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GBS-based map construction and QTL identification for major agronomic traits in mungbean

By

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GENERAL ABSTRACT

Mungbean is an important crop grown in South, East and Southeast Asia. As mungbean contains a high protein content, along with other nutritional befinits such as carboyhoydrates, lipids, minerals, and vitamins, it is important to investigate genomics regions controlling specific agronomic traits for the improvement in breeding programs, where most of the major traits are quantitatively inherited. The general objective is to identify quantitative trait loci (QTLs) of mungbean associated with days to first flowering (DFF), days to 50% flowering

(DF), 100-seed weight (100-SW), lodging resistance (Ldge), and cercospora leaf spot resistance (CLS). Flowering is one of the most crucial trait to be studied in mungbean because of its direct correlation with crop yield. It has previously been reported that a higher number of pod yield produces after the two to three weeks of first flowering and its consequences of a high yielding mungbean. Therefore, it is of great interest to investigate the genetic loci underlying flowering time and its associated traits, allowing breeders to develop novel varieties with efficient altered flowering times. In this study, a total of 190 F_6 recombinant inbred lines (RILs) developed from the cross of VC 1973A (Sunhwa nokdu) and VC 2984 (Gyeonggi Jaerae #5) were used to identify quantitative trait loci (QTLs) for DFF, DF, 100-SW, Ldge, CLS, plant height (PH), and number of branches (NB).

A genetic map was constructed using 798 single nucleotide polymorphism (SNP) markers developed by a genotyping-by-sequencing (GBS) method from a segregating a RIL population. The generated markers were distributed over all 11 chromosomes (Chrs), covering 757.39 centiMorgans (cM) of mungbean genome with the average distance between adjacent markers of 0.95 cM. Using composite interval mapping (CIM), we identified six QTLs were

identified for DFF and DF, four for 100-SW, two for PH, two for NB, one for Ldge, and two for CLS. Among them, one QTL located on Chr4 was identified to be associated with three agronomics traits with significant LOD scores, of which they were DFF, DF and PH. This genomic region then was deployed in the soybean genome for comparative analysis to identify synteny relationships between mungbean and soybean, whether this region co-localized with the soybean flowering QTL regions.

Keywords: Mungbean; quantitative trait loci (QTL); agronomic traits, recombinant inbred lines; genetic mapping; synteny;

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LIST OF ABBREVIATIONS

QTL   Quantitative trait loci

GBS   Genotyping by sequencing

DFF   Days to first flowering

DF   Days to 50% flowering

PH   Plant height (cm)

100-SW   100-seed weight (g)

NB   Number of branches (ea)

LDGE   Lodging resistance

CLS   Cercospora leaf spot resistance

GENERAL INTRODUCTION

Mungbean (*Vigna radiata*) is an important crop in developing countries of South, East, and Southeast Asia, mainly cultivated by small scale famers, as it attributes nutritional needs of protein, minerals, and vitamins, (Kang et al. 2014; Lambrides and Goodwin 2007). Besides the nutritional facts, mungbean has the ability to fix atmospheric nitrogen through root rhizobial symbiosis and convert it into a form that enriches soil fertility (Graham et al. 2003). Its economic importance across Asia has been high, resulting in a corresponding increase of production from 2.3 million tons to 30.1 million tons from 1985-2000 (Shanmugasundaram, 2009). Based on yield projections, the world's demand for mungbean is expected to rise in the future (Chauhan et al. 2010). Currently, the major leading producing countries are India, China, Myanmar, Pakistan, Thailand, and Vietnam (Nair et al. 2012). As mungbean has become one of the valuable commercial crops in Asian countries, its genomes have been sequenced recently, covering 80% of the estimate genome (Kang et al. 2014). Not only would it be used as a reference for genomics research in mungbean,

but as well as it would ultimately accelerate future breeding programs for development of improved cultivars.

Days to first flowering (DFF), days to 50% flowering (DF), plant height (PH), 100-seed weight (SW), number of branches (NB), lodging resistance (Ldge), cercospora leaf spot resistance (CLS) in mungbean are important traits and valuable to develop mungbean cultivars with superior performance and adaptation. Many agronomic traits are controlled by multiple genomic regions known as QTLs. To improve mungbean cultivation, some of the QTLs have been positioned on the mungbean genetic linkage map using RFLP, RAPD, and SSR markers to identify a variety of the traits. These traits are 100-seed weight (Humphry et al., 2005; Mei et al., 2009; Takehisa et al., 2012), seed color (Lambrides et al., 2004), bruchid resistance (Mei et al., 2009), cercospora leaf spot resistance (Chankaew et al., 2011), seed dormancy, pod dehiscence, and phenology-related traits (Takehisa et al., 2012). For the trait of days to first flowering, four QTLs were mapped to LG2, GL4, LG6, and LG11 using 430 SSR and EST-SSR markers from a BC_1F_1 mapping population derived from the cross between wild and cultivated mungbean (JP211874 X JP229096) (Isemura et al, 2012). Recently, a density genetic linkage map of

mungbean was constructed, consisting of 1321 single nucleotide polymorphisms (SNP) markers developed through genotyping by sequencing (GBS) (Kang et al., 2014). Therefore, further extension of QTL analysis and identification of genomic regions containing yield-associated traits would hence enable potential yield increase.

Flowering is the transition from vegetative to reproductive growth that is a critical stage in determining potential yield for crop plants, and successful reproduction occurs when flowers bloom synchronously at the optimal time (Simpson and Dean 2002). In mungbean, its seed yield is positively correlated with number of flowers, percentage of pod set, number of seeds per pod, and seed size (Mondal 2007). Even with the breeding efforts in mungbean for efficient yield improvement, the seed production still remains low and has been fluctuating (Mondal et al. 2012) because of the occurrence of flower abscission and immature pods (Mondal et al. 2011). Like most legumes, despite many flower numbers are produced, only a few of them develops into pods (Fakir et al. 2011; Mondal et al. 2007); about 60-92% flowers are shed in soybean (*Glycine max*) (Nahar and Ikeda 2002; Saitoh et al. 2004) and 70-90% flowers in mungbean (*Vigna radiata*) (Kumari and Verma, 1983; Mondal et al. 2011). Abscission of flowers

reportedly takes place in most of later-formed flowers (Isobe et al. 1995; Kuroda et al. 1998; Mondal et al. 2007), indicating that prevention of late flowering would increase the yield. In fact, a higher number of pod yield has resulted in the bloom within two to three weeks after first flowering (Mondal and Hamid 1998; Mondal et al. 2011b). Thus, for high yielding mungbean, it is of great interest to investigate the genetics underlying flowering time and its associated traits, allowing breeders to develop novel varieties with altered flowering times.

In this study, we conducted QTL analysis of mungbean agronomic traits using RIL derived from a cross between VC 1973A (Sunhwa nokdu) and VC 2984 (Gyeonggi Jaerae). The objective of this study were to identify QTLs for days to first flowering (DFF), days to 50% flowering (DF), plant height (PH), 100-seed weight (SW), number of branches (NB), lodging resistance (Ldge), cercospora leaf spot resistance (CLS). We found several genomic regions of QTL for these agronomic traits in this population.

LITERATURAL REVIEWS

Genotyping by Sequencing

Advances in next generation technologies have driven the costs of DNA sequencing down to the point that genotyping-by-sequencing (GBS) is now flexible for large population, high diversity genome species. GBS approach is simple, quick, extremely specific, highly reproducible, and may reach important regions of the genome that are inaccessible to sequence capture approaches. A procedure for constructing GBS libraries based on reducing genome complexity with methylation-sensitive restriction enzymes (Res). By using methylation-sensitive Res, repetitive regions of genomes can be avoided and lower copy regions targeted with two to three fold higher efficiency (Robert J. Elshire et al., 2011). GBS is the latest application of next-generation sequencing protocols for the purpose of discovering and genotyping SNPs in a variety of crop species and populations. (Jennifer Spindel et al., 2013). Although GBS is fairly straightforward for small genomes, target enrichment or reduction of genome complexity must be employed to ensure sufficient overlap in sequence coverage for species with large genomes. Enrichment strategies including long

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range PCR amplification of specific genomic regions, use of molecular inversion probes, and various DNA hybridization/ sequence capture methods (Lira Mamanova et al., 2010) are time-consuming, technologically challenging, and can be cost-prohibitive for assaying large number of samples. Reducing genome complexity with restriction enzymes(Res), however, is easy, quick, extremely specific, highly reproducible, and may reach important regionsof the genome that are inaccessible to sequence capture approaches. By choosing appropriate Res, repetitive regions of genomes can be avoided and lower copy regions can be targeted with two to three fold higher efficiency (Gore MA et al., 2007 and 2009), which tremendously simplifies computationally challenging alignment problems in species with high levels of genetic diversity(Robert J. Elshire et al., 2011).

