Three biological replicates of every sample were measured. The reaction procedures were as follows: pre-denaturation at 95 °C for 30 s; denaturation at 95 °C for 5 s, annealing at 60 °C for 30 s, 40 cycles. Each reaction was performed in biological triplicates and the relative gene expression levels were analyzed using \( 2^{-\Delta\Delta CT} \) method with the GmActin (Glyma18g52780) gene as an internal control.

4.5. Haplotype Analysis of Soybean PRR Genes

The 207 whole-genome resequencing soybean varieties were obtained from our previous study [58] (Supplementary Table S2), and the sequence data have been deposited in the NCBI database under Short Read Archive (SRA) Accession Number SRP062560 and PRJNA589345. The SNPs and insertion and deletion polymorphism were extracted for further haplotype analysis. The flowering time across six environments of 207 soybean varieties was recorded as days from emergence to beginning to bloom [62]. The flowering
time phenotype of each variety in a single environment was determined by taking the average from two replications. The association analysis of GmPRR haplotypes with flowering time was determined by Duncan’s multiple range test using GraphPad Prism 8 (p < 0.05).

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms23179970/s1.

**Author Contributions:** Conceptualization, T.H., J.B. and S.S.; methodology, L.W., B.J. and F.C.; software, L.W. and P.W.; validation, L.W. and P.W.; resources, T.W., S.Y., B.J., C.W., W.H. and S.S.; writing—original draft preparation, P.W. and L.W.; writing—review and editing, T.H., L.W., L.Z., J.Z. and E.S.; visualization, P.W.; funding acquisition, T.H. and S.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was funded by grants from the National Key R&D Program of China (2021YFF1001203), China Agriculture Research System (CARS-04) and CAAS Agricultural Science and Technology Innovation Project.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The sequence data used in this study are available in the NCBI database under Short Read Archive (SRA) Accession Number SRP062560 and PRJNA589345.

**Acknowledgments:** We thank Lijuan Qiu and Zhangxiong Liu for supplying some germplasms, and we thank Jinlu Tao, Haiyan Zeng, Yanfeng Zhou and Xuegang Sun for their field management.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Garner, W.W.; Allard, H.A. Effect of the Relative Length of Day and Night and Other Factors of the Environment on Growth and Reproduction in Plants. *J. Agric. Res.* **1920**, *18*, 553–606. [CrossRef]

2. Garner, W.W.; Allard, H.A. Further Studies in Photoperiodism: The Response of the Plant to Relative Length of Day and Night. *J. Agric. Res.* **1923**, *23*, 871–920.

3. Wang, J.; Wu, Y.; Wu, H.; Sun, S. Analysis on Photoperiod Ecotypes of Cultivated Soybean Originating from Different Locations of China. *Acta. Agric. Sin.* **1956**, *7*, 169–180. (In Chinese)

4. Han, T.; Wang, J.; Fan, B.; Yao, W.; Yang, Q. Effects of Post-Flowering Daylength on Agronomic Characters of Soybean. *Chin. J. Appl. Ecol.* **1996**, *7*, 167–173, (In Chinese with English Abstract).

5. Watanabe, S.; Harada, K.; Abe, J. Genetic and Molecular Bases of Photoperiod Responses of Flowering in Soybean. *Breed Sci.* **2012**, *61*, 531–543. [CrossRef]

6. Cao, D.; Takeshima, R.; Zhao, C.; Liu, B.; Jun, A.; Kong, F. Molecular Mechanisms of Flowering under Long Days and Stem Growth Habit in Soybean. *J. Exp. Bot.* **2017**, *68*, 1873–1884. [CrossRef]

7. Fu, M.; Wang, Y.; Ren, H.; Du, W.; Wang, D.; Bao, R.; Yang, X.; Tian, Z.; Fu, L.; Cheng, Y.; et al. Genetic Dynamics of Earlier Maturity Group Emergence in South-to-North Extension of Northeast China Soybeans. *Theor. Appl. Genet.* **2020**, *133*, 1839–1857. [CrossRef]

8. Xia, Z.; Watanabe, S.; Yamada, T.; Tsubokura, Y.; Nakashima, H.; Zhai, H.; Anai, T.; Sato, S.; Yamazaki, T.; Lü, S.; et al. Positional Cloning and Characterization Reveal the Molecular Basis for Soybean Maturity Locus E1 that Regulates Photoperiodic Flowering. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, E2155–E2164. [CrossRef]

