博 士 論 文 の 要 約

博士の専攻分野の名称：　博　士（農学）　　　　　　氏名　　竹島　亮馬

学 位 論 文 題 名

Gene network and molecular diversity of a *FLOWERING LOCUS T* orthologue, *FT5a*, in the control of flowering and stem termination in soybean

(ダイズの開花および伸育性の制御における *FLOWERING LOCUS T* オルソログ *FT5a* の遺伝子ネットワークと分子的多様性)

Hokkaido University, Graduate School of Agriculture

Division of Agrobiology and Bioresources　Doctor Course

Ryoma Takeshima

Summary

Chapter 1. Introduction

Time to flowering and maturation influences the productivity, adaptability, and quality of seed crops. Flowering time is determined by integration of signals from external stimuli (such as photoperiod and temperature) and internal conditions (such as plant age and the amount of gibberellic acid). In soybean, two *FLOWERING LOCUS T* (*FT*) orthologues, *FT2a* and *FT5a*, play a major role in floral induction. Their expression in response to different photoperiods is controlled by allelic combinations at the maturity loci *E1* to *E4* and *E9*, generating variation in flowering time among cultivars. However, the molecular basis of natural variation in time to flowering and maturation is still poorly understood. Identification of a responsible gene for a novel genetic variation(s) for flowering time is important for better understanding of the molecular mechanisms underlying natural variations of flowering time in soybean, and also for marker-assisted breeding for flowering time. Furthermore, investigation on gene networks in the control of flowering provides more valuable information for understanding the molecular mechanisms underlying wide adaptability of soybean. The aims of my thesis are to identify a responsible gene for a novel quantitative trait locus (QTL) for flowering time and determine the gene network which controls flowering and post-flowering development in soybean.

Chapter 2. Review on molecular mechanism of flowering and stem growth habit in soybean

As soybean is a short day (SD) plant, an essential trait for soybean to adapt to higher latitude environments is a reduced or absent inhibition of flowering by long day-lengths. In Chapter 2, I reviewed recent studies on molecular mechanisms of flowering under long day (LD) conditions and stem growth habit in soybean as five subchapters; 1) Molecular basis of soybean maturity loci – *E1* to *E10* and *J* – for flowering, 2) Functions and divergence of soybean orthologues of *FT,* 3) PHYA-E1 module, 4) Stem growth habit and 5) Molecular mechanisms for adaptation to long days at high latitudes.

Chapter 3. Identification of a responsible gene for a quantitative trait locus that promotes flowering under long days

To expand soybean cultivation areas toward northern regions of higher latitudes, we need to develop breeding lines with early flowering and maturity adapted to a short growing season, by accumulating early-maturity alleles at many loci. Eleven maturity loci – *E1* to *E10* and *J* – have been reported to be involved in the control of both times of flowering and maturity. Among then, *E1*, *E3*, *E4*, *E7*, and *E8* are related to photoperiod sensitivity, in particular, to artificially induced long LD of different light qualities. On the other hand, there are large numbers of unidentified QTLs in different linkage groups which have been reported to be involved in the control both time of flowering and maturity. The aim of this study was to identify a novel gene which controls the reduced photoperiod-sensitivity. In this study, I described the molecular dissection of a QTL for flowering time detected in two independent crosses between early-maturing soybean cultivars. Fine-mapping and subsequent sequencing and expression analyses had identified genetic variation of *FT5a* as a novel gene which controls reduce photoperiod-sensitivity under LD condition.

In this study, I used segregating populations of two early-maturing soybean crosses, Toyoharuka × 1532-1 (cross A), and a near-isogenic line (NIL) of Harosoy for *e3* allele (Harosoy-*e3*) × Jiagedaqi-02 (cross B). Using this two segregating populations, I performed 1) QTL analysis for flowering time, 2) fine-mapping to delimit the QTL, 3) sequence analysis of candidate gene (*FT5a*) for the QTL, 4) expression analysis of *FT5a* by NILs for the QTL. Furthermore, I confirmed 5) the geographical distribution of the novel genetic variation of *FT5a* by using single nucleotide polymorphisms (SNPs) calling data which were called from the re-sequencing data of 302 worldwide cultivated and wild soybean collections and 137 early-maturing landraces and improved cultivars developed in northeast China.

1) In QTL analysis, I detected the largest effect for flowering time on linkage group J (Chromosome 16) in both crosses. I tentatively designated this QTL as *qDTF-J*. 2) By fine-mapping, I delimited the *qDTF-J* to a genomic region of 107-kb that harbored nine genes; four genes for apyrase proteins, one gene each for tetratricopeptide repeat-like superfamily protein, an aquaporin-like superfamily protein, a transmembrane protein of unknown function with a DUF106 domain, an unannotated protein, and FT5a. Because *FT5a* is a functional *FT* ortholog and promotes flowering of soybean under non-inductive conditions when ectopically expressed, *FT5a* was the most likely candidate for *qDTF-J*. 3) In sequence analysis of *FT5a* genomic region, I detected 15 DNA polymorphisms between

parents with the early-flowering (*ef*) and late-flowering (*lf*) alleles in the promoter region, an intron, and the 3′ untranslated region. 4) Then I developed the NILs for *ef* and *lf* alleles to investigate the relationship between flowering time and expression profile of *FT5a*. Flowering was earlier in NILs for *ef* than in those for *lf*. The expression levels of *FT5a* were higher in NILs for the *ef* allele than in those for the *lf* allele. Those results suggested that the differences in flowering times between NILs were closely associated with the transcript abundances of *FT5a*. 5) To confirm the geographical distribution of *ef* allele, I carried out SNP calling from re-sequencing data of 439 soybean accessions and identified 22 haplotypes in the cultivated accessions and 7 haplotypes in the wild accessions. Among them, the *ef* allele was rarely observed (frequency, 4%), whereas *lf* allele was most common (86 %). Furthermore, all of the *ef* accessions were originated in northern Japan and northern China. Those results suggested that *ef* allele is a rare haplotype distinct from the haplotypes most common in the cultivated soybean population; it is also present in the wild soybean population.

Based on the results, I concluded that the most likely responsible gene of this QTL (*qDTF-J*) is *FT5a* and that the elevated expression level, which is most likely caused by the DNA polymorphisms of the genomic region, confers the early flowering phenotype of *ef* allele. Furthermore, the *ef* allele might have been introgressed from wild soybean during domestication and/or subsequent genetic diversification. The *ef* allele at *FT5a* may play an adaptive role at latitudes where early flowering is desirable.

Chapter 4. Gene network and functional divergence of *FLOWERING LOCUS T* orthologues

The contents of this chapter will be published in a scientific journal within the next five years. So, I cannot publish this chapter in this summary on the internet.

Chapter 5. General Discussion

In this study, I found that 1) the different transcript abundances of *FT5a* control time to flowering under LD conditions (Chapter 3), 2) *FT5a* and *FT2a* are most likely direct targets of floral repressor *E1*, and 3) *FT5a* controls post-flowering stem termination through a different pathway from *FT2a* (Chapter 4). Based on the results obtained, I discussed a role of the *ef* allele of *FT5a* with elevated transcript abundances in the control of flowering under LD conditions, and functions of *FT5a* and *FT2a* in the control of post-flowering stem termination. The findings obtained in this study may contribute not only to our better-understanding on molecular mechanisms of flowering and post-flowering reproductive growths but also to soybean breeding for improving the adaptability to LD conditions in high latitudes and the yield by ideal combinations of genes responsible for the stem growth habit.