# CLONING OF FLOWERING GENES (*WJFLC* AND *WJFT*) IN WASABI (JAPANESE HORSERADISH) AND MONITORING OF FLOWERING RESPONSE WITH THEIR EXPRESSION

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**ABSTRACT**

Wasabi (*Wasabia japonica*) is a commercially important crop in Japan. We isolated a *FLC* ortholog (*WjFLC*) and *FT* ortholog (*WjFT*) from wasabi. Predicted amino acid sequence encoded by *WjFLC* and *WjFT* showed 89% and 85% identities with *FLC* and *FT* of Arabidopsis, respectively. The expression of *WjFLC* was high in October and reduced in November when flower buds are formed in wasabi. On the other hand, expression of *WjFT* was not detected in October and was slightly detected in November. Thereafter, *WjFT* was highly expressed later in February. We examined the best condition for initiation of flower bud formation under various artificial environments by monitoring the flowering response of wasabi using *WjFLC* and *WjFT*.

***Keywords:*** expression, flowering genes, flowering response, wasabi

## INTRODUCTION

Wasabi (*Wasabia japonica*), a member of the Brassicaceae, is a commercially important crop in Japan. Wasabi is cultivated in restricted regions such a cool mountain streams or fields of highland because wasabi grows at relatively low temperature and low light. Growth conditions such light and temperature, have been examined from a horticultural point of view in wasabi (Tanaka *et al*., 2008; Tanaka *et al*., 2009). However, the physiological characters in flowering are poorly understood. The expression pattern of flowering-related genes could be used as a monitor in order to determine the best condition for flowering under artificial environment.

Flowering is controlled by various environmental factors, including low temperature and photoperiod (Jung and Müller, 2009; Amasino and Michaels, 2010). The molecular mechanism of the flowering has been well examined in the winter-annual plant Arabidopsis. Vernalization induces flowering by suppressing the expression of *FLOWERING LOCUS C* (*FLC*), which is a key repressor of flowering (Michaels and Amasino, 1999; Sheldon *et al*., 2000). In Arabidopsis, *CONSTANS* (*CO*) is a key transcriptional regulator of the photoperiod pathway (Suárez-López *et al*., 2001). *CO* induces the expression of *FLOWERING LOCUS T* (*FT*), which is an integrator of floral-inductive signals from various genetic pathways (Helliwell *et al*., 2006). The expression of *FT* occurs in the leaf phloem, and FT protein moves to the meristem (Corbesier *et al*., 2007) and promotes floral transition through interaction with *FLOWERING LOCUS D(FD*) (Abe *et al*., 2005).

Under natural conditions, differentiation of flower buds in wasabi occurs between October and January and ceases in February. Inflorescence starts to grow in early spring and flowers open. After flowering, wasabi grows vegetatively until the following autumn. This perennial feature of flowering is distinct from that of annual and biennial Brassicaceae, which die after flowering. Although flowering is an interesting subject, there is little information on flowering genes in wasabi. In this study, we cloned *FLC* and *FT* orthologs from wasabi and examined their expression in the plants grown in the conditions both field and artificial environment.

## MATERIALS AND METHODS

Rosette leaves of wasabi plantswere harvested from a field at Nakatou in Matsumoto, Japan on July 2, October 9, November 18, 2009 and February 13, 2010 and used for isolating RNA. The average temperature in early July, early October, middle of November and middle of February was 22°C, 14°C, 6°C and 0°C, respectively. Total RNA was isolated by the standard phenol-SDS methods.

Cloning of *FLC* ortholog (*WjFLC*) and *FT* ortholog (*WjFT*) from wasabi carried out usingthe procedures described in previous papers (Kubo *et al*., 2011; 2012). Semi-quantitative RT-PCR was performed to detect the expression of *WjFLC* and *WjFT* (Kubo *et al*., 2011; 2012).

For heterologous expression of *WjFT* in Arabidopsis, the cDNA fragment between the start codon and the stop codon was amplified by PCR. The PCR product was inserted into a modified version of binary vector pIG121-Hm. The plasmid was transferred to *Agrobacterium tumefaciens* C58C1 by electroporation method. *Arabidopsis thaliana* Columbia was grown as described previously (Kubo *et al*., 1999). Arabidopsis was transformed by vacuum infiltration. Transformed plants were selected with an agar medium containing kanamycin and carbenicillin. T1 seedlings were grown in soil and used to count leaf numbers.

## RESULTS AND DISCUSSION

We isolated a *FLC* ortholog (*WjFLC*) from wasabi (DDBJ/GenBank/EMBL accession no. ADK92387). The coding region was 594 bp, which encoded a protein with 198 amino acid residues. The amino acid sequence predicted from the cDNA showed 89% identity with AtFLC (Fig. 1). MADS box domain signature was found between 3rd and 57th amino acids by PROSITE search. All the amino acid residues in the MADS box domain were conserved between AtFLC and its ortholog in wasabi.

