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IN SILICO IDENTIFICATION OF THE GENES THAT CONTROL FLOWERING TIME IN WALNUT (*Juglans regia*)

Muhammad SAMEEULLAH[1] **Turan KARADENİZ**[1]

SUMMARY

Walnut (*Juglans regia*) is an important source of fatty acids consisting of saturate and unsaturated fats. Recently, genome sequencing of walnut has been completed with size of 606 Mbp and chromosome numbers (2n) 32. Very little information has been known about flowering genes of walnut because of its perennial growth habit. With the whole genome sequencing, studies of flowering genes will led to accelerate the finding of key genes responsible for late or early flowering habit in walnut. We have found putative key genes related to control of flowering time. These genes would act as functional marker to identify early and late flowering cultivars of walnut. Ten gene families has been found in walnut genome which controlling flowering timing in the flowering pathway. The gene families are (1) Agamous–like MADS–box protein AGL3 (2) Floral homeotic protein APETALA 3, (3) Flowering time control protein I (4) Photoperiod independent protein (5) Early flowering protein (6) Flowering time control protein II (7) Protein TERMINAL FLOWER 1 (8) Vernalization (9) Polycomb group protein. These genes would be important source of selection of cultivars for breeding new cultivars of walnut.

Key words: Walnut, flowering time genes, gene families, breeding, cultivar, genome

INTRODUCTION

Walnut (*Juglans regia*) is an important nut tree which is rich in saturated and unsaturated fatty acids [4, 5]. Flowering is transition of vegetative tissues to reproductive tissues in the life cycle of the angiosperms. The flowering is tightly controlled by internal and external environment cues. These internal and external cues determine the timing of flowering in the plant's life cycle. A little information about walnut flowering genes is reported so far [6, 9]. In *Arabidopsis thaliana* the transition of secondary inflorescne to flowering is mediated by endogenous and environmental cues which regulate the expression of transcription factor encoding floral meristem identity gene *LEAFY* (*LFY*) [2, 3]. Other flowering regulators Agamous–like MADS–box protein AGL3, Floral homeotic protein APETALA 3, Flowering time control protein I, Photoperiod independent protein, Early flowering protein, Flowering time control protein II, Protein terminal flower 1, Vernalization and Polycomb group protein.

Such as which have been characterized in *Arabidopsis thaliana* not yet determined in walnut. The whole genome sequence of walnut (*Juglans regia*) has been sequenced and the genome annotation information is available publicly [8]. The aim of this study was to identify in silico based genes related to flowering in walnut genome and their evolutionary relationship with Arabidopsis flowering genes.

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[1] Abant İzzet Baysal Üniversitesi, Ziraat ve Doğa Bilimleri Fakültesi, Bahçe Bitkileri Bölümü, BOLU

MATERIALS AND METHODS

The flowering genes were identified by the key words like floral, flowering in the annotated genome. The corresponding protein sequences of the flowering genes were then retrieved from the walnut genome database [8]. The Arabidopsis homologues of the walnut flowering genes were used to construct phylogenetic tree using neighbor joining method by MEGA 7.0 [10].

Conserved Domain Sequence Determination

Conserved domain sequence of walnut flowering genes were determined by using proteins sequences in NCBI conserved domain database (CDD) [7].

RESULTS AND DISCUSSION

Agamous–Like MADS–Box Protein AGL3

In this gene family walnut gene *JrAGL4* found which is homologue to *Arabidopsis thaliana AtAGL3*, *AtAGL4*. This gene family belongs to MADS superfamily and contains conserved domain MADS_MEF2_like domain–containing protein.

Floral Homeotic Protein Apetala 3

In this gene family two walnut genes *JrAP3.1* and *JrAP3.2* found which are homologue to *Arabidopsis thaliana AtAP3*. This gene family belongs to MADS and K–box superfamily and contains conserved domains MADS_MEF2_like and K–box.

Flowering Time Control Protein I FY

In this gene family walnut gene *JrFY*, found which is homologue to *Arabidopsis thaliana AtFY*. This gene family belongs to WD40 superfamily and contains conserved domain WD40.