9. Watanabe, S.; Xia, Z.; Hideshima, R.; Tsubokura, Y.; Sato, S.; Yamanaka, N.; Takahashi, R.; Anai, T.; Tabata, S.; Kitamura, K.; et al. A Map-based Cloning Strategy Employing a Residual Heterozygous Line Reveals that the GIGANTEA Gene Is Involved in Soybean Maturity and Flowering. *Genetics* **2011**, *188*, 395–407. [CrossRef]

10. Watanabe, S.; Hideshima, R.; Xia, Z.; Tsubokura, Y.; Sato, S.; Nakamoto, Y.; Yamanaka, N.; Takahashi, R.; Ishimoto, M.; Anai, T.; et al. Map-based Cloning of the Gene Associated with the Soybean Maturity Locus E3. *Genetics* **2009**, *182*, 1251–1262. [CrossRef]

11. Liu, B.; Kanazawa, A.; Matsumura, H.; Takahashi, R.; Harada, K.; Abe, J. Genetic Redundancy in Soybean Photoresponses Associated with Duplication of the Phytochrome a Gene. *Genetics* **2008**, *180*, 995–1007. [CrossRef] [PubMed]

12. Liu, W.; Jiang, B.; Ma, L.; Zhang, S.; Zhai, H.; Xu, X.; Hou, W.; Xia, Z.; Wu, C.; Sun, S.; et al. Functional Diversification of Flowering Locus T Homologs in Soybean: GmFT1a and GmFT2a/Sa Have Opposite Roles in Controlling Flowering and Maturation. *New Phytol.* **2018**, *217*, 1335–1345. [CrossRef] [PubMed]

13. Bonato, E.R.; Vello, N.A. E6, a Dominant Gene Conditioning Earlier Flowering and Maturity in Soybeans. *Genet. Mol. Biol.* **1999**, *22*, 229–232. [CrossRef]
14. Sun, H.; Jia, Z.; Cao, D.; Jiang, B.; Wu, C.; Hou, W.; Liu, Y.; Fei, Z.; Zhao, D.; Han, T. GmFT2a, a Soybean Homolog of FLOWERING LOCUS T, Is Involved in Flowering Transition and Maintenance. *PLoS ONE* **2011**, *6*, e29238. [CrossRef]

15. Kong, F.; Nan, H.; Cao, F.; Wang, J.; Yuan, S. A New Dominant Gene E9 Conditions Early Flowering and Maturity in Soybean. *Crop Sci.* **2014**, *54*, 2529–2535. [CrossRef]

16. Kong, F.; Liu, B.; Xia, Z.; Sato, S.; Kim, B.M.; Watanabe, S.; Yamada, T.; Tabata, S.; Kanazawa, A.; Harada, K.; et al. Two Cooperatively Regulated Homologs of FLOWERING LOCUS T Are Involved in the Control of Photoperiodic Flowering in Soybean. *Plant Physiol.* **2010**, *154*, 1220–1231. [CrossRef]

17. Zhao, C.; Takeshima, R.; Zhu, J.; Xu, M.; Sato, M.; Watanabe, S.; Kanazawa, A.; Liu, B.; Kong, F.; Yamada, T.; et al. A Recessive Allele for Delayed Flowering at the Soybean Maturity Locus E9 Is a Leaky Allele of FT7a, a FLOWERING LOCUS T Ortholog. *BMC Plant Biol.* **2016**, *16*, 20. [CrossRef]

18. Samanfar, B.; Molnar, S.J.; Charette, M.; Schoenrock, A.; Dehne, F.; Golshani, A.; Belzile, F.; Cober, E.R. Mapping and Identification of a Potential Candidate Gene for a Novel Maturity Locus, E10, in Soybean. *Theor. Appl. Genet.* **2017**, *130*, 377–390. [CrossRef]

19. Takeshima, R.; Hayashi, T.; Zhu, J.; Zhao, C.; Xu, M.; Yamaguchi, N.; Sayama, T.; Ishimoto, M.; Kong, L.; Shi, X.; et al. A Soybean Quantitative Trait Locus that Promotes Flowering under Long Days Is Identified as FT5a, a FLOWERING LOCUS T Ortholog. *J. Exp. Bot.* **2016**, *67*, 5247–5258. [CrossRef]

20. Lu, S.; Zhao, X.; Hu, Y.; Liu, S.; Nan, H.; Li, X.; Fang, C.; Cao, D.; Shi, X.; Kong, L.; et al. Natural Variation at the Soybean J Locus Improves Adaptation to the Tropics and Enhances Yield. *Nat. Genet.* **2017**, *49*, 773–779. [CrossRef]