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WjFLC   1 MGRKKLEIKRIENKSSRQVTFSKRRNGLIEKARQLSVLCDASVALLVVSASGKLYSFSSG
          ************************************************************
AtFLC   1 MGRKKLEIKRIENKSSRQVTFSKRRNGLIEKARQLSVLCDASVALLVVSASGKLYSFSSG

WjFLC  61 DNLVKILDRYGKQHVDDLKALDLQSKALNYGSHHELLEVVESKLVESNVDNVSVDFLAQL
          **************.******* **********.****.*.****.***.***.* *.**
AtFLC  61 DNLVKILDRYGKQHADDLKALDHQSKALNYGSHYELLELVDSKLVGSNVKNVSIDALVQL

WjFLC 121 EDHLETALSLTRARKTELMLKLVDSLKEKEKMLKEENQVLASQMEKNHHVGAEADNMEIS
          *.*******.***.*********..********************.********. **.*
AtFLC 121 EEHLETALSVTRAKKTELMLKLVENLKEKEKMLKEENQVLASQMENNHHVGAEAE-MEMS

WjFLC 181 PRQISDINLPVTLPLLN
          *    . **********
AtFLC 180 PAGQISDNLPVTLPLLN
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**Fig. 1.** Comparison of amino acid sequences of WjFLC and AtFLC (Kubo *et al*., 2012). Identical and similar residues are indicated by asterisks and dots, respectively.

A *FT* ortholog (*WjFT*) was also identified in wasabi (DDBJ/GenBank/EMBL accession no. HQ667761). A cDNA encoding a protein with 175 amino acid residues was identified. Eighty–five percent of the amino acids was identical as between WjFT and AtFT.

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WjFT    1 MSISPRDPLVVGRVVTDVLEPPTRSISLRVTYVQRVVTNGLDLRPSQLLNKPRVEIGGED
          ***. ****.*.***.***.**.***.*.*** ** ***********. ***********
AtFT    1 MSINIRDPLIVSRVVGDVLDPFNRSITLKVTYGQREVTNGLDLRPSQVQNKPRVEIGGED

WjFT   61 LRNFYTLVMVDPDVPSPSNPHLREYLHWLVTDIPATTGTNFGNEIVSYESPRPTSGIHRI
          ************************************.******.**.*.**.****.
AtFT   61 LRNFYTLVMVDPDVPSPSNPHLREYLHWLVTDIPATTGTTFGNEIVCYENPSPTAGIHRV