Photoperiod Independent Protein

In this gene, family walnut gene *JrPIE1* found which is homologue to *Arabidopsis thaliana AtPIE1*. This gene family belongs to HSA superfamily and contains conserved domain HAS.

Early Flowering Protein

In early flowering gene family two walnut genes *JrELF3.1*, *JrELF3.2* were found. Arabidopsis homologue to this gene family is *AtELF3*. This gene family belongs to SPOC superfamily with conserved domain of SPOC [11].

Flowering Time Control Protein II

In flowering time control protein II gene family one walnut genes *JrFCA* was found. Arabidopsis homologues to this gene family are *AtFPA*, *AtFCA*. This gene family belongs to RRM_SF and WW superfamily with conserved domain of RRM2_FCA and WW [12].

Protein Terminal Flower 1

In protein terminal flower I gene family two walnut *JrTFL1.1*, *JrTFL1.2* genes were found. Arabidopsis homologue to this gene family is *AtFL1*. This gene family belongs to PEBP superfamily protein [13].

Vernalization

In vernalization gene family one walnut genes *JrVRN2* was found. Arabidopsis homologue to this gene family is *AtVRN2*. This gene family belongs to VEFS–Box superfamily with conserved domain of VEFS–Box [14].

Polycomb Group Protein Embryonic Flower2

In Polycomb group protein EMBRYONIC FLOWER 2 gene family two walnut *JrEMF2.1*, *JrEMF2.2* genes were found. Arabidopsis homologue to this gene family is *ATEMF2*. This gene family also belongs to VEFS–Box superfamily with conserved domain of VEFS–Box as vernalization gene family [14].

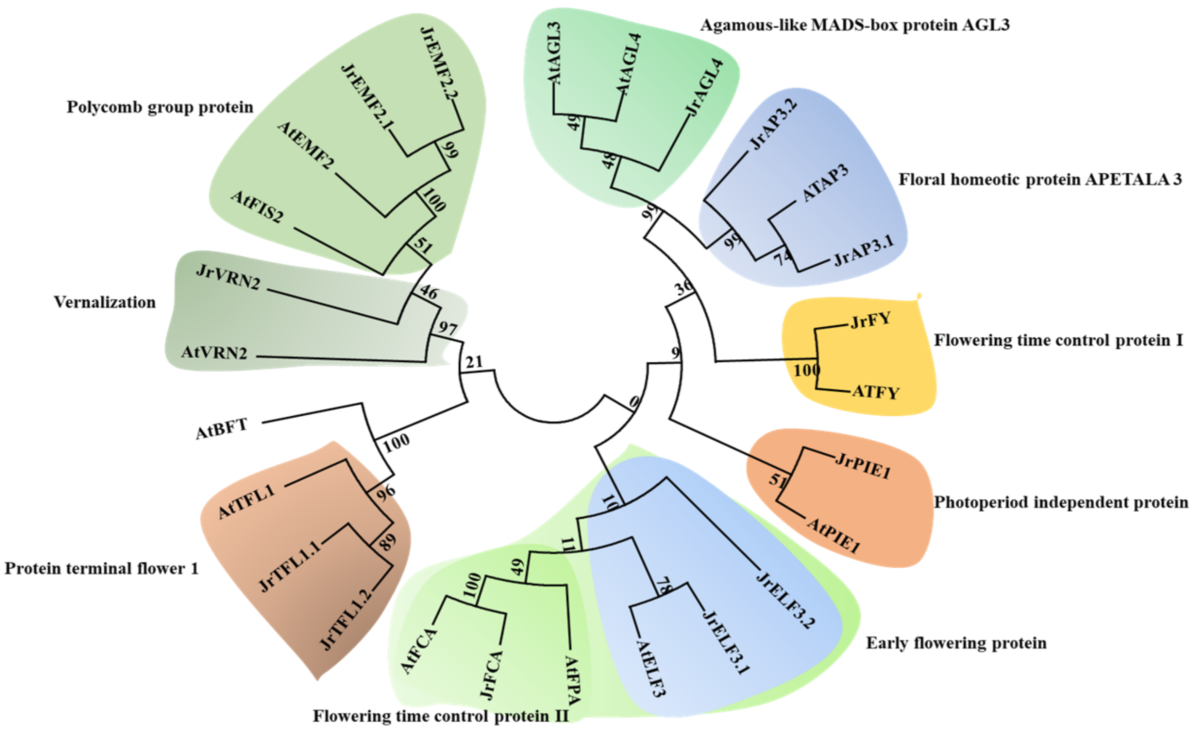

Figure 1. Phylogenetic tree of walnut flowering gene families. The tree was constructed by neighbor joining methods with 1000 repeats