QTL Analysis

A quantitiative trait is traditionally defined as a trait with a continuous distribution, in contrast to a discrete distribution. Many agronomic traits are controlled by multiple genomic regions known as QTLs. Continuous phenotypic variation observed for many important traits in agriculture is caused by the segregation of independent polygenes with small effects (Paterson et al. 1990). The genes responsible for trait variation, called QTLs, were identified by their linkage to previously mapped quantitative genetic markers (Dudley et al. 1993). With the advent of molecular markers, like RFLP, AFLP, RAPD, SSR and SNP, together with the convenience of the advanced analytical techniques, the molecular study of quantitative traits becomes facility in many plnat species (Wang et al. 1999). QTL mapping involves construction of genomic maps and searching for a relationship between traits and polymorphic markers.

Mungbean is an important pulse crop in South-East Asia, South Asia. The production area of pulses is decreasing in treid in South and South-East Asia due to increase in cultivation of cereals and vegetable crops after 1970s (FAO, 2011; Munir et al. 2012). To improve

mungbean cultivation, some of the QTLs have been positioned on the mungbean genetic linkage map using RFLP, RAPD, and SSR markers to identify a variety of the traits. These traits are seed weight (Humphry et al. 2005; Mei et al. 2009; Isemura et al. 2012), seed color (Lambrides et al. 2004), bruchid resistance (Mei et al. 2009), Cercospora leaf spot resistance (Chankaew et al. 2011), seed dormancy, pod dehiscence, and phenology-related traits (Isemura et al. 2012). For the trait of days to first flowering, four QTLs were mapped to LG2, GL4, LG6, and LG11 using 430 SSR and EST-SSR markers from a BC_1F_1 mapping population derived from the cross between wild and cultivated mungbean (JP211874 X JP229096) (Isemura et al, 2012).

Genetic linkage map of mungbean

A warm-season legume mungbean (*Vigna radiata*) is an important crop in developing countries of South, East, and Southeast Asia, mainly cultivated by small scale famers, as it attributes nutritional needs of protein, minerals, and vitamins, (Kang et al. 2014; Lambrides and Goodwin 2007). Besides the nutritional facts, mungbean has the ability to fix atmospheric nitrogen through root rhizobial symbiosis and convert it into a form that enriches soil fertility (Graham et al. 2003). Its economic importance across Asia has been high, resulting in a corresponding increase of production from 2.3 million tons to 30.1 million tons from 1985-2000 (AVRDC, 2009). Based on yield projections, the world's demand for mungbean is expected to rise in the future (Chauhan et al. 2010). Currently, the major leading producing countries are India, China, Myanmar, Pakistan, Thailand, and Vietnam (Nair et al. 2012). As mungbean has become one of the valuable commercial crops in Asian countries, its genomes have been sequenced recently, covering 80% of the estimate genome (Kang et al. 2014). Not only would it be used as a reference for genomics research in mungbean, but as well as it would ultimately accelerate future

breeding programs for development of improved cultivars.

Recently, a high-density genetic linkage of mungbean was constructed, consisting of 1,321 single nucleotide polymorphisms (SNP) markers developed through genotyping by sequencing (GBS) (Kang et al. 2014). Therefore, further extension of QTL analysis and identification of genomic regions containing yield-associated traits would hence enable potential yield increase.

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CHAPTER I

GBS-based map construction and QTL identification for major agronomic traits in mungbean

ABSTRACT

Mapping for major agronomic traits is important to assist breeding strategies for high yielding varieties of mungbean. The major agronomic traits are quantitatively inherited in mungbean (*Vigna radiata*) including flowering time, 100-seed weight, plant height, cercospora leaf spot resistance, lodging resistance; thus, the mapping of genomic regions conferring these traits is essential. In this chapter, a total of 190 F_6 recombinant inbred lines (RILs) developed from the cross of VC1973A (Seonhwanogdu) x VC2984 (Kyung-Ki Jaerae #5) were used to identify quantitative trait loci (QTLs) for plant height (PH), number of branches (NB), 100-seed weight (100-SW), lodging resistance (Ldge), and cercospora leaf spot resistance (CLS). A density genetic map of an F_6 RIL population was generated from 798 single nucleotide polymorphism (SNP) markers created by the use of genotyping by sequencing (GBS). These markers were well-distributed over 11 chromosome (Chr), covering 757.39 centiMorgans (cM) of mungbean genome with the average distance between adjacent markers of 0.95 cM. Based on Composite interval mapping (CIM), four QTLs were identified for 100-SW, two for PH, six for DFF and DF, two

for NB, one for Ldge, and two for CLS, of which one QTL mapped on Chr4 was associated with more than one agronomic traits including DFF, DF and PH. Thus, the identified traits can be valuable resouces that contribute to the biological control of the major QTLs in this mungbean RIL population.

INTRODUCTION

Mungbean (*Vigna radiata*) is one of the important legume crop in developing countries of South, East, and Southeast Asia, cultivated by small scale famers, because of its nutritional values of protein, minerals, and vitamins, (Kang et al. 2014; Lambrides and Goodwin 2007). Besides the nutritional facts, mungbean has the ability to fix atmospheric nitrogen through root rhizobial symbiosis and convert it into a form that enriches soil fertility (Graham et al. 2003). Its economic importance across Asia has been high, resulting in a corresponding increase of production from 2.3 million tons to 30.1 million tons from 1985-2000 (Shanmugasundaram, 2009). Based on yield projections, the world's demand for mungbean is expected to rise in the future (Chauhan et al. 2010). Currently, the major leading producing countries are India, China, Myanmar, Pakistan, Thailand, and Vietnam (Nair et al. 2012). As mungbean has become one of the economically commercial crops in Asian countries, its genomes have been sequenced recently, covering 80% of the estimate genome (Kang et al. 2014). Not only would it be used as a reference for genomics research in mungbean, but as well as it would ultimately accelerate future breeding programs

for development of improved cultivars.

The agronomic traits of plant height (PH), 100-seed weight (100-SW), number of branches (NB), lodging resistance (Ldge), and cercospora leaf spot resistance (CLS) are important traits to be studied to develop mungbean improved cultivars with superior performance and adaptation. Many agronomic traits are known to be controlled by multiple genomic regions containing quantitative trait loci (QTLs). According to previous studies, some of the QTLs have been positioned on the mungbean genetic linkage map using RFLP, RAPD, and SSR markers to identify a variety of the traits. These traits are seed weight (Humphry et al., 2005; Mei et al., 2009; Takehisa et al., 2012), seed color (Lambrides et al., 2004), bruchid resistance (Mei et al., 2009), Cercospora leaf spot resistance (Chankaew et al., 2011), seed dormancy, pod dehiscence, and phenology-related traits (Takehisa et al., 2012). For the trait of days to first flowering, four QTLs were mapped to LG2, GL4, LG6, and LG11 using 430 SSR and EST-SSR markers from a BC_1F_1 mapping population derived from the cross between wild and cultivated mungbean (JP211874 X JP229096) (Isemura et al, 2012). Recently, a high-density genetic linkage of mungbean was constructed, consisting of 1,321 single nucleotide

polymorphisms (SNP) markers developed through genotyping by sequencing (GBS) (Kang et al., 2014). Therefore, further extension of QTL analysis and identification of genomic regions containing yield-associated traits would hence enable potential yield increase.

The objectives of this study were to identify QTLs for 100-seed weight (100-SW), plant height (PH), number of branches (NB), lodging (Ldge) resistance and cercospora leaf spot (CLS) resistance and perform correlation analysis among these traits. We observed several genomic regions of QTLs underlying these agronomic traits and verified the correlation in this population.

MATERIAL AND METHODS

Plant population and agronomic traits

A total of 190 F6 RILs population derived from the cross between VC1973A (Seonhwanogdu) x VC2984 (Kyung-Ki Jaerae #5), was used to construct a genetic map and for QTL analysis of the agronomic traits. Two mungbean cultivars VC1973A (Seonhwanogdu), which was developed at the AVRDC-The World Vegetable Center in 1982, and the Korean landrace VC2984 (Kyung-Ki Jaerae #5) were used. The maternal parent of VC1973A has desirable agronomic characteristics such as disease resistance and high yield. Crosses were made during the summer 2011, and F_2 generation progeny were generated via selfing the offspring of the hybrids in a greenhouse. RILs were created from the F_2 population for four generations using a SSD method from winter 2011 to spring 2013, resulting in F_6 mapping population consisted of 190 RILs. The RILs and their parents were planted in the field at the experiment farm of Seoul National University in Suwon. Five agronomic traits were measured, such as 100-seed weight (100-SW), number of branched (NB), cercospora leaf spot resistance (CLS),

lodging resistance (Ldge), and plant height (PH). Three plants per line were selected and measured for PH and NB. For CLS, the severity of the symptom was scored from scale 1 to 5 where 1 = no symptom and 5 = ~75-100% symptom observed on leaves. Lodiging (Ldge) was also rated on a 1-5 scale: 1 = 100% erect and 5 = prostrate.