21. Yue, Y.; Liu, N.; Jiang, B.; Li, M.; Wang, H.; Jiang, Z.; Pan, H.; Xia, Q.; Ma, Q.; Han, T.; et al. A Single Nucleotide Deletion in *OsELF3* Confers Long Juvenility and Is Associated with Adaptation of Tropic Soybean. *Mol. Plant.* **2017**, *10*, 656–658. [CrossRef]

22. Bu, T.; Lu, S.; Wang, K.; Dong, L.; Li, S.; Xie, Q.; Xu, X.; Cheng, Q.; Chen, L.; Fang, C.; et al. A Critical Role of the Soybean Evening Complex in the Control of Photoperiod Sensitivity and Adaptation. *Proc. Natl. Acad. Sci. USA* **2021**, *23*, e2010241118. [CrossRef]

23. Li, Y.; Dong, Y.; Wu, H.; Hu, B.; Zhai, H.; Yang, J.; Xia, Z. Positional Cloning of the Flowering Time QTL qFTT2-1 Reveals the Link between the Clock Related PRR Homolog with Photoperiodic Response in Soybeans. *Front. Plant Sci.* **2019**, *10*, 1303. [CrossRef]

24. Wang, L.; Sun, S.; Wu, T.; Liu, L.; Sun, X.; Cai, Y.; Li, J.; Jia, H.; Yuan, S.; Chen, L.; et al. Natural Variation and CRISPR/Cas9-Mediated Mutation in GmPRR37 Affect Photoperiodic Flowering and Contribute to Regional Adaptation of Soybean. *Plant Biotechnol. J.* **2020**, *18*, 1869–1881. [CrossRef]

25. Li, C.; Li, Y.; Li, Y.; Hu, L.; Hong, H.; Tian, Y.; Li, H.; Zhao, T.; Zhou, X.; Liu, J.; et al. A Domestication-Associated Gene GmPRR3b Regulates the Circadian Clock and Enhances Yield in Soybean. *Mol. Plant.* **2020**, *13*, 745–759. [CrossRef]

26. Lu, S.; Dong, L.; Fang, C.; Liu, S.; Kong, L.; Cheng, Q.; Chen, L.; Su, T.; Nan, H.; Zhang, D.; et al. Stepwise Selection on Homeologous *PRR* Genes Controlling Flowering and Maturity during Soybean Domestication. *Nat. Genet.* **2020**, *52*, 428–436. [CrossRef]

27. Dong, L.; Fang, C.; Cheng, Q.; Su, T.; Kou, K.; Kong, L.; Zhang, C.; Li, H.; Hou, Z.; Zhang, Y.; et al. Genetic Basis and Adaptation Trajectory of Soybean from Its Temperate Origin to Tropics. *Nat. Commun.* **2021**, *12*, 5445. [CrossRef]

28. Cheng, Q.; Dong, L.; Su, T.; Li, T.; Gan, Z.; Nan, H.; Lu, S.; Fang, C.; Kong, L.; Li, H.; et al. CRISPR/Cas9-Mediated Targeted Mutagenesis of GmLHY Genes Alters Plant Height and Internode Length in Soybean. *BMC Plant Biol.* **2019**, *19*, 562. [CrossRef]

29. Wang, Y.; Yuan, L.; Su, T.; Wang, G.; Qiao, Y.; Zhang, S.; Jia, Q.; Yu, G.; Fu, Y.; Cheng, Q.; et al. Light and Temperature Entrainable Circadian Clock in Soybean Development. *Plant Cell Environ.* **2020**, *43*, 637–648. [CrossRef]

30. Creux, N.; Harmer, S. Circadian Rhythms in Plants. *Cold Spring Harb. Perspect. Biol.* **2019**, *11*, a034611. [CrossRef]

31. Farré, E.M.; Liu, T. The PRR Family of Transcriptional Regulators Reflects the Complexity and Evolution of Plant Circadian Clocks. *Curr. Opin. Plant Biol.* **2013**, *16*, 621–629. [CrossRef] [PubMed]

32. Gil, K.E.; Park, C.M. Thermal Adaptation and Plasticity of the Plant Circadian Clock. *New Phytol.* **2019**, *221*, 1215–1229. [CrossRef] [PubMed]

33. Matsuhashiga, A.; Makino, S.; Kojima, M.; Mizuno, T. Circadian Waves of Expression of the APRR1/TOCI Family of Pseudo-Response Regulators in Arabidopsis Thaliana: Insight into the Plant Circadian Clock. *Plant Cell Physiol.* **2000**, *41*, 1002–1012. [CrossRef]