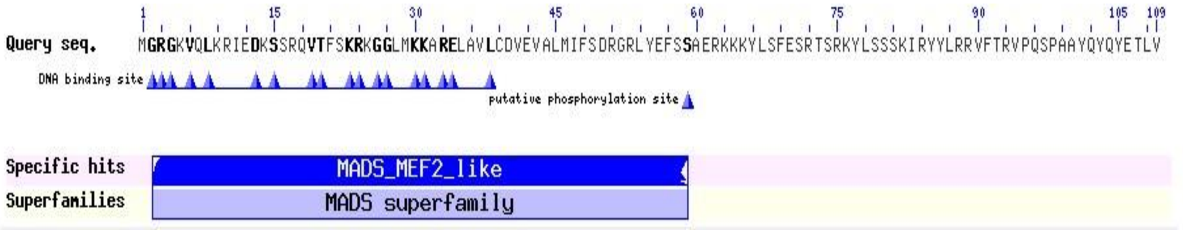

Figure 2. Conserved domain sequence of the walnut *JrAGL4* gene

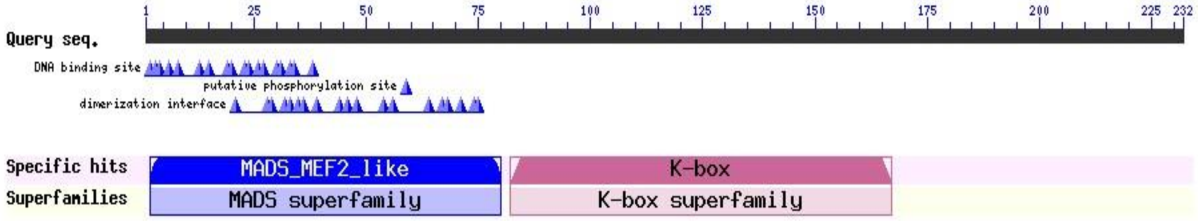

Figure 3. Conserved domain sequence of the walnut genes *JrAP3.1 and JrAP3.2*

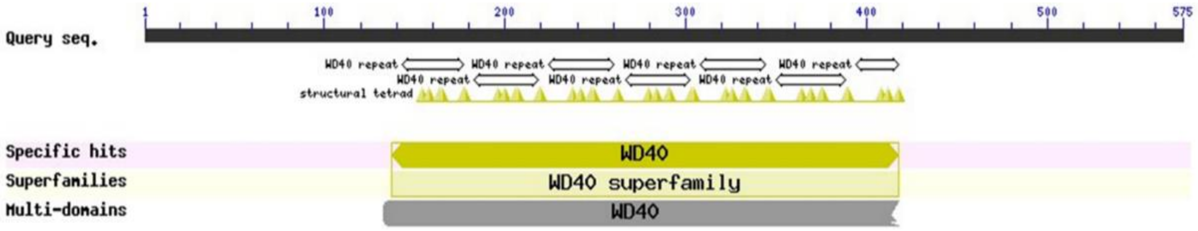

Figure 4. Conserved domain sequence of the walnut *JrFY* gene

Figure 5. Conserved domain sequence of *JrPIE1* gene

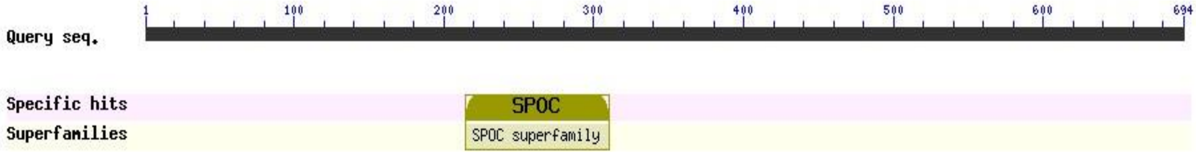

Figure 6. Conserved domain sequence of genes *JrELF3.1*, *JrELF3.2*.