Single nucleotide polymorphism (SNP) markers development

SNP markers were used to identify allelic variations of different mungbean linkage groups at SNP loci. All of the SNP markers were developed by the following GBS methods. Genomic DNA was digested with a restriction enzyme called ApeKI. Digested DNA fragments were ligated to the P1 and P2 adapters at both primer sites and the end sites were repaired with addition of A. The P1 adapter contained a forward amplification primer site, an Illumina sequencing primer site and a barcode sequence. After ligation, the fragments were PCR amplified with P1 and P2 specific primers. The library was validated on the Agilent Technologies 2100 Bio-analyzer and the ABI StepOnePlus Real-Time PCR system. About 154.7G bases were generated for 190 samples without adapter sequence and low quality of reads. All

sequencing reads were aligned to the reference genome sequence using BOWTI2. Polymorphic loci against the reference sequence were selected and filtered by quality value must be greater than 20 and supported over 6 reads.

Statistical analysis

We constructed a mungbean genetic map from an F_6 population of 190 recombinant inbred lines with join map 4.0 software and the Kosambi mapping function was used to convert recombination frequencies to genetic distances. The segregation of each SNP markers in the 190 RILs was evaluated by a chi-square test. QTL positions of agronomic traits were identified in the 190 F_6 RIL populations with composite interval mapping using the software Q Gene 4.3.10 (Joehanes and Nelson, 2008). In order to determine the significance thresholds for QTL, a permutation test with 1000 replications was used each trait at $p = 0.05$ and $p = 0.01$ with a logarithm of odds (LOD) value for each QTL. Simple correlation coefficients among six agronomic traits within the RIL population were calculated from mean values using statistica 7.1 software (Statsoft, Inc.

2013).

RESULTS

Variation of agronomic traits

From the parents and RILs generated by SSD from a cross between VC1973A and VC2984, a high degree of phenotypic variation in response to agronomic traits was screened (Figure I-1). Frequency distributions of phenotypes for 100-SW, PH, and NB were ranged between 3.4 g and 6.2 g, 40 cm and 90, and 1 EA and 6.5 EA, respectively. In all cases, the maternal parent VC1973A had the higher values for all agronomic traits compared to the paternal parent VC2984, varying by 1.8 g for SW, 15 cm for PH and 2 EA for NB. The mean range of SW in the RILs was 4.98g, and for PH was 60.48cm, and for NB was 4.01EA (Fig. I-1). For all agronomic traits, the RIL population showed transgressive segregation, having the RILs distributed beyond the mean value as a substantial number of the lines exhibited earlier than the maternal VC1973A and later than the paternal VC2984.
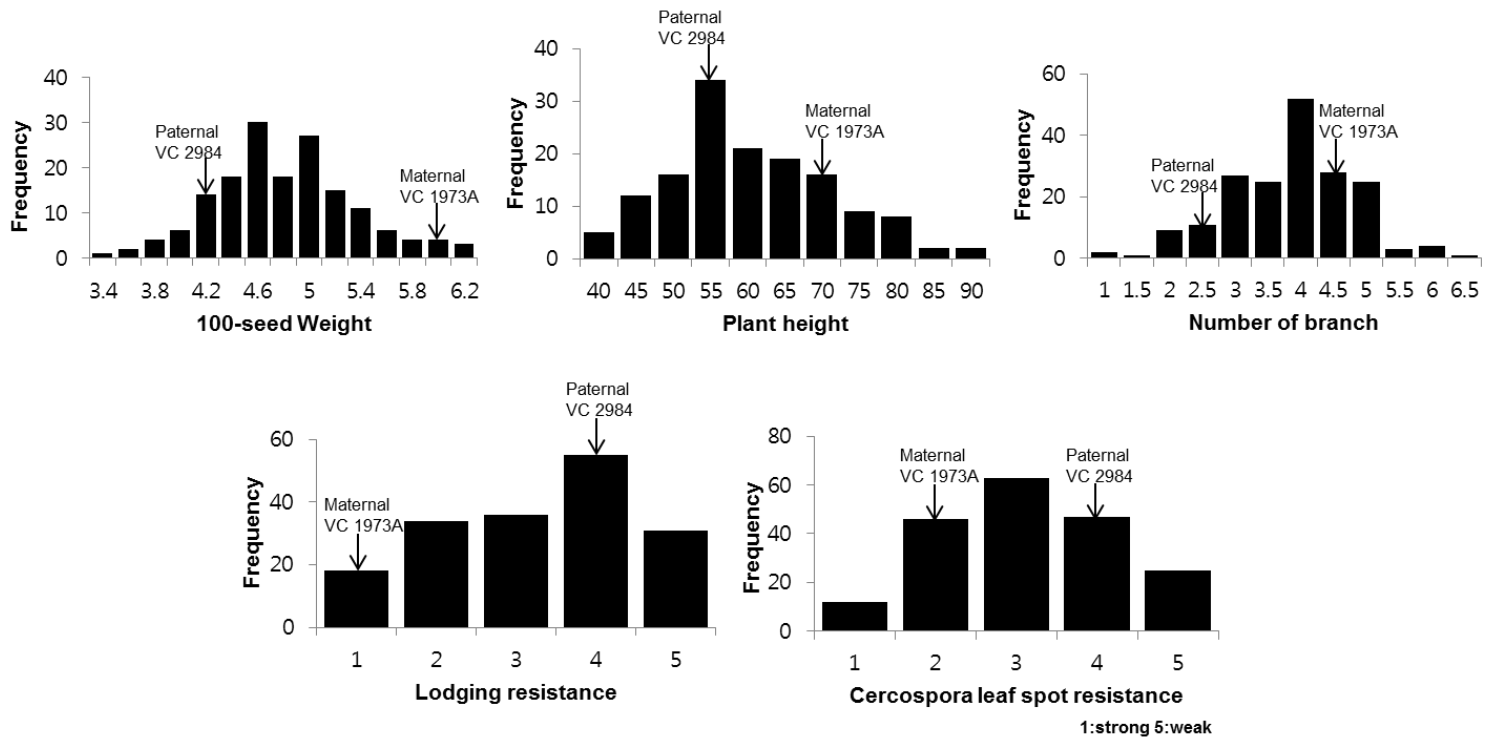
Figure I-1. Frequency distribution of agronomic traits for 190 RILs derived from VC1973A X VC 2984

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Table I-1 variation of agronomic traits for the parents and the RIL population

Traits	Parent		RIL population		h^2
	VC1973A	VC2984	Mean ± S.D	Range	
Days to first flowering	44	46	39.86 ± 2.39	34 ~ 49	92.1
Days to 50% flowering	45	50	41.48 ± 2.39	36 ~ 50	93.2
100-seed weight 2013 (g)	6.1	3.45	5.41 ± 7.62	3.4 ~ 6.2	91.7
100-seed weight 2014 (g)	6.19	4.31	4.98 ± 4.45	3.3 ~ 6.19	-
Plant height (cm)	70.4	55.25	60.48 ± 10.90	40 ~ 90	89.6
Number of branch (EA)	4.3	2.3	4.01 ± 0.96	1 ~ 6.5	-
Cercospora leaf spot resistance	2	5	3.16 ± 1.09	1 ~ 5	-
Lodging resistance	1	4	3.24 ± 1.24	1 ~ 5	-

Correlations among major agronomic traits

All of the agronomic traits, DF, 100-SW, PH, CLS and NB, were significant (P<0.05) in an RIL population. The result of correlation coefficient (r) analysis revealed to be positive, showing r > 0.52 for DF vs. PH. When significant at P<0.01, DF had a negative correlation with FYD while a postivie correlation occurred between PH. No correlations were detected for SW vs. DF and DF vs. CLS and NB in this study.

QTL analysis of agronomic traits

QTL mapping was conducted for agronomic traits using CIM. A total of two QTL peaks for cercospora leaf spot resistance, one for lodging resistance, two for plant height, two for number of branch were observed. To detect QTLs, a threshold value of the LOD scores determined by permutation tests and phenotypic effect (R^2) were obtained (Table I-2). For CLS, QTLs located in Chr 5 and Chr 11 were detected with a LOD score of 3.34 and 17.72 between flanking SNP markers   97_756706 ~ 17_3170881 and 15_2959889 ~ 15_2508102, respectively, accounted for   5.14 and 32.43 of the R^2. For PH, two QTLs were located on chromosome 3 and the other was located on chromosome 4. In addition, two QTLs for NB were distributed over chromosome 2 and 3. One QTL for Ldge was located on the chromosome 10, respectively. Among the QTLs detected in this study, the QTL for CLS and PH between 15_2450010 ~ 15_2508977 and 38_761680 ~ 38_1197095 accounted for a maximum value for 30.0% of total phenotypic variance. For 100-SW, four QTLs were identified in 2013 and four QTLs were detected 2014 (Table I-3). QTLs for 100-SW were distributed over chromosome 1, 3, 8 and 9. Three QTLs for 100-SW, *sw 1-1, sw 3-1* and *sw 8-1* were all detected in both 2013 and 2014.