34. Matsuhashiga, A.; Makino, S.; Kojima, M.; Yamashina, T.; Mizuno, T. The APRR1/TOCI Quintet Implicated in Circadian Rhythms of Arabidopsis Thaliana: II. Characterization with CCA1-Overexpressing Plants. *Plant Cell Physiol.* **2002**, *43*, 118–122. [CrossRef]

35. Nakamichi, N.; Kita, M.; Niinuma, K.; Ito, S.; Yamashina, T.; Mizoguchi, T.; Mizuno, T. Arabidopsis Clock-Associated Pseudo-Response Regulators PRR9, PRR7 and PRR5 Cooperatively and Positively Regulate Flowering Time through the Canonical CONSTANS-Dependent Photoperiodic Pathway. *Plant Cell Physiol.* **2007**, *48*, 822–832. [CrossRef]

36. Koo, B.H.; Yoo, S.C.; Park, J.W.; Kwon, C.T.; Lee, B.D.; An, G.; Zhang, Z.; Li, J.; Li, Z.; Paek, N.C. Natural Variation in OsPRR37 Regulates Heading Date and Contributes to Rice Cultivation at a Wide Range of Latitudes. *Mol. Plant* **2013**, *6*, 1877–1888. [CrossRef] [PubMed]

37. Yan, W.; Liu, H.; Zhou, X.; Li, Q.; Zhang, J.; Lu, L.; Liu, T.; Liu, H.; Zhang, C.; Zhang, Z.; et al. Natural Variation in Ghd7.1 Plays an Important Role in Grain Yield and Adaptation in *Rice*. *Cell Res.* **2013**, *23*, 969–971. [CrossRef] [PubMed]

