Exploring apple specialized metabolites across the DREAM agroecosystem in the Mediterranean basin

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Context of the DREAM project

Climate change is increasingly challenging fruit production in the Mediterranean basin, where intensive orchard systems rely on high chemical inputs and low plant diversity, reducing ecosystem. The **DREAM project (Diversified Orchards for REsilient and sustAinable Mediterranean farming systems; PRIMA, EU)** (*Figure 1*) promotes an agroecological approach that integrates: (i) scalar multi-variety orchard with a range of modern and old genotypes; (ii) association with crop mixture, attracting natural enemies and repelling phytophagous insects, and (iii) the adoption of regulated deficit irrigation strategies.

Beyond agronomic performance, fruit organoleptic quality is closely linked to **specialized metabolite** composition, such as polyphenols and vitamins, which also play key roles in plant defence, stress tolerance and human health benefits. In this study, we investigated the specialized metabolome of apple fruits cultivated in Bologna (Italy) under conventional and DREAM agroecosystems using an **untargeted LC-MS/MS approach,** comparing peel and flesh tissues. This work provides insights into how innovative orchard systems influence fruit metabolite composition and contribute to more resilient and sustainable fruit production

DREAM agroecological farming system

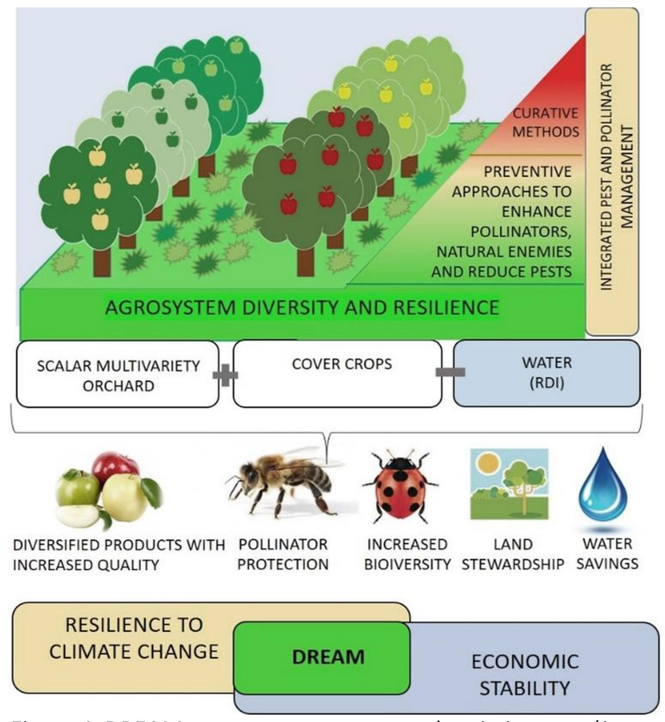
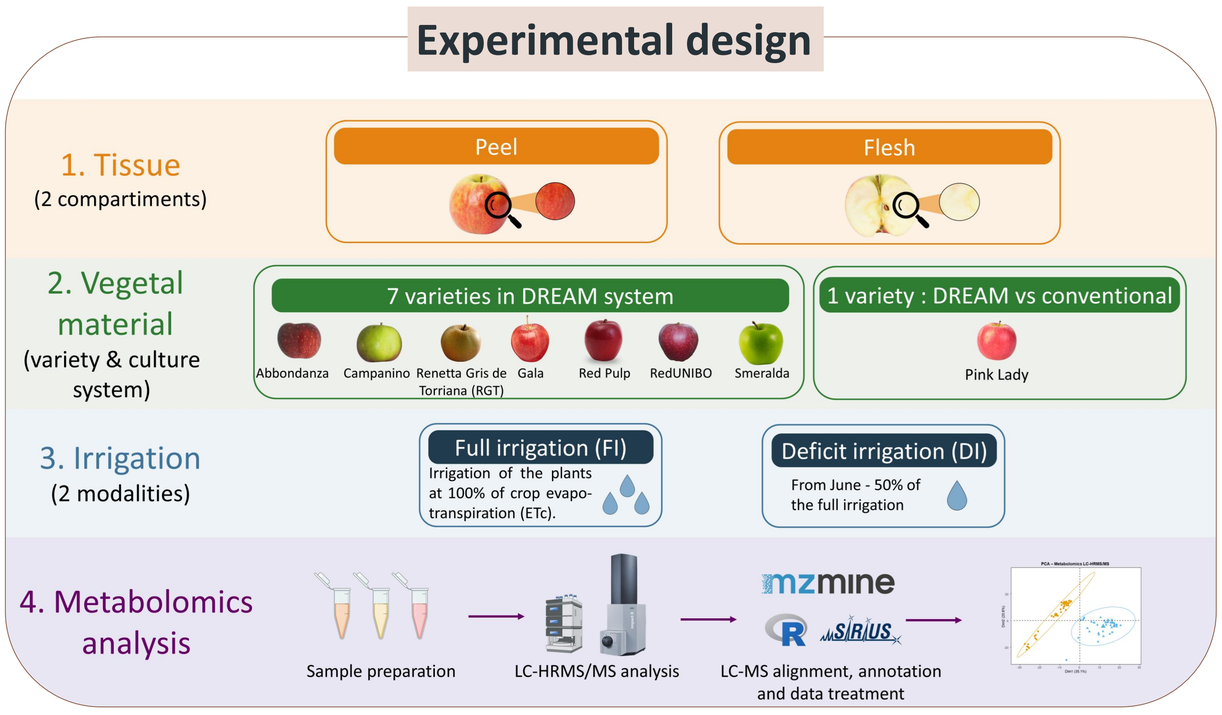