Other QTLs for *sw 9-1* was detected in 2013 and *sw 9-2* was identified in 2014, indication that these QTLs expressed stably across environments.

Table I-2. QTLs for agronomic traits in the RIL population

Trait	Chromosome	Locus	SNP marker interval	Position (cM)	LOD	ADD	R^2
Cercospora leaf spot resistance	5	*CLS 5-1*	97_756706 ~ 17_3170881	57.37	3.34	0.25	5.14
	11	*CLS 11-1*	15_2959889 ~ 15_2508102	49.23	17.72	-0.63	32.43
Lodging resistance	10	*LD 10-1*	32_2548609 ~ 32_2193266	47.1	3.4	-0.46	8.3
Plant height	3	*PH 3-1*	5_819939 ~ 138_608043	60.5	3.11	2.74	6.01
	4	*PH 4-1*	38_696318 ~ 38_688531	14.84	12.89	6.68	30.02
Number of branch	2	*NB 2-1*	135_850714 ~ 49_325096	54.5	2.67	-0.23	5.8
	3	*NB 3-1*	109_1259968 ~ 5_2360306	48.39	2.56	0.24	5.6

Table 3. QTLs for 100-seed weight in the RIL population

Trait	Chromosome	Locus	SNP marker interval	Position (cM)	LOD	ADD	R^2
2013 (100-seed weight)	1	*SW 1-1*	96_1187948 ~ 96_965903	21.04	5.34	-0.19	11.1
	3	*SW 3-1*	336_136701 ~ 76_928050	80.47	5.73	-0.19	13.2
	8	*SW 8-1*	85_1354789 ~ 181_680878	46.92	3.98	-0.17	10.5
	9	*SW 9-1*	25_2188690 ~ 25_915297	15.22	2.83	-0.13	5.9
2014 (100-seed weight)	1	*SW 1-1*	96_1096320 ~ 242_192748	20.47	4.77	-0.18	10.1
	3	*SW 3-1*	244_273344 ~ 131_966862	74.47	5.51	-0.16	10.4
	8	*SW 8-1*	85_1354789 ~ 181_680878	44.92	4.75	-0.18	9.8
	9	*SW 9-2*	217_243771 ~ 25_2517363	8.24	3.8	-0.15	9.4

DISCUSSION

Previous linkage mapping constructed by 430 SSR and EST-SSR makers has identified that a region associated 100-seed weight trait on mungbean was located on LG1, LG2, LG3, LG7, LG8 and LG11 (Isemura et al. 2012). Recently, a high-density genetic linkage of mungbean was constructed, consisting of 1,321 single nucleotide polymorphisms (SNP) markers developed through genotyping by sequencing (GBS) (Kang et al. 2014). Therefore, further extension of QTL analysis and identification of genomic regions containing yield-associated traits would hence enable potential yield increase. In this paper, we identified QTL regions of agronomic traits using a mungbean genetic map consisting of 798 SNP markers, allowing more efficient on identification of QTLs conferring to agronomic traits. Our result suggesting that the use of high density markers narrowed down the putative QLT region associated with agronomic traits in mungbean.

For 100-SW, two QTLs with *sw 2-1* and *sw 10-1* at the marker interval 26_2674269 ~ 26_2956758 and 217_158264 ~ 217_235147 on chromosome 2 and 10 was freshly identified in this study. But the other QTLs on chromosome 1, 7 and 8 were mapped near the QTLs

reported by (Isemura et al. 2012). In mungbean, its seed yield is positively correlated with number of flowers, percentage of pod set, number of seeds per pod, and seed weight (Mondal 2007). Even with the breeding efforts in mungbean for efficient yield improvement, the seed production still remains low and has been fluctuating (Mondal et al. 2012).

In conclusion, chromosome 1, 2, 3, 4, 5, 8, 9, 10 and 11 had the QTLs for CLS, Ldge, PH, NB and SW. It was possible to relate one QTL to more than one trait (Zhang et al. 2004). Several QTLs of various traits were mapped on the same loci. In this study, two QTLs, one for PH and another for DFF, were located at the same marker interval 38_696318 ~ 38_688531 on chromosome 4. These QTLs were inferred as being related to two or more agronomic traits. The QTLs mapped at same marker loci on chromosome 4 were likely related more agronomic traits in this mungbean population.

CHAPTER II

QTL for flowering traits in mungbean was associated with soybean flowering genes

ABSTRACT

As mungbean has irregular bloom dates that resulted in asynchronous maternity, identifying and understanding genes involved in the control of flowering time are essential for higher yields. However, not much of studies have been conducted for mapping QTL associated with flowering time. The objectives were to investigate quantitative trait loci (QTLs) for first flower and flowering time in mungbean, and to examine syntenic relationships of the detected QTLs with soybean using comparative analysis. A F_6 recombinant inbred line (RIL) population from a cross between two mungbean Korean cultivars developed from single seed decent (SSD) was used to evaluate the phenotypic data for days to first flowering (DFF) and days to 50% flowering (DF). The QTL on chromosome (Chr) 4 was detected to be a major QTL for both DFF and DF with a LOD score of 16.64 and 20.53, accounting for a 26.96 and 33.14% of phenotypic variance, respectively. Using a mungbean genetic map consisted of 798 SNPs, three QTLs for DFF on Chrs 4, 6 and 8, as well as three QTLs for DF on Chrs 4, 5 and 8 were identified, where both DFF and DF QTLs were located on Chr 4 and 8. Comparative analysis of its syntenic region in soybean revealed the co-

localization of QTLs for first flower with *E3* and *E4* flowering genes encoding phytochrome A (phyA). Identification of QTLs and genes underlying DFF and DF will facilitate breeding programs significantly and hence increasing yields in mungbean by controlling the time of flowering.

INTRODUCTION

A warm-season legume mungbean (*Vigna radiata*) is an important crop in developing countries of South, East, and Southeast Asia, mainly cultivated by small scale famers, as it attributes nutritional needs of protein, minerals, and vitamins, (Kang et al. 2014; Lambrides and Goodwin 2007). Besides the nutritional facts, mungbean has the ability to fix atmospheric nitrogen through root rhizobial symbiosis and convert it into a form that enriches soil fertility (Graham et al. 2003). Its economic importance across Asia has been high, resulting in a corresponding increase of production from 2.3 million tons to 30.1 million tons from 1985-2000 (Shanmugasundaram, 2009). Based on yield projections, the world's demand for mungbean is expected to rise in the future (Chauhan et al. 2010). Currently, the major leading producing countries are India, China, Myanmar, Pakistan, Thailand, and Vietnam (Nair et al. 2012). As mungbean has become one of the valuable commercial crops in Asian countries, its genomes have been sequenced recently, covering 80% of the estimate genome (Kang et al. 2014). Not only would it be used as a reference for genomics research in mungbean, but as well as it would ultimately accelerate future

breeding programs for development of improved cultivars.

Flowering is the transition from vegetative to reproductive growth that is a critical stage in determining potential yield for crop plants, and successful reproduction occurs when flowers bloom synchronously at the optimal time (Simpson and Dean 2002). In mungbean, its seed yield is positively correlated with number of flowers, percentage of pod set, number of seeds per pod, and seed size (Mondal 2007). Even with the breeding efforts in mungbean for efficient yield improvement, the seed production still remains low and has been fluctuating (Mondal et al. 2012) because of the occurrence of flower abscission and immature pods (Mondal et al. 2011). Like most legumes, despite many flower numbers are produced, only a few of them develops into pods (Fakir et al. 2011; Mondal et al. 2007); about 60-92% flowers are shed in soybean (*Glycine max*) (Nahar and Ikeda 2002; Saitoh et al. 2004) and 70-90% flowers in mungbean (*Vigna radiata*) (Kumari and Verma, 1983; Mondal et al. 2011). Abscission of flowers reportedly takes place in most of later-formed flowers (Isobe et al. 1995; Kuroda et al. 1998; Mondal et al. 2007), indicating that prevention of late flowering would increase the yield. In fact, a higher number of pod yield has resulted in the bloom within two to three weeks after first

flowering (Mondal and Hamid 1998; Mondal et al. 2011b). Thus, for high yielding mungbean, it is of great interest to investigate the genetics underlying flowering time and its associated traits, allowing breeders to develop novel varieties with altered flowering times.