38. Gao, H.; Jin, M.; Zheng, X.; Chen, J.; Yuan, D.; Yin, X.; Wang, M.; Huang, D.; Zhang, Z.; Zhou, K.; et al. Days to Heading 7, a Major Quantitative Locus Determining Photoperiod Sensitivity and Regional Adaptation in *Rice*. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 16337–16342. [CrossRef]
39. Beales, J.; Turner, A.; Griffiths, S.; Snape, J.W.; Laurie, D.A. A Pseudo-Response Regulator is Mis-expressed in the Photoperiod Insensitive Ppd-D1a Mutant of Wheat (Triticum aestivum L.). Theor. Appl. Genet. 2007, 115, 721–733. [CrossRef]
40. Turner, A.; Beales, J.; Faure, S.; Dunford, R.P.; Laurie, D.A. The Pseudo-Response Regulator Ppd-H1 Provides Adaptation to Photoperiod in Barley. Science 2005, 310, 1031–1034. [CrossRef]
41. Murphy, R.L.; Klein, R.R.; Morishige, D.T.; Brady, J.A.; Rooney, W.L.; Miller, F.R.; Dugas, D.V.; Klein, P.E.; Mullet, J.E. Coincident Light and Clock Regulation of Pseudo-Response Regulator Protein 37 (PRR37) Controls Photoperiodic Flowering in Sorghum. Proc. Natl. Acad. Sci. USA 2011, 108, 16469–16474. [CrossRef]
42. Yang, J.; Huang, X. A New High-Quality Genome Sequence in Soybean. Sci. China Life Sci. 2018, 61, 1604–1605. [CrossRef]
43. Shen, Y.; Du, H.; Liu, Y.; Ni, L.; Wang, Z.; Liang, C.; Tian, Z. Update Soybean Zhonghuang 13 Genome to a Golden Reference. Sci. China Life Sci. 2019, 62, 1257–1260. [CrossRef]
44. Wang, L.; Sun, J.; Wang, L.; Li, B.; Zhao, R. Breeding and Application of Widely Adapted, High-yield and High-protein Soybean Variety Zhonghuang 13. Soybean Sci. 2019, 1–6, (In Chinese with English Abstract).
45. Bendix, C.; Marshall, C.; Harmon, F. Circadian Clock Genes Universally Control Key Agricultural Traits. Mol. Plant 2015, 8, 1135–1152. [CrossRef]
46. Strayer, C.; Oyama, T.; Schultz, T.F.; Raman, R.; Somers, D.E.; Más, P.; Panda, S.; Kreps, J.A.; Kay, S.A. Cloning of the Arabidopsis Clock Gene TOC1, an Autoregulatory Response Regulator Homolog. Science 2000, 289, 768–771. [CrossRef]
47. Salomé, P.A.; McClung, C.R. PSEUDO-RESPONSE REGULATOR 7 and 9 Are Partially Redundant Genes Essential for the Temperature Responsiveness of the Arabidopsis Circadian Clock. Plant Cell 2005, 17, 791–803. [CrossRef]
48. Murakami, M.; Ashikari, M.; Miura, K.; Yamashino, T.; Mizuno, T. The Evolutionarily Conserved OsPRR Quintet: Rice Pseudo-Response Regulators Implicated in Circadian Clock Function in Natural and Agricultural Settings. Plant Cell Physiol. 2003, 44, 1229–1236. [CrossRef]
49. Shu, Y.; Yu, D.; Wang, D.; Guo, D.; Guo, C. Genome-wide Survey and Expression Analysis of the MADS-Box Gene Family in Soybean. Mol. Biol. Rep. 2013, 40, 3901–3911. [CrossRef]
50. Gendron, J.M.; Pruneda-Paz, J.L.; Doherty, C.J.; Gross, A.M.; Kang, S.E.; Kay, S.A. Arabidopsis Circadian Clock Protein, TOC1, Is a DNA-binding Transcription Factor. Proc. Natl. Acad. Sci. USA 2012, 109, 3167–3172. [CrossRef]
51. Nakamichi, N.; Ito, T.; Kamioka, M.; Suzuki, T.; Yamashino, T.; Higashiyama, T.; Sakakibara, H.; Mizuno, T. Transcriptional Repressor PRR5 Directly Regulates Clock-Output Pathways. Proc. Natl. Acad. Sci. USA 2012, 109, 17123–17128. [CrossRef]
52. Michael, T.P.; McClung, C.R. Enhanced Fitness Confounded by Naturally Occurring Clock Variations in the Arabidopsis Circadian Clock. Science 2003, 302, 1049–1053. [CrossRef] [PubMed]
53. Müller, N.A.; Wijnen, C.L.; Srinivasan, A.; Ryngajlo, M.; Oher, I.; Lin, T.; Ranjan, A.; West, D.; Maloof, J.N.; Sinha, N.R.; et al. Domestication Selected for Deceleration of the Circadian Clock in Cultivated Tomato. Nat. Genet. 2016, 48, 89–93. [CrossRef] [PubMed]
54. Greenham, K.; Lou, P.; Puzeys, J.R.; Kumar, G.; Arnevik, C.; Farid, H.; Willis, J.H.; McClung, C.R. Geographic Variation of Plant Circadian Clock Function in Natural and Agricultural Settings. J. Biol. Rhythm. 2017, 32, 26–34. [CrossRef] [PubMed]
55. Muranaka, T.; Ito, S.; Kudoh, H.; Oyama, T. Circadian-Period Variability Underlies the Local Adaptation of Photoperiodism in the Short-Day Plant Lemma aquatilis. iScience 2022, 25, 104634. [CrossRef] [PubMed]
56. Forni, D.; Pozzoli, U.; Caglioni, R.; Tresoldi, C.; Menozzi, G.; Riva, S.; Guerini, F.R.; Comi, G.P.; Bolognesi, E.; Bresolin, N.; et al. Genetic Adaptation of the Human Circadian Clock to Day-Length Latitudinal Variations and Relevance for Affective Disorders. Genome Biol. 2014, 15, 499. [CrossRef] [PubMed]
57. Lemay, M.A.; Russell, M.A. Latitudinal Cline in Allele Length Provides Evidence for Selection in a Circadian Rhythm Gene. Biol. J. Linn. Soc. 2014, 111, 869–877. [CrossRef]
58. Zhang, T.; Wu, T.; Wang, L.; Jiang, B.; Zhen, C.; Yuan, S.; Hou, W.; Wu, C.; Han, T.; Sun, S. A Combined Linkage and GWAS Analysis Identifies QTls Linked to Soybean Seed Protein and Oil Content. Int. J. Mol. Sci. 2019, 20, 5915. [CrossRef]
59. Chen, C.; Cai, Y.; Qu, M.; Wang, L.; Sun, H.; Jiang, B.; Wu, T.; Liu, L.; Sun, S.; Wu, C.; et al. Soybean Adaptation to High-latitude Regions Is Associated with Natural Variations of GmFT2b, An Ortholog of FLOWERING LOCUS T. Plant Cell Environ. 2020, 43, 934–944. [CrossRef]
60. Chen, C.; Chen, H.; Zhang, Y.; Thomas, H.R.; Frank, M.H.; He, Y.; Xia, R. TBoots: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. Mol. Plant 2020, 13, 1194–1202. [CrossRef]
61. Kumar, S.; Stecher, G.; Tamure, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol. Biol. Evol. 2016, 33, 1870–1874. [CrossRef]