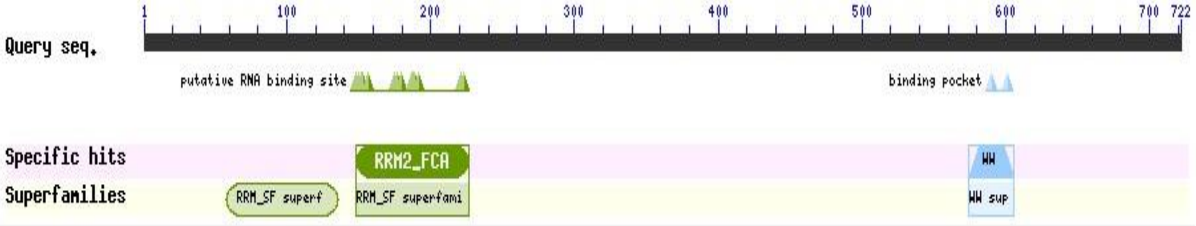

Figure 7. Conserved domain sequence of gene *JrFCA*.

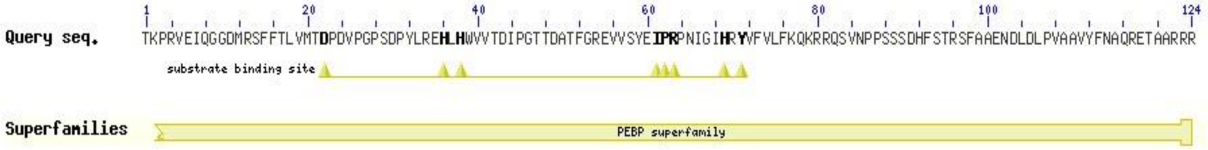

Figure 8. Conserved domain sequence of genes *JrTFL1.1*, *JrTFL1.2*.

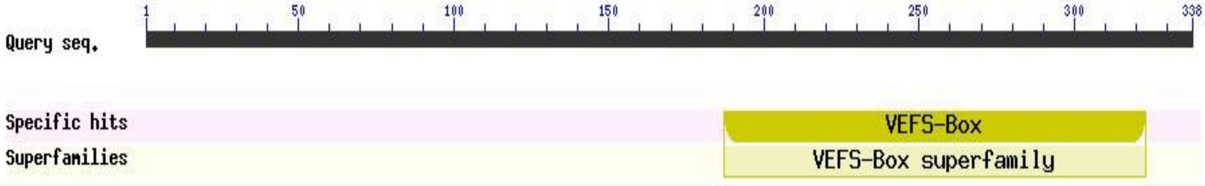

Figure 9. Conserved domain sequence of gene *JrVRN2*.

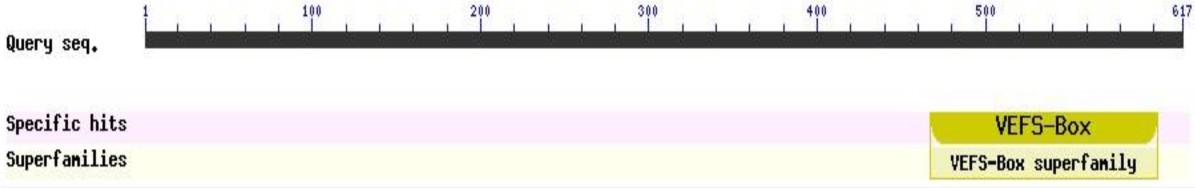

Figure 10. Conserved domain sequence of genes *JrEMF2.1*, *JrEMF2.2*.

CONCLUSION

Based on datamining of walnut genome sequence we could able to identify 9 gene families of walnut belong to flowering gene families based on close relation with *Arabidopsis thaliana* flowering genes. These genes will be important assets to dissect early or late flowering mechanism in walnut. Since timing of flowering in walnut is crucial to escape from late frost in early spring. Therefore, these genes would be beneficial to understand the flowering time and further to screen the early or late flowering walnut genotypes.

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