In legume crops, knowledge as to what genes play a role in regulating flowering time and their mechanisms was limited in the past. However, because of the availability of advanced genome sequence technologies, a computational approach has been performed to investigate such genes with ease using those sequenced legume species, including *Lotus japonicas*, *Medicago truncatula*, pigeonpea, chickpea, soybean and mungbean (Jain et al. 2013; Kang et al. 2014; Sato et al. 2008; Schmutz et al. 2010; Young et al. 2011; Varshney et al. 2012). Recently, putative flowering genes have been identified on a genome-wide level using homologs of a set of 207 *Arabidopsis thaliana* genes involved in the control of lowering time in three legume species, *L. Japonicus*, *M. truncatula*, and soybean (Kim et al. 2013). Similar numbers of the *A. thaliana* flowering-related genes were counted in *L. Japonicus* and *M. truncatula*, 96 and 98 genes, respectively, whereas 304 genes with a three times higher abundance were detected in soybean. This could be explained by two different genome duplication

events since *L. Japonicus* and *M. truncatula* have underwent a single round of ancient duplication in contrast to soybean with two rounds of ancient and recent duplications (Ono et al., 2010; Young et al., 2011). In addition, the function of the putative soybean flowering-related genes was investigated by co-localization of quantitative trait loci (QTL) analysis. The QTL regions of chromosome (Chr) 6, 7, and 19 were detected to be highly associated with flowering time-associated traits including pod maturity, first flower, reproductive period, and flowering time, containing mainly those genes homologous to *SVP* and *CRY1*, *AGL*, *AP2* /*TOE3*/*SMZ*, *FT*/*TSF*/*TFL1*, *PHY A*/*B*/*C*, *RF12* and *LHY*/*CCA1* (Kim et al., 2012).

To improve mungbean cultivation, some of the QTLs have been positioned on the mungbean genetic linkage map using RFLP, RAPD, and SSR markers to identify a variety of the traits. These traits are seed weight (Humphry et al., 2005; Mei et al., 2009; Takehisa et al., 2012), seed color (Lambrides et al., 2004), bruchid resistance (Mei et al., 2009), Cercospora leaf spot resistance (Chankaew et al., 2011), seed dormancy, pod dehiscence, and phenology-related traits (Takehisa et al., 2012). For the trait of days to first flowering, four QTLs were mapped to LG2, GL4, LG6, and LG11 using 430 SSR and EST-SSR

markers from a BC_1F_1 mapping population derived from the cross between wild and cultivated mungbean (JP211874 X JP229096) (Isemura et al, 2012). Recently, a high-density genetic linkage of mungbean was constructed, consisting of 1,321 single nucleotide polymorphisms (SNP) markers developed through genotyping by sequencing (GBS) (Kang et al., 2014). Therefore, further extension of QTL analysis and identification of genomic regions containing yield-associated traits would hence enable potential yield increase.

In this study, we identified QTLs conferring days to first flowering (DFF) and days to 50% flowering (DF) using the 190 F_6 recombinant inbred line (RILs) populations with three repetitions. The genomic region of QTLs underlying both DF and DFF traits with significant LOD scores was deployed in the soybean genome for comparative analysis to identify synteny relationships between mungbean and soybean, whether this region co-localized with the soybean flowering QTL regions.

MATERIALS AND METHODS

Plant population

To develop a RIL population for genetic mapping and QTL analysis, two mungbean cultivars VC1973A (Seonhwanogdu), which was developed at the AVRDC-The World Vegetable Center in 1982, and the Korean landrace VC2984 (Kyung-Ki Jaerae #5) were used. The maternal parent of VC1973A has desirable agronomic characteristics such as disease resistance and high yield. Crosses were made during the summer 2011, and F_2 generation progeny were generated via selfing the offspring of the hybrids in a greenhouse. RILs were created from the F_2 population for four generations using a SSD method from winter 2011 to spring 2013, resulting in F_6 mapping population consisted of 190 RILs.

Phenotypic evaluation

In May 2013, the generated F_6 RIL population comprised 190 lines were planted initially in the field at the Seoul National University experimental farm in Suwon, Korea, for the evaluation of DF and DFF, as well as for

the isolation of DNA. In addition, two repetitions of the RILs were conducted. Each line was planted in one row, where one seed per planting hole was sown with nine seeds per row. Optimization of planting density and cultivation practices was performed depending on the local conditions. Both of DF and DFF were evaluated by measuring the number of days from germination to opening of the first flower of a plant in each line, and to opening of half of the plants in each line came into bloom, respectively.

Genetic map and QTL analysis

Our high-density mungbean genetic map was constructed in a previous study from the F_6 RIL population developed by a cross between VC1973A and VC2984 through genotyping by sequencing (GBS) (Kang et al., 2014). To explain briefly about the genetic map, it consisted of 798 SNPs covering all 11 linkage groups of mungbean. These SNPs spanned 757.39 centiMorgans (cM) of the genome with an average distance between markers of 0.95 cM (Figure II-1). The distribution of the mapped markers among the mungbean chromosomes was not uniform, with the lowest number of markers of

24 on Chr 9 and the highest of 133 on Chr 1. The genetic linkage map with respect to chromosome length ranged from 44.93 cM to 107.89 cM located on Chr 7 and Chr 4, respectively.

The Kosambi map function (Kosambi, 1944) was used to convert recombination frequencies into genetic distances between markers, and the segregation of each SNP markers was evaluated by a chi-square test at the $P < 0.05$ level of significance. Putative QTL positions of DF and DFF were identified in the 190 F_6 RIL populations with composite interval mapping using the software Q Gene 4.3.10 (Joehanes and Nelson, 2008). In order to determine the significance thresholds for QTL, a permutation test with 1000 replications was used with a logarithm of odds (LOD) value for each QTL ranged from 2.34 to 3.06 with 99% confidence.
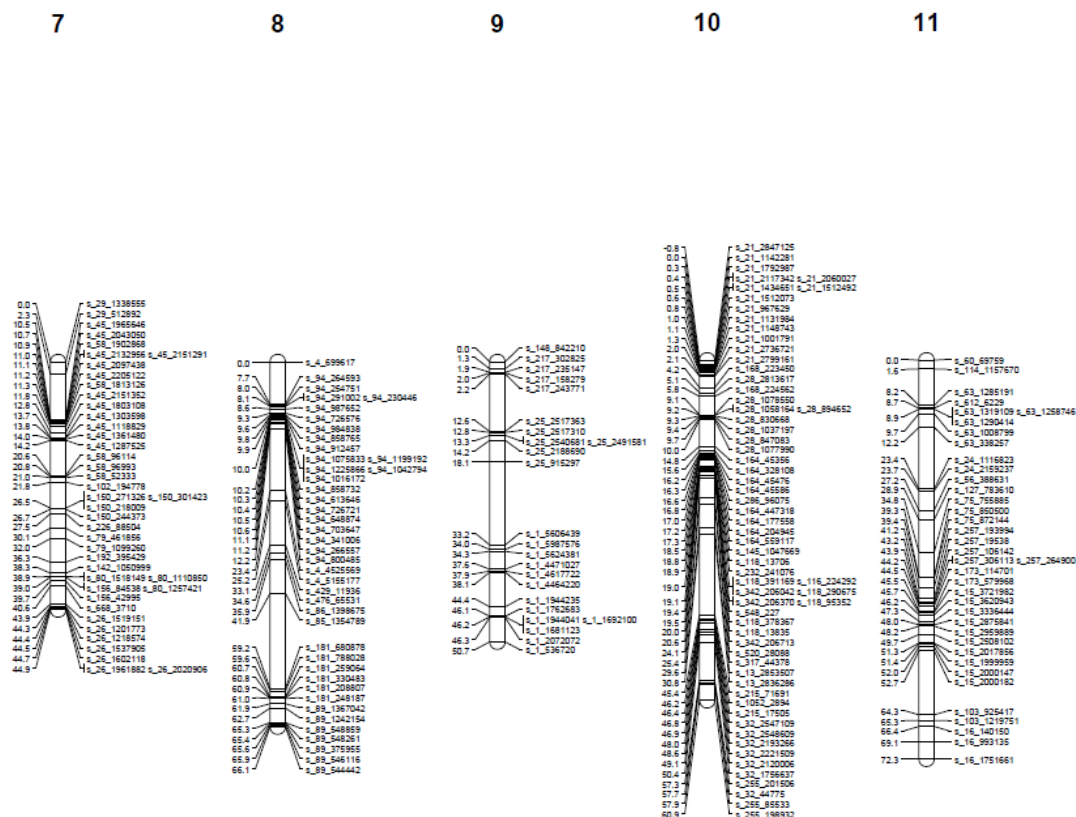
Figure II-1. Genetic linkage map of mungbean based on GBS

Comparative analysis of the QTL regions for DFF and DF

A comparative genomic analysis between mungbean and soybean of the putative QTL regions conferring DFF and DF identified by the composite interval mapping (CIM). Genomic regions around the putative DFF and DF QTLs within the 1 Mbp up- and down-stream flanked by closest SNP markers were used as synteny blocks to investigate co-localization of flowering time (FT) and first flower (Fflr) QTL in the soybean genome. Sequences in all observed putative QTL regions were downloaded from http://plantgenomics.snu.ac.kr/. All of the identified flowering time associated QTLs, including flowering time, first flower, pod maturity, beginning of pod, reproductive period and seed filling period (Kim et al. 2012) were retrieved from the Soybase Web site (*http://www.soybase.org/dlpages/index.php*). Based on the macrosyntenic comparison between mungbean and soybean using MCScanX with default parameters searches (Wang et al. 2012), the co-localized QTLs were positioned on the mungbean genome.