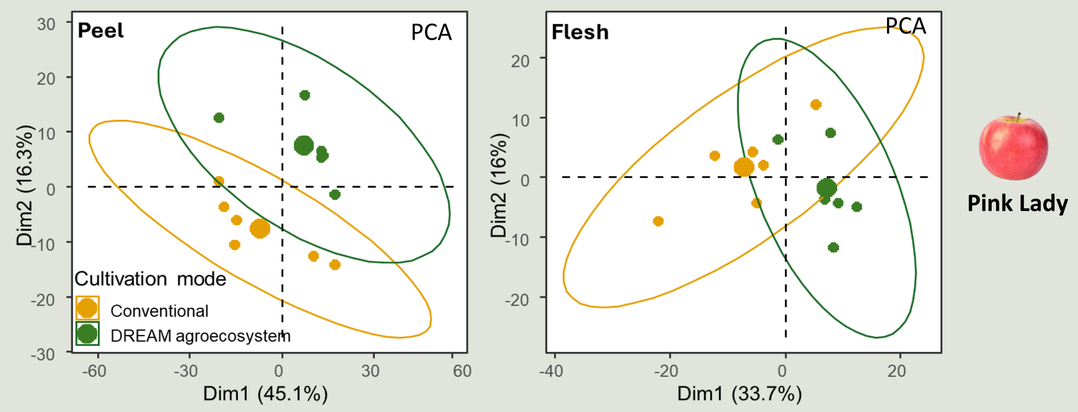
Figure 1. DREAM agrosystem concept and main impacts (*image from https://www.dream4fruit.eu Copyright © 2023 DREAM*)

Experimental design



Is the DREAM system impact the metabolisme of the apple ?

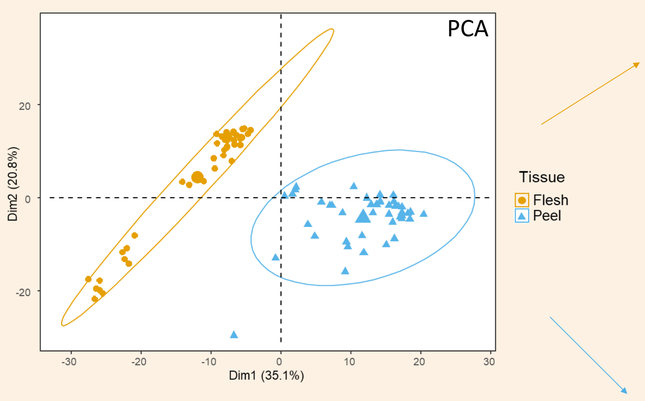
To assess whether the DREAM system impacts apple metabolism, the metabolite composition of Pink Lady apples was compared between DREAM agroecological system and conventional conditions.



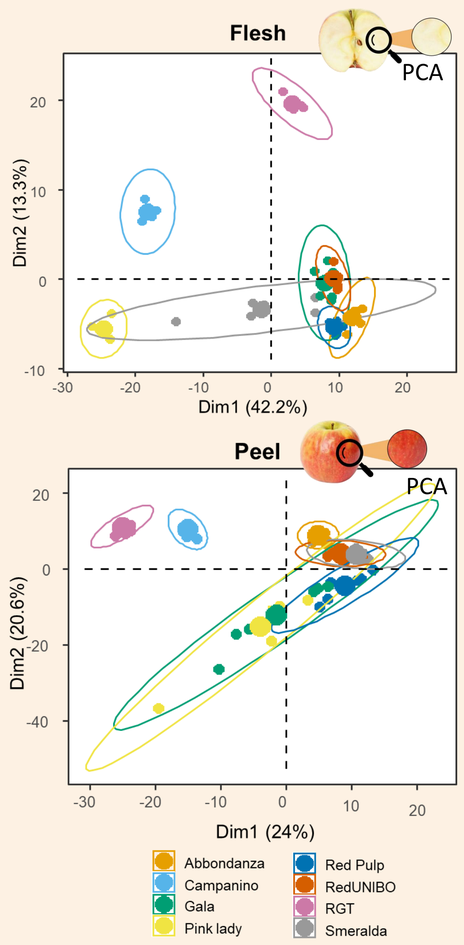
Comparison of Pink Lady apples with and without the DREAM systems showed no clear metabolic effect in the flesh, whereas the peel appeared more affected suggesting a tissue-dependent response

