Final Report

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Title: Understanding the control of perpetual flowering and continuous runnering in strawberry

Dissecting the control of continuous cropping in strawberry

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1. Industry Summary

Consumer demand for year-round strawberry fruit has increased the need to extend the cropping season. Perpetual flowering cultivars, which produce fruit continuously throughout the growing season, offer a promising solution. However, these cultivars typically exhibit reduced runner production, due to a genetic trade-off between flowering and runnering, presenting challenges for propagation and breeding efforts. Additionally, limited understanding of the mechanisms controlling these traits in the cultivated strawberry (*Fragaria* × *ananassa*) has hindered the application of advanced breeding or crop improvement tools. This PhD work aimed to better understand the genetic and molecular mechanisms underlying perpetual flowering and continuous runnering in strawberry, to contribute to the development of perpetual flowering cultivars with extended fruiting periods and improved propagation potential.

In this work, the control of perpetual flowering was explored by functionally studying key flowering genes *TFL1* and *FT1* in *F. x ananassa* seasonal flowering cultivar ‘Malling Centenary’ and perpetual flowering cultivar ‘Calypso’ using CRISPR/Cas9 genome editing and phenotyping. *FaTFL1* mutant lines exhibited early flowering and reduced runner production under long day conditions, validating its role as a floral repressor and demonstrating the regulatory link between these traits. *FaFT1* mutant lines presented alteration in floral and fruit morphology suggesting its role in floral development. Furthermore, the genetic regulation of the trade-off between flowering and runnering was studied through genotyping-by-sequencing based QTL mapping using a woodland strawberry (*Fragaria vesca*) F_2 mapping population developed from a cross between an ecotype from Spain with continuous runnering habit and an accession from Iceland with a ‘normal’ runnering habit. Two additive QTLs associated with continuous runnering were identified using interval mapping. The most significant QTL was narrowed and validated using cleave amplified polymorphic sequences (CAPS) for genotyping in a second validation population. Candidate genes associated with dormancy and axillary bud outgrowth were identified in both QTLs. Gene expression analysis showed a significant downregulation of two candidate genes in continuous runnering individuals supporting their role in the control of this trait. Finally, the functional role of the two main candidate genes for continuous runnering was explored through CRISPR/Cas9 targeted mutagenesis. Two mutant lines for one of the main candidate genes were obtained, however the editing efficiency was very low and consequently this part of the study was not pursued to completion. The work developed within this thesis provides valuable insight into the mechanisms controlling perpetual flowering and the developmental trade-off between generative and vegetative reproduction that could inform future crop improvement strategies.