学位論文の要旨

Abstract of Thesis

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学位論文題目 Title of Thesis（学位論文題目が英語の場合は和訳を付記）

PHYTOCLOCK 1 confer extreme early flowering in wheat
コムギの極早生性の原因遺伝子は *PHYTOCLOCK 1* である

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Wheat (*Triticum aestivum* L.) is one of the most important cereal crops as a staple food worldwide. However, the current production trend is not enough for the predicted demand in future due to rapid population growth and dietary changes (FAO, 2018). Flowering time is one of the vital agricultural traits of wheat that can contribute to meet the future demand through higher yield and stable production even under adverse global climate conditions. In Japan, early flowering cultivars, which enable harvesting before rainy season (from beginning of June to middle of July), have been developed to avoid pre-harvest sprouting, Fusarium head blight, as well as to create a space for double cropping system with rice in south-western part of Japan. Flowering time in wheat is a complex character which is controlled by vernalization requirement, photoperiodic response, and earliness *per se*. These three characters are known as major determinants of flowering time which enable the adaptation to diverse cultivation regions. Among these three, the earliness *per se* is controlled by many genes with smaller effect and is effective for fine tuning of flowering time. However, till today, the candidate region of earliness gene was not narrowed down with a single causal gene. Therefore, we conducted high density mapping of the earliness gene and validate a single causal gene for the earliness.

A Japanese breeding line ‘Chogokuwase’ was developed by selecting an extremely early segregant in a segregating population of ‘Minaminokomugi’ (a Japanese middle early cultivar) x ‘Geurumil’ (a Korean middle early cultivar), and headed earlier by three weeks compared to two parental lines whose heading date is around 10 April in normal year. To identify the causal gene(s) for its extreme earliness, recombinant inbred lines (RILs) were developed by crossing ‘Chogokuwase’ with another middle early cultivar ‘Kinuiroha’. Genetic analysis using 152 RILs showed a segregation of extremely early and middle early plants at a ratio of 1:3, indicating the involvement of two major genes (QTLs, Quantitative Trait Loci). The earliest 20 RILs and the latest 20 RILs were selected and allele frequency was compared between two bulks using 61 SSR markers covering 21 chromosomes of wheat. This analysis showed that two SSR markers *Xgwm340* and *Xbarc71* located on distal part of chromosomes 3BL and 3DL, respectively, linked with earliness QTLs. Since wheat is hexaploid, it was considered that earliness gene homolog was also located on distal part of chromosomes 3AL.

To identify causal genes (QTLs) by map-based study, we focused on the distal part of 3BL, since genome sequence necessary for SSR marker development was publicly available (Choulet et al., 2014). Mapping populations, in which only *QTL-3B* segregated, were derived from a cross of ‘Chogokuwase’ x ‘CKRIL54’ (a RIL carrying the late allele of *QTL-3B* and early alleles of *QTL-3A* and *QTL-3D*). In these populations, segregation of extremely early and middle early plants fitted to a ratio of 1:3, clearly confirming the involvement

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of a single gene. The earliness effect of *QTL-3B* was estimated to be around ten days in the field. In the initial mapping using SSR and DArT markers, *QTL-3B* was mapped to a 10.8cM region in the distal part of 3BL. Subsequently, additional SSR and SNP markers were developed based on the genome sequence of 3B, and this QTL region was narrowed down to a 445kb region through the screening of recombinants and their progeny tests. According to gene annotation in 'Chinese Spring' genome sequence, this region contained seven predicted genes including wheat *PHYTOCLOCK1* (*PCL1*), an orthologue of Arabidopsis *LUX/PCL1*. By RNA-seq analysis, it was confirmed that *PCL1-B1* (3B homolog of *PCL1*) was the only gene which transcribed intact mRNA. Our research group also analyzed sequence polymorphism of *PCL1* homologs, and reported that 'Chogokuwase' carries non-functional alleles for three homoeologs, while 'Kinuiroha' carries functional alleles of *PCL1-B1* and *PCL1-D1* (Mizuno et al, 2016). Thus, we finally concluded that *QTL-3B* is *PCL1-B1*.

To evaluate the effect of *PCL1* homoeologs on heading time, doubled haploid (DH) lines were developed from F1 hybrid of 'Chogokuwase' x 'Kinuiroha' by pollinating maize pollen. Since both parents carry early allele of *PCL1-A1*, 109 DHs and 152 RILs were classified into four groups by *PCL1-B1* and *PCL1-D1* genotype and heading time was compared among genotype groups. Compared to 'Kinuiroha' type RILs, carrying early allele of *PCL1-A1*, and late alleles of *PCL1-B1* and *PCL1-D1* (designated as ELL type), ELE and EEE types headed earlier by 6.2 and 16.3 days, respectively ($P<0.01$). Although EEL type headed earlier by 2.1, the difference was statistically insignificant. The same result was obtained in DH population. These results indicated that non-functional early allele of *PCL1-D1* accelerated heading in the presence of functional allele of *PCL1-B1*. In contrast, earliness effect of non-functional early allele of *PCL1-B1* was masked in the presence of functional allele of *PCL1-D1*, though non-functional allele of *PCL1-B1* was essential for extreme earliness. These results clearly indicated that early heading cultivar can be developed by introducing non-functional allele of *PCL1*. Moreover, heading time can be fine-tuned by the combination of *PCL1* alleles.

To know the geographical distribution and the origin of non-functional early allele of *PCL1* genes, a total of 274 local landraces and 105 improved cultivars covering various regions of the world were analyzed using allele specific markers. Among local landraces, only those from Japan have early allele of *PCL1-A1*, strongly indicating its origin in Japan. As to improved cultivars, 25 of 105 cultivars carry early allele of *PCL1-A1*, and the frequency is quite high in Japan. Good example was seen in cultivars on the pedigree of 'Kinuiroha' (ELL type), showing its early allele of *PCL1-A1* derived from local landrace, 'Eshima' and/or 'Shinrikikomugi'. Early allele of *PCL1-A1* in six cultivars outside Japan was also derived from a Japanese landrace, 'Akakomugi'. Therefore, it was concluded that SNP mutation of *PCL1-A1* occurred in Japan. In contrast, early allele of *PCL1-B1* distributed frequently and widely, and it was difficult to conclude the origin of its early allele. Pedigree analysis showed early allele of *PCL1-D1* of 'Geurumil' (EEE type) could be traced back to US old cultivar 'Early Blackhull' (LEE type). 'Early Blackhull' is known to be selected as an early off-type from 'Blackhull' (LEL type) in 1928. It was clearly indicated that early allele of *PCL1-D1* originated by SNP mutation in the field of 'Blackhull' in USA. In addition, the analysis of 'Extra Early Blackhull', selected as an early off-type from 'Early Blackhull' (LEE type) in 1951, showed that *PCL1-A1* gene region was deleted. Therefore, it is rational to conclude that 'Extra Early Blackhull' (EEE type) have null early allele of *PCL1-A1* which caused earlier heading compared with 'Early Blackhull'.

Non-functional early alleles of *PCL1* homoeologs showed different magnitude of effect on early heading, and thus the desirable combination of the alleles can be a better opportunity for fine tuning of flowering time in wheat. In addition, the diagnostic DNA markers established in this study can be utilized in practical breeding program which can contribute partly to fulfill the increasing food demand in future and to ensure the food security.