WjFT  121 VLVLFRQLGRQTVYEPGWRQHFNTREFAAIYNLGLPVAAVFFNCQRESGCGGRRS
          *..***********.*****.*******.************.************
AtFT  121 VFILFRQLGRQTVYAPGWRQNFNTREFAEIYNLGLPVAAVFYNCQRESGCGGRRL
```

**Fig. 2**. Comparison of deduced amino acid sequences of WjFT and AtFT (Kubo *et al*., 2011). Identical and similar residues are indicated by asterisks and dots, respectively.

Flowering was promoted remarkably when *35S::WjFT* was introduced into Arabidopsis (Fig. 3). In some transformed plants, inflorescence appeared when four rosette leaves formed (Fig. 3A). The wild type plants never formed inflorescence at the same stage. The average number of rosette leaves for the wild type was 12.4 under continuous light, but that of the T1 plants of the *WjFT* overexpressor (WjFTOX) was 5.4 (Fig. 3B). Strong expression of *WjFT* was observed in three representative WjFTOXlines (lines 25, 27 and 30) that showed the early-flowering phenotype (Fig. 3C). This indicates that *WjFT* is a promoter of flowering.

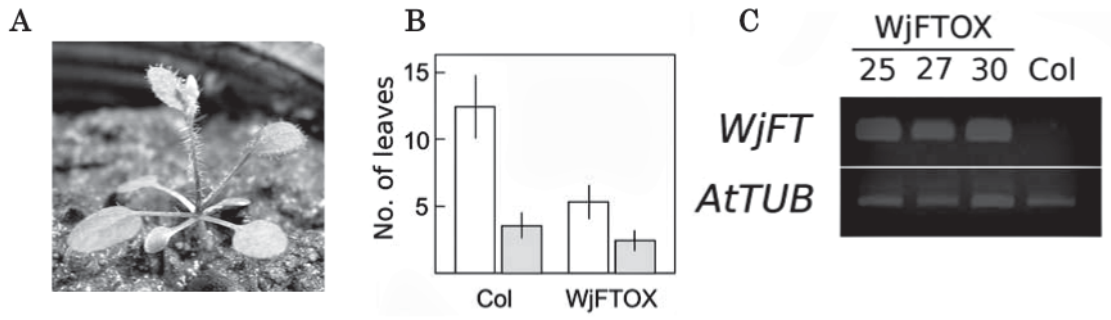


**Fig. 3.** Heterologous expression of *WjFT* in Arabidopsis (Kubo *et al*., 2011). A, This photograph shows a representative line that has two cotyledons, four rosette leaves, and two cauline leaves. B, Average numbers of rosette leaves (hollow box) and cauline leaves (solid box) were calculated from 32 wild type plants (Col) and 49 independent T1 plants of *WjFT*overexpressor (WjFTOX). Transformed plants selected with kanamycin (T1 seedlings) were grown in soil, and leaf numbers were counted. Bars indicate SD. C, The expression of *WjFT* in three WjFTOX lines (line 25, 27 and 30) and Col were examined by semi-quantitative RT-PCR. The expression of tubulin (*AtTUB*) was used as control.

The expression of *WjFLC* was examined in various parts of plant. Wasabi was harvested on July 2 to extract RNA from root, apical part of lateral bud, young leaf and mature leaf. RNA was also extracted from young leaf, rhizome and root of plants grown in a growth chamber, too. *WjFLC* was expressed all the tissues examined (Fig. 4).

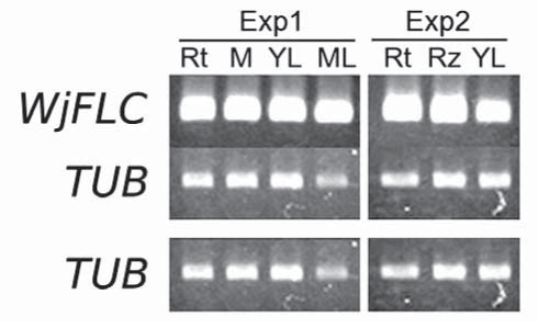


**Fig. 4**. Expression of *WjFLC* in various organs of wasabi (Kubo *et al*., 2012). Total RNA was extracted from root (Rt), apical region of lateral bud (M), young leaf (YL) and mature leaf (ML) of the plants grown in fields (Exp1), and root (Rt), rhizome (Rz) and young leaf (YL) of the plants grown in growth chamber (Exp2). The expression of *WjFLC* was examined by semi-quantitative RT-PCR. Tubulin (*WjTUB*) was used for control.

The expressions of *WjFT* and *WjFLC* were examined in the flowering process using wasabi grown in Nakatou area in Matsumoto, Japan. Wasabi grown in a field usually started to form flower buds in October when the average temperature was lower than 15°C. Therefore, we expected that flower buds would be formed between October and November, because the average temperature around Nakatou area was 14°C in early October and 6°C in the middle of November. Rosette leaves were harvested from the plant grown in the field at Nakatou on October 9, 2009, November 18, 2009, and February 13, 2010 to examine the expression of *WjFT* and *WjFLC* (Fig. 5). The expression of *WjFLC* was high in October but reduced in November. In February, the expression of *WjFLC* could not be detected. Thus, the suppression of *WjFLC* expression seemed to be correlated with the induction of flowering, indicating *WjFLC* functions as a repressor as shown in *AtFLC*. On the other hand, *WjFT* was not detected in October, but increase in November. In February, the expression of *WjFT* was high (Fig. 5).

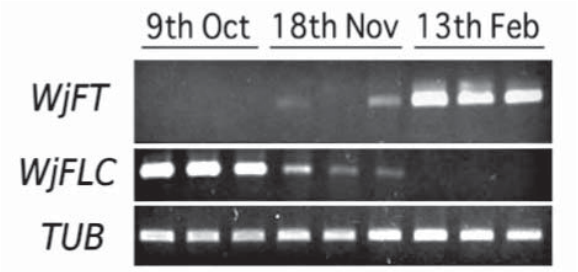