Analysis of whole genome duplication (WGD)

A homology search was conducted for the mungbean QTL region

affecting both DF and DFF between soybean and mungbean. The mungbean protein sequences were compared to within and among species using BLASTP searches with a threshold E-value of $1e^{-10}$ and parsed out top 5 hits. We calculated synonymous (Ks) values of all putative homologs in a pairwise manner using the perl script, add_ka_and_ks_to_collinearity.pl, from MCScanX package.

RESULTS

Phenotype data analysis

From the parents and RILs generated by SSD from a cross between VC1973A and VC2984, a high degree of phenotypic variation in response to DFF and DF was screened (Figure II-2). Frequency distributions of phenotypes for DFF and DF were ranged between 34 and 49 days and between 36 days and 50 days, respectively. In all cases, the maternal parent VC1973A had the earlier DFF and DF dates compared to the paternal parent VC2984, varied by up to 4 and 5 days, i.e., from 39 to 43 days for DFF and from 40 to 45 days for DF. The mean range of DFF in the RILs was 40 days, and for DF was 41 days (Figure II-2). For both DFF and DF, the RIL population showed transgressive segregation, having the RILs distributed beyond the mean value as a substantial number of the lines exhibited earlier than the maternal VC1973A and later than the paternal VC2984. Moreover, the frequency showed a near normal distribution across the DFF and DF, in which these quantitative traits are controlled by multiple genes. More than half of the plants in VC1973A and VC2984 were in bloom after two days of the appearance of first flowering. Frequency

50

distributions of DFF and DF showed similar patterns of increase and decrease as the number of days increase. The frequencies of DFF and DF increased until 40 days but significantly decreased during days after 41 days. Interestingly, a mild increase was observed at 42 days for both DFF and DF (Figure II-2).
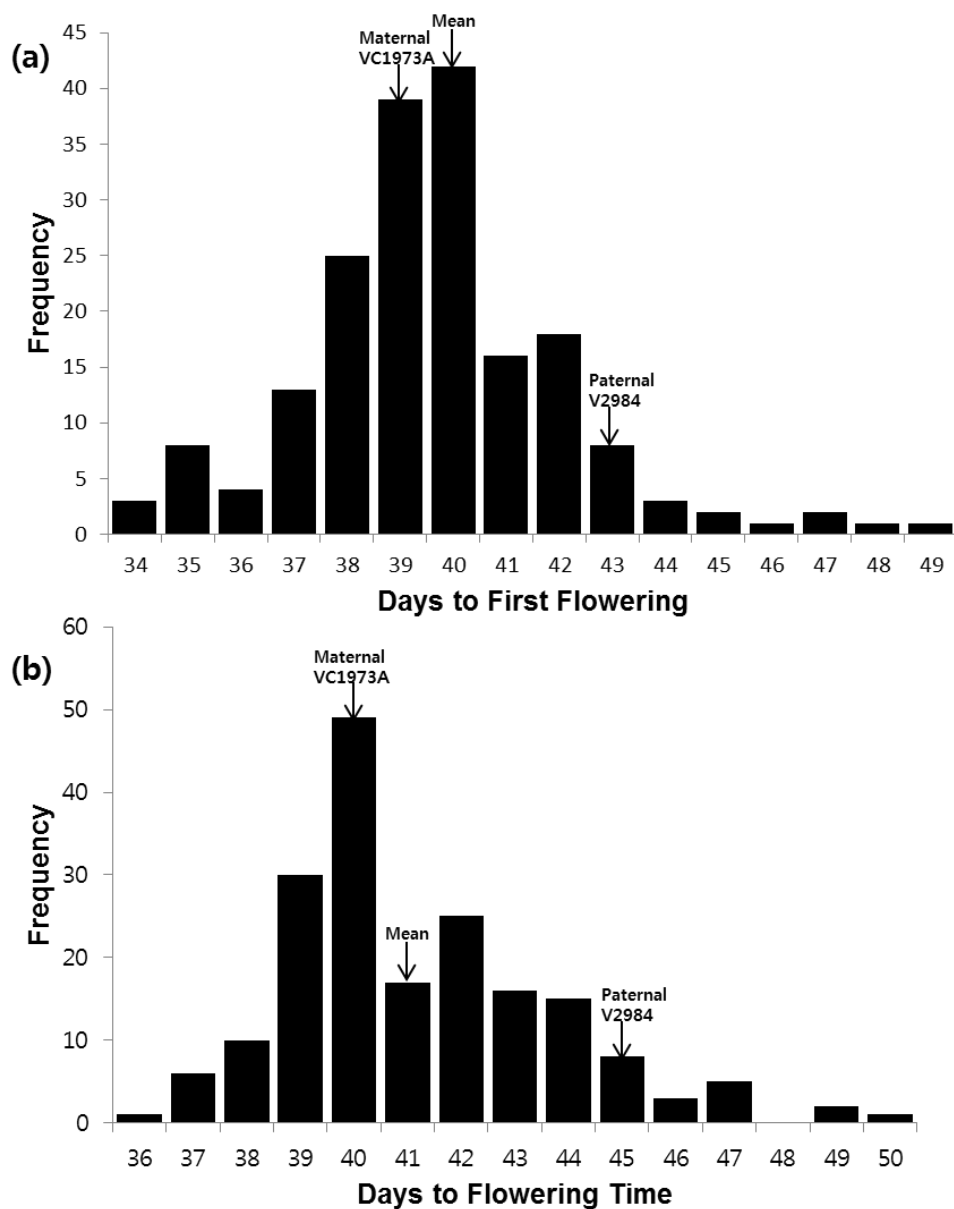
Figure II-2. Frequency distribution of days to first flowering and days to flowering time for 190 RILs derived from VC1973A X VC 2984

QTL analysis of DFF and DF

QTL mapping was conducted for DFF and DF using CIM. A total of two QTL peaks for DFF and three QTL peaks for DF were observed, of which one QTL peak on Chr 4 and 8 were corresponding to both DFF and DF QTLs (Figure II-2). To detect QTLs, a threshold value of the LOD scores determined by permutation tests and phenotypic effect (R^2) were obtained (Table II-1). The positions for which LOD peaks were above the threshold (LOD > 2.5) were identified as the QTLs. For DFF, QTLs located in Chr 3, Chr6 and Chr 8 were detected with a LOD score of 16.64, 2.73 and 4.13 between flanking SNP markers   38_696318 ~ 38_688531, 172_168481 ~ 110_239083 and 429_11936 ~ 476_65531, respectively, accounted for   26.96, 3.49 and 5.3 of the R^2. The QTL identified on Chr 4 displayed a positive additive effect, and the latter had a negative additive effect. Three QTLs in Chr 4, Chr 5, and Chr 8 were identified that affected DF, accounting 33.14%, 5.8%, and 4.6% of the R^2, located near the SNP marker intervals including 38_696318 ~ 38_688531, 98_940230 ~ 98_469646, and 429_11936 ~ 476_65531. The QTL mapped in Chr 4 had the highest LOD score (20.53) compared to the other two QTLs of Chr 5 and Chr8 (4.9 and 3.96,

respectively). Of the three QTLs, two located in Chr 3 and Chr 5 had positive additive effects, whereas one QTL in Chr 8 had a positive additive effect (Table II-1). Interestingly, the QTLs that were detected for both DFF and DF were co-located on Chr 4 and 8, in the region between the marker intervals of 38_696318 ~ 38_688531 and 429_11936 ~ 476_65531 and had the highest phenotypic effects, indicating a significant association between DFF and DF.

