BUD DORMANCY AND FLOWERING REGULATION MECHANISMS IN *CASTANEA SATIVA* MILL.

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INTRODUCTION

The sweet chestnut tree (*Castanea sativa* Mill.) is a tree from the Fagaceae family and one of the most significant Mediterranean tree species, being an important natural resource for the wood and fruit industries. *C. sativa* yearly cycle follows a perennial habit: in fall, entrance into dormancy occurs concomitantly with the shortening of day length and decrease in temperature. In Spring, dormancy break is followed by vegetative and reproductive growth. It is a monoecious tree with accentuated protrandry: in spring, unisexual male catkins emerge in the axils of new leaves, whereas bisexual catkins emerge one month later in the terminal end of the shoots.

The development of flowers is dependent on several genetic pathways that signal the plant the appropriate timing for flowering, and are well known in some model herbaceous species. *FLOWERING LOCUS T* (*FT*), whose gene product is known as the florigen, is of particular importance in these pathways, as it is an universal flowering promoter in several plant species (Golembeski *et al.*, 2014). In the Fagaceae *Quercus suber* and *Fagus crenata*, *QsFT* and *FcFT* signal flowering induction events the year before flowering occurs (Sobral *et al.*, 2020; Satake *et al.*, 2019). Thus, these trees display a long anthesis period. Despite the overall importance of *C. sativa*, the molecular mechanisms that control its flower induction and development, and dormancy transitions, are still elusive.

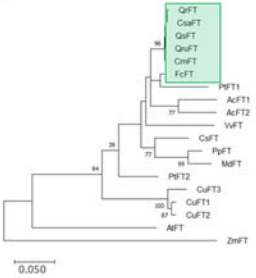
C. SATIVA 'JUDIA' CULTIVAR PHENOLOGY

C. sativa follows a perennial habit: after a period of dormancy in winter, bud break occurs in early spring. New leaves and male and female flowers emerge from the newly formed branches, and fruits develop during summer. Growth cessation and dormancy establishment occur in autumn, concomitantly with leaf and fruit fall.

Blue arrows - male flowers Pink arrows - female flowers Scale bar: 1 cm

FLOWERING INDUCTION OCCURS IN SUMMER

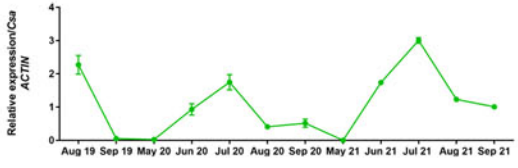
CsaFT phylogeny



Fagaceae FT-like proteins cluster together in the same clade, suggesting a conserved role in these species.

The analysed species include *Q. robur* (Qr), *Q. rubra* (Qru), *C. mollissima* (Cm), *Q. suber* (Qs), *C. sativa* (Csa), *F. crenata* (Fc), *Populus trichocarpa* (Pt), *Actinidia chinensis* (Ac), *Vitis vinifera* (Vv), *Cucumis sativus* (Cs), *Prunus persica* (Pp), *Malus domestica* (Md), *Citrus unshiu* (Cu), *Arabidopsis thaliana* (At), *Zea mays* (Zm)

CsaFT expression in leaves



CsaFT expression in *C. sativa* leaves during three consecutive years suggests that flowering induction occurs in summer, in the year prior to flowering.

TRANSCRIPTOMICS OF BUD DORMANCY REGULATION

Pipeline

RNA extraction from buds (1 year)

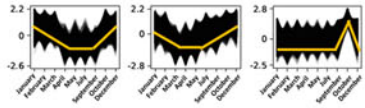
Sequencing (Illumina)

de novo transcriptome (Trinity)

Transcript quantification (Salmon)

Co-expression networks (Clust)

Transcripts upregulated during dormancy

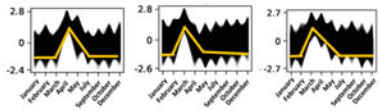


Positive regulators of dormancy: TCP12, TCP18, HUB2, DYL, MAX2

Negative regulation of flowering: FLC, FRI, SVP

Hormones: Abscisic acid biosynthesis, giberellins and citokinins catabolism

Transcripts upregulated during active growth

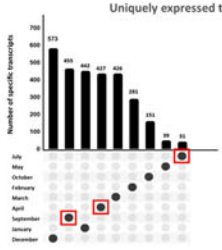


Dormancy break: EBB1, TCP2, DME

Positive regulation of flowering: FD, FVE, FY, FPA, AGL6

Hormones: Abscisic acid catabolism, giberellins and citokinins biosynthesis

Uniquely expressed transcripts during one growing season



July (flowering induction):
Floral organ development and nitrate transport

September (dormancy establishment):
Negative regulation of flowering and starch synthesis

April (bud break):
Starch catabolism and carbohydrate transport

RNA-Seq analysis allowed the identification key regulatory transcripts and pathways involved in the dormancy progression and flowering of *C. sativa*

CONCLUSIONS

- Flowering induction in *C. sativa* occurs the year prior to the emergence of flowers, similarly to other trees from the Fagaceae family
- Factors such as dormancy regulators, hormones, carbohydrate metabolism and flowering time genes may be involved in the different stages of *C. sativa* perennial development.

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Acknowledgements

This work was supported by co-financed by the European Regional Development Fund (ERDF), through the Operational Programme for Competitiveness and Internationalization (COMPETE 2020), under Portugal 2020, and by the Fundação para a Ciência e a Tecnologia – FCT I.P.(National Agency for Science and Technology) with a project grant PTDC/ASP-SIL/27980/2017, and "Contrato-Programa" UIDB/04050/2020

A.T.A was funded by the grant SFRH/BD/136834/2018 .