**Fig. 5.** Expression of *WjFT* and *WjFLC* in field grown wasabi plants (Kubo *et al*., 2011; 2012). Leaves were harvested on October 9 and November 18, 2009, and February 13, 2010. Different plants were used in different sampling dates. The expression of *WjFT* and *WjFLC* were examined by semi-quantitative RT-PCR. The expression of tubulin (*WjTUB*) was used as control. Experiments were triplicated from three different plants.

*AtFLC* has a central role in regulating the response to vernalization but several closely related homologs are identified in Arabidopsis (Ratcliffe et al., 2003). It is not known whether wasabi has paralogous genes other than the *WjFLC*. Because *WjFLC* was the most similar to *AtFLC* than the other *FLC* homologs of Arabidopsis, *WjFLC* may be a counterpart of *AtFLC* and play a central role in regulating the response to vernalization.

There is a possibility that wasabi has other *WjFT* paralogs. In a preliminary experiment, several putative *FT* homologs were identified in wasabi when PCR was performed using genome DNA as template. However, *WjFT* may play a central role in flowering because *WjFT* is the most similar to *FT* among the *FT* homologs of Arabidopsis. Overexpression of *WjFT* remarkably promoted the flowering of Arabidopsis. Furthermore, the expression of *WjFT* increased in the season when wasabi forms flower buds. As the environmental condition for flowering has not been established in wasabi, *WjFLC* and *WjFT* could be useful in determining the best condition for flowering.

The expression of *WjFLC* was examined in 2-month–old and 6-month-old wasabi plants after seeding to determine the best condition for initiation of flower bud formation under artificial environment in the closed growing system. The suppression of *WjFLC* expression was not observed in 2-month-old plant grown at 10°C during 4 months (Fig. 6A), and no flower bud formation occurred. On the other hand, *WjFLC*

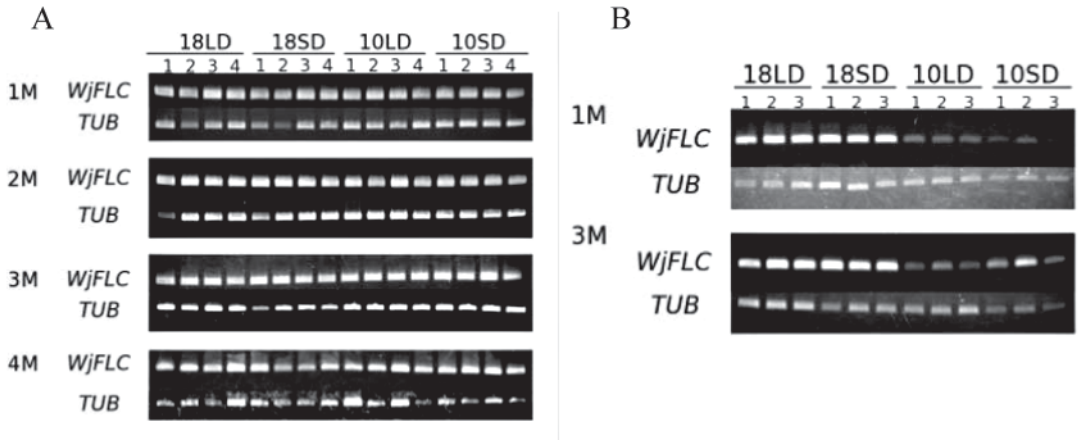


**Fig. 6.** Expression of *WjFLC* in wasabi plants grown under various artificial conditions in the closed growing system. Plants were grown for 2 months (A) and 6 months (B) at 18°C under 12hL:12hD after seeding, and then transferred to 18°C under long day (16hL:8hD) (18LD), 18°C under short day (8hL:16hD) (18SD), 10°C under long day (16hL:8hD) (10LD) and 10°C under short day (8hL:16hD) (10SD) for 1 to 4 months, respectively. Total RNA was extracted from leaves of four (A) or three different plants (B). The expression of *WjFLC* was examined by semi-quantitative RT-PCR. The expression of tubulin (*WjTUB*) was used as control.

**Table 1.** Number of Flower buds formed in wasabi under various artificial environmental conditions during 3 months.

| | Artificial environmental condition | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 18LD | | | 18SD | | | 10LD | | | 10SD | | |
| Plant* | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Number of flower bud | 2 | 0 | 0 | 0 | 0 | 0 | 7 | 9 | 11 | 0 | 0 | 1 |

*Plants were grown for 6 months at 18°C under 12hL:12hD after seeding, and then three plants in each experiment were transferred to at 18°C under long day (16hL:8hD) (18LD), 18°C under short day (8hL:16hD) (18SD), 10°C under long day (16hL:8hD) (10LD) and 10°C under short day (8hL:16hD) (10SD) for 3 months, respectively.

expression in 6-month-old plant was markedly reduced at 10°C (Fig. 6B), and flower buds were formed at 10°C under long day (16hL:8hD) rather than short day (8hL:16hD) (Table 1). These results indicated that wasabi plant must reach a certain developmental stage to sense low temperature for initiation of flower bud formation. Exposure to low temperature more than one month is essential to suppress the expression of *WjFLC* in the flowering response in wasabi. The long day condition is also important for the expression of *WjFT* that is the strong promoter of flowering.

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