Table II-1. QTLs for days to days to first flowering and days to 50% flowering in the RIL population

Trait	Chromosome	Locus	SNP marker interval	Position (cM)	LOD	ADD	R^2
Days to First flowering	4	Fflr 4-1	38_696318 ~ 38_688531	14.84	16.64	1.38	26.96
	6	Fflr 6-1	172_168481 ~ 110_239083	62.66	2.73	-0.51	3.49
	8	Fflr 8-1	429_11936 ~ 476_65531	34.61	4.1	-0.61	5.3
Days to Flowering time	4	FT 4-1	38_696318 ~ 38_688531	14.84	20.53	1.6	33.14
	5	FT 5-1	98_940230 ~ s_98_469646	38.03	4.9	0.68	5.8
	8	FT8-1	429_11936 ~ 476_65531	34.61	3.96	-0.6	4.6

Chr 4

Chr 5

Chr 6

Chr 8
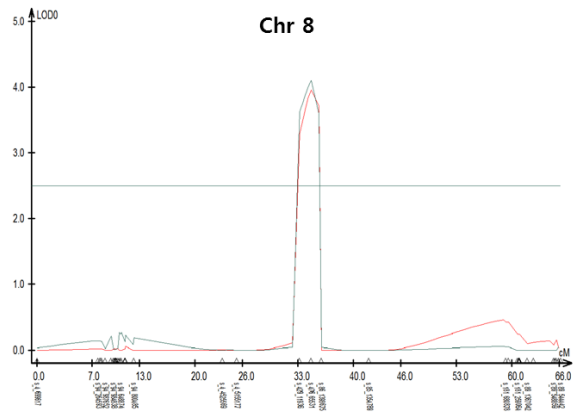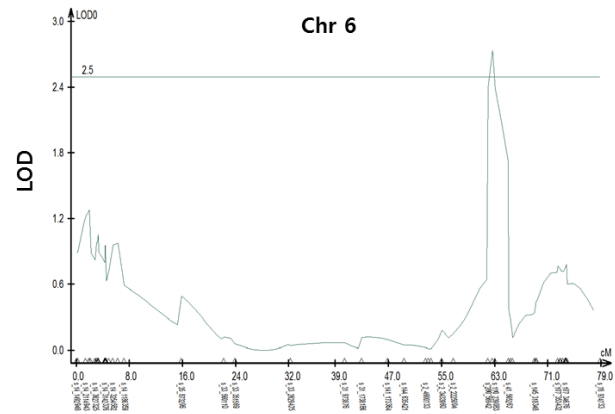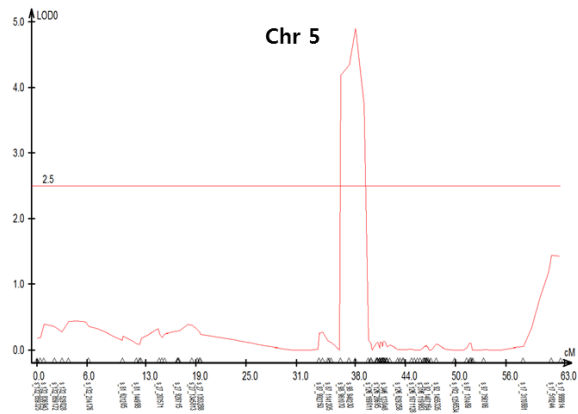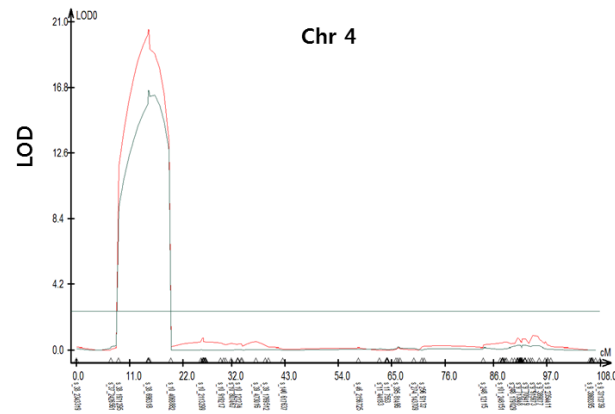
Days to first flowering
Days to flowering time

56

Figure II-3. Identifications of QTLs conferred Fflr and FT on chromosome 4, 5, 6 and 8. Positions of QTLs identified with red bar on each chromosome. The purple lines represent days to first flowering and green lines represent days to flowering time

Comparative analysis of QTL region for DFF and DF

Using genomic regions of the intervals containing the five QTLs conferring DFF and DF including 38_696318 ~ 38_688531, 172_168481 ~ 110_239083, 429_11936 ~ 476_65531, and 98_940230 ~ 98_469646, synteny relationships were revealed by comparative analysis between *G. max* and *V. radiata*. A few QTLs associated with flowering-related trait were co-localized on Chr 11 and Chr 19 in soybean, and moreover, Chr 3 that influenced DFF and DF in mungbean was co-localized with soybean QTL region for first flower (Fflr). The mungbean putative QTL of DF between 98_940230 ~ 98_469646 was associated with the trait controlling flower number (Flr_num) on Chr 11 in the soybean genome.

An alignment of chromosome Vr04 underlying both DFF and DF in *V. radiata* was carried out to detect synteny blocks. In *G. max*, there were two distinctive pairs of homeologous regions (Gm19 versus Gm03, and Gm20 versus Gm10), and one paralogous region (Vr08) in *V. radiata* (Figure II-4). Pairs of the synteny blocks between Gm19 and Gm03 showed a higher level of similarity to one another compared with another pair between Gm20 and Gm10, which indicated genome

duplication. In *G. max*, QTLs in homeologous syntenic regions of Gm19 and Gm20 were detected to have involved in first flower (Fflr16-4 and Fflr16-3, respectively). Moreover, the genes, *Glyma19g41210* and *Glyma20g22160*, encoded by the flowering *E3* and *E4* loci were identified on chromosomes Gm19 and Gm20, respectively. The duplicated copies of these genes were investigated, in which two pairs of paralogs were present in the soybean genome, *Glyma19g41210-Glyma03g38620* and *Glyma20g22160-Glyma10g28170*. The homologous gene of both *E3* and *E4* was detected on the putative mungbean flowering region Vr04, which was *Vradi04g07170* (Figure II-4). Scrutinizing of rates of synonymous-site changes (Ks) between these genes led to date the duplication event. The small Ks values of *Glyma19g41210-Glyma03g38620* (0.154) and *Glyma20g22160-Glyma10g28170* (0.117) indicated that they were produced by the recent polyploidy event, as compared to the higher Ks values observed between combinations of *Glyma03g38620- Glyma20g22160* (0.520) and *Glyma19g41210-Glyma10g28170* (0.516). The duplicates of *Glyma19g41210-Glyma10g28170* had a smaller Ks value, suggesting that these paralogs may be resulted from the ancient polyploidy event. For mungbean, a paralog of the putative flowering gene *Vradi03g07170*

was detected on Vr08 with a Ks value of 0.661, as mungbean has only
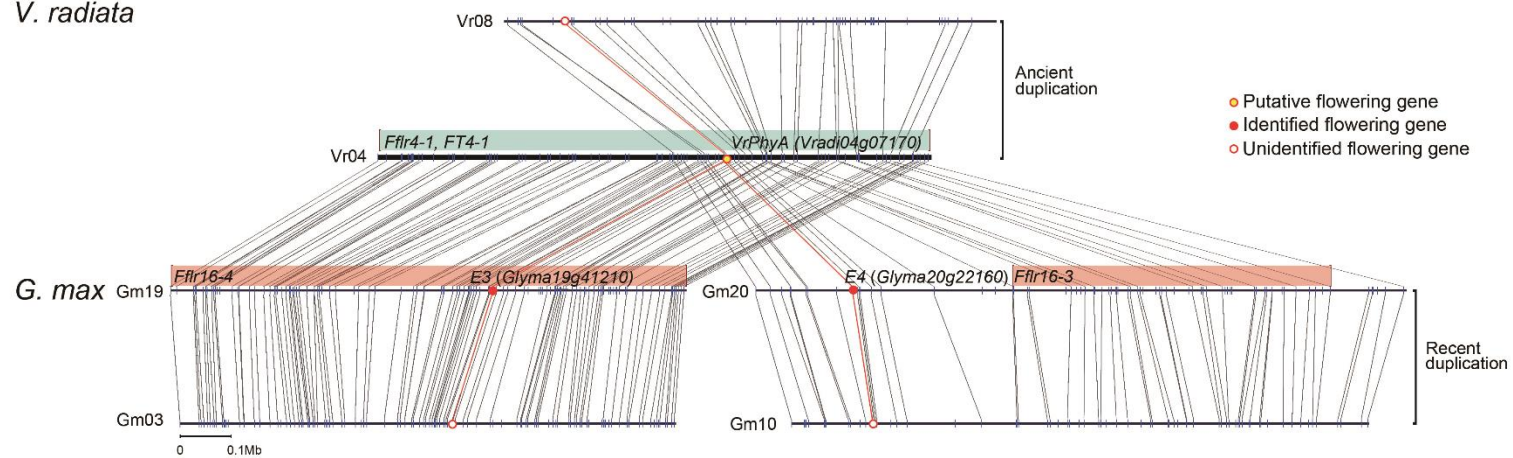
underwent one round ancient duplication.