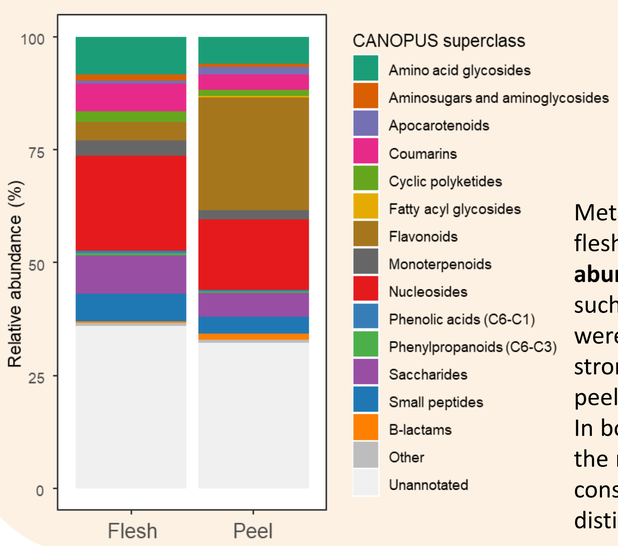
Comparison of varieties according the tissue (peel and flesh)

Metabolomic data were processing using a workflow including filtering, weight normalization, log-transformation and Pareto scaling prior to statistical analysis.



PCA of the LC-HRMS/MS dataset revealed a clear separation driven by tissue (flesh vs skin). As expected, fruit tissues showed contrasting metabolite landscapes. Based on this strong structuring effect, downstream analysis were conducted separatly for each tissue to better resolve additional effect.

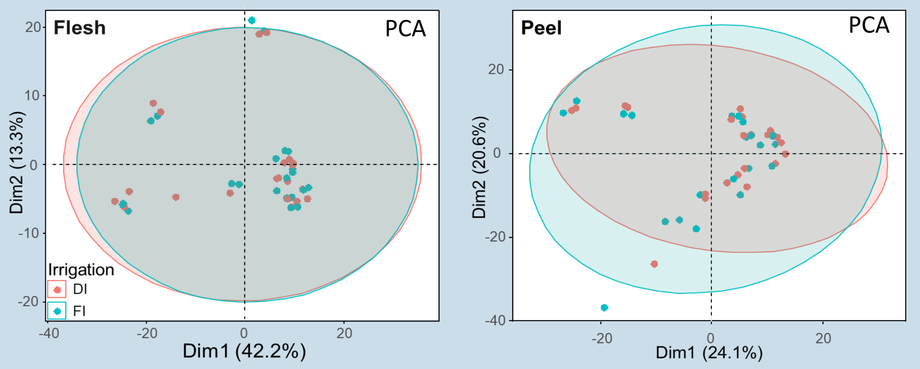




Metabolite composition differed between peel and flesh, with the **peel showing a higher relative abundance of flavonoids**, whereas primary metabolites such as sugars, nucleosides and amino acid glycosides were more reprensented in the flesh. This suggests a stronger enrichment of specialized metabolites in the peel compared to the flesh.

In both peel and flesh, two varieties do not cluster with the remaining six varieties. RGT and Campanino appear consistenly separated from the main group, suggesting distinct metabolic profiles

Impact of the irrigation of the plants on the fruit metabolites



No clear metabolic differences were observed between irrigation conditions (FI: full irrigation and DI: deficit irrigation) in either peel or flesh, as also indicated by poor classification performance in PLS-DA between irrigation groups and high cross-validation error rates. This suggests that, under the tested conditions, reducing water input does not significatyl alter the overall metabolite composition of the fruit. These results indicate a potential for water-saving irrigation strategies without major impact on fruit metabolic profile

Conclusions and Perspectives

The specialized metabolites of both apple flesh and peel cultivated in Italia using the DREAM agroecosytem is not significantly affected, suggesting encouraging prospects for the adoption of this system as an alternative to conventional orchard management.

To further asses the impact of the DREAM system, samples from multiple locations and accros three consecutive years of cultivation will be analyzed.

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