Figure II-4. Alignment of homologous regions in mungbean on chromosome 3 and soybean on chromosome 10 and 20

Discussion

As there are many reports on the soybean QTLs and genes involved in flowering (N Yamanaka et al., 2001, Liu et al., 2011, Zhang et al., 2013), syntenic comparison of the region that was identified as a QTL of large effect on both DFF and DF near the SNP markers (38_696318 and 38_688531) on chr 4 was conducted between mungbean and soybean. There were four synteny regions detected on Gm19, Gm03, Gm20, and Gm10 in soybean, of which the regions of Gm19 and Gm20 were co-localized with soybean QTLs (*flr16-4* on Gm19 and *flr16-3* on Gm20). The detected QTL for DFF and DF was named as *Fflr3-1* and *FT3-1* (Figure II-4). Interestingly, out of known eight E loci responsible for flowering, *E1* and *E2* (Bernard 1971), *E3* (Buzzel 1973), *E4* (Buzzel and Voldeng 1983), *E5* (McBlain and Bernard 1987), *E6* (Bonato and Vello 1999), *E7* (Cober and Voldeng 2001), and *E8* (Cober et al. 2010), the two flowering genes *E3* and *E4* were located within the co-localized synteny regions of Gm19 and Gm20 (Figure II-4), consistent with locations identified in previous linkage mapping analyses: L (Gm19) for *E3* (Molnar et al. 2003) and I (Gm20) for *E4* (Abe et al. 2003; Molnar et

al. 2003).

Previous linkage mapping constructed by 430 SSR and EST-SSR makers has identified that a region associated flowering-related trait on mungbean was located on LG2, LG4, LG6 and LG11 (Isemura et al. 2012). Using the comparative analysis between mungbean and soybean, chromosomal locations of the flowering-related QTLs were detected by the nearest EST-SSR markers, of which four EST-SSR markers were tracked at Chr 1 for CEDG281 (position: 14044856-14045055), Chr 2 for CEDG245 (position: 7010514-7010642), Chr 3 for MBSSR015 (position: 9286515-9286670), and Chr 5 for CEDG026 (position: 2534969-2535115) (Kim et al. 2014). In this paper, we identified QTL regions of flowering traits using a mungbean genetic map consisting of 798 SNP markers, allowing more efficient on identification of genes and QTLs conferring to DFF and DF. Our putative flowering gene (*Vradi04g07170*) was found to be located near the flowering QTL region of MBSSR015 on chr 4, suggesting that the use of high density markers narrowed down the putative QLT region associated with DFF and DF in mungbean.

The mungbean putative flowering gene was identified that was

homologous to both *E3* and *E4*. The *E3* and *E4* have been reported to be involved in the sensitive to photoperiod under different light conditions by encoding the light receptor phytochrome A (phyA) proteins known as *GmPhyA3* (Watanabe et al., 2009) and *GmPhyA2* (Liu et al. 2008). To investigate the mungbean putative gene was closely related to other homologous genes in soybean, protein sequences of all six flowering genes including a pair of paralogous genes in mungbean (*Vradi04g07170-Vradi08g11890*) and two pairs of homologous genes in soybean (*Glyma03g38620- Glyma20g22160* and *Glyma19g41210-Glyma10g28170*) were used to construct a phylogenetic tree (Figure II-5). Phylogenic analysis showed that the putative mungbean flowering gene *Vradi04g07170* was more closely related to soybean flowering gene of *E3* rather than its homologous gene of *Vradi08g11890*. A protein alignment showed that they were highly conserved in all six genes, except that there was a missing region in the putative mungbean flowering gene *Vradi04g07170* (Figure II-5). Interestingly, this missing region identified comprising a PAS2 (Per-Arnt-Sim) domain in which it is known as a signal sensor that can monitor changes in light, redox potential, oxygen, and small ligands (Taylor and Zhulin 1999). The proper function of PAS domain is

important as it facilitates interaction with putative signaling partners (Krall and Reed 1999). Other features of phyA including a PAS1 domain and a histidine kinase domain were conserved in all six genes (Figure II-5). It was suggested that PAS2 is essential for dimerization of phyA (Kim et al. 2006). Thus, it is worth nothing that further research on this putative mungbean gene and the effect of PAS2 will help us to understand the mechanisms that control flowering time and could ultimately improve flowering synchronization in mungbean.

In conclusion, identification of QTLs underlying DFF and DF will provide opportunities for breeders to develop new cultivars with synchronous flowering time in order to increase yields. SNP markers that were closely linked to the DFF and DF QTLs will be very efficient for marker-assisted selection of flowering time in mungbean. Because *E3* and *E4* are known as phytochrome genes *GmPhyA3* and *GmPhyA2*, the detected mungbean flowering gene *Vradi04g07170* on Chr 3 was named as *VrPhA* (Figure II-4). This may be a key gene that involves the time of flowering; thus, more studies are needed to understand the mechanisms associated with flowering time in mungbean.
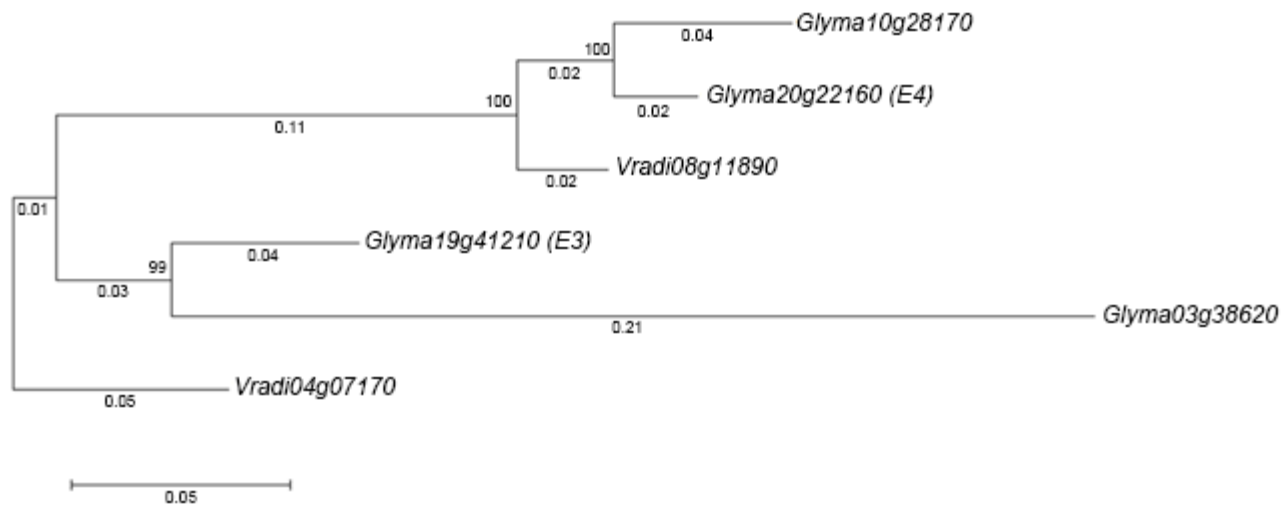
Figure II-4. Phylogenetic tree of the mungbean putative flowering gene, *Vradi04g07170*, along with its homologous

and paralogous genes obtained by protein sequences

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국문 초록

녹두는 주로 남아시아와 동남아시아에서 재배 및 생산되는 주요 작물로 숙주 나물의 재료 및 다양한 음식에 널리 이용되고 있다. 하지만 숙기가 일정하지 않아 재배 및 수확에 많은 어려움을 겪고 있다. 본 연구는 선화녹두 (VC 1973A)와 경기재래 (VC 2984) 를 모본과 부본으로 생성한 RIL 집단을 이용하여 녹두의 경장, 백립중, 분지수, 도복성, 개화시, 개화기, 반점병, 수확량 등의 주요 농업형질의 양적형질 유전자좌를 밝히고자 한다. GBS 방법을 사용하여 11개의 염색체에 걸쳐 798개의 SNP 마커를 개발하여 녹두 유전체 지도를 작성하였으며 수원 농장에서 수집한 주요 농업형질의 피노타입 자료를 바탕으로 각 농업형질들의 양적형질 유전자좌를 규명하였으며 이는 앞으로 녹두의 새로운 품종 개발에 유용하게 사용될 것이다.

주요어: 녹두, GBS, 양적형질 유전자좌, 농업형질

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