

# Genetic and molecular determinism of diterpenes metabolism in *Coffea* spp

**Cafestol** and **kahweol** are two coffee-specific diterpenes. Although various effects of these compounds have been shown or hypothesized on human health, little information is currently available on the genetic and molecular bases of their metabolism. This work presents data on diterpenes accumulation during fruit development and analysis of gene expression for three key enzymes of the diterpenes biosynthesis pathway.

## Phenotypic diversity

- Diterpenes contents were analyzed in green beans of:
  - 3 *C. canephora*, and 3 *C. arabusta* genotypes. For each genotype, 3 clonal replicates were analyzed separately.
  - *C. congensis*, *C. sessiliflora*, *C. liberica* var *liberica* and *C. liberica* var *dewevrei*. For each species, 3 plants were analyzed separately.
- Diterpenes quantification was carried out according to the method of Dias *et al.* (2007).
- Significant intraspecific (within *C. canephora* and *C. arabusta*) and interspecific variability were observed (Fig. 1).
- According to these results analysis of genotypes representative of the diversity of *C. canephora* and *C. arabica* is on-going.

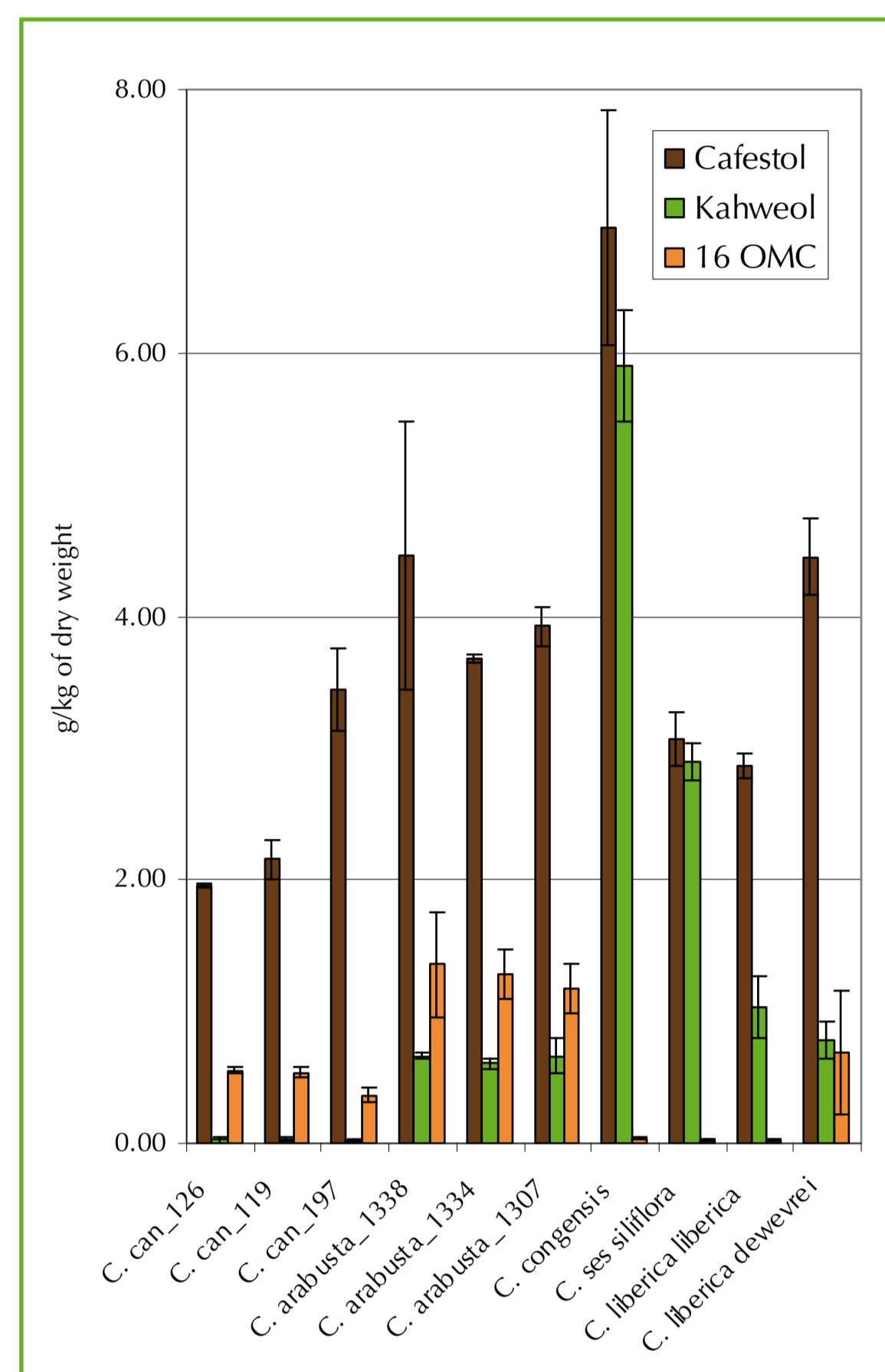


Figure 1. Diterpenes concentrations in a few genetic units of the *Coffea* genus.

## Evolution of diterpenes concentrations during fruit development

- Fruits of *Coffea arabica* cv Iapar59 (3 plants) and *Coffea canephora* cv RL R2 4-I-6 (3 plants) were harvested every 4 weeks between flowering and complete maturation and immediately stored in liquid nitrogen.
- Perisperm, and endosperm of each stage/plant were dissected and analyzed separately for kahweol and cafestol contents using the method of Dias *et al.* (2007) (Fig. 2).
- Kahweol concentration increases in the perisperm of *C. arabica* between 60 and 100 DAF. Following this peak, a continuous decrease simultaneous to an increase in endosperm was observed.
- Almost the same patterns were observed for cafestol in *C. arabica* and *C. canephora*: rapid increase between 60 and 120 DAF (150 for *C. canephora*) followed by a gradual decrease in perisperm accompanied by an accumulation in the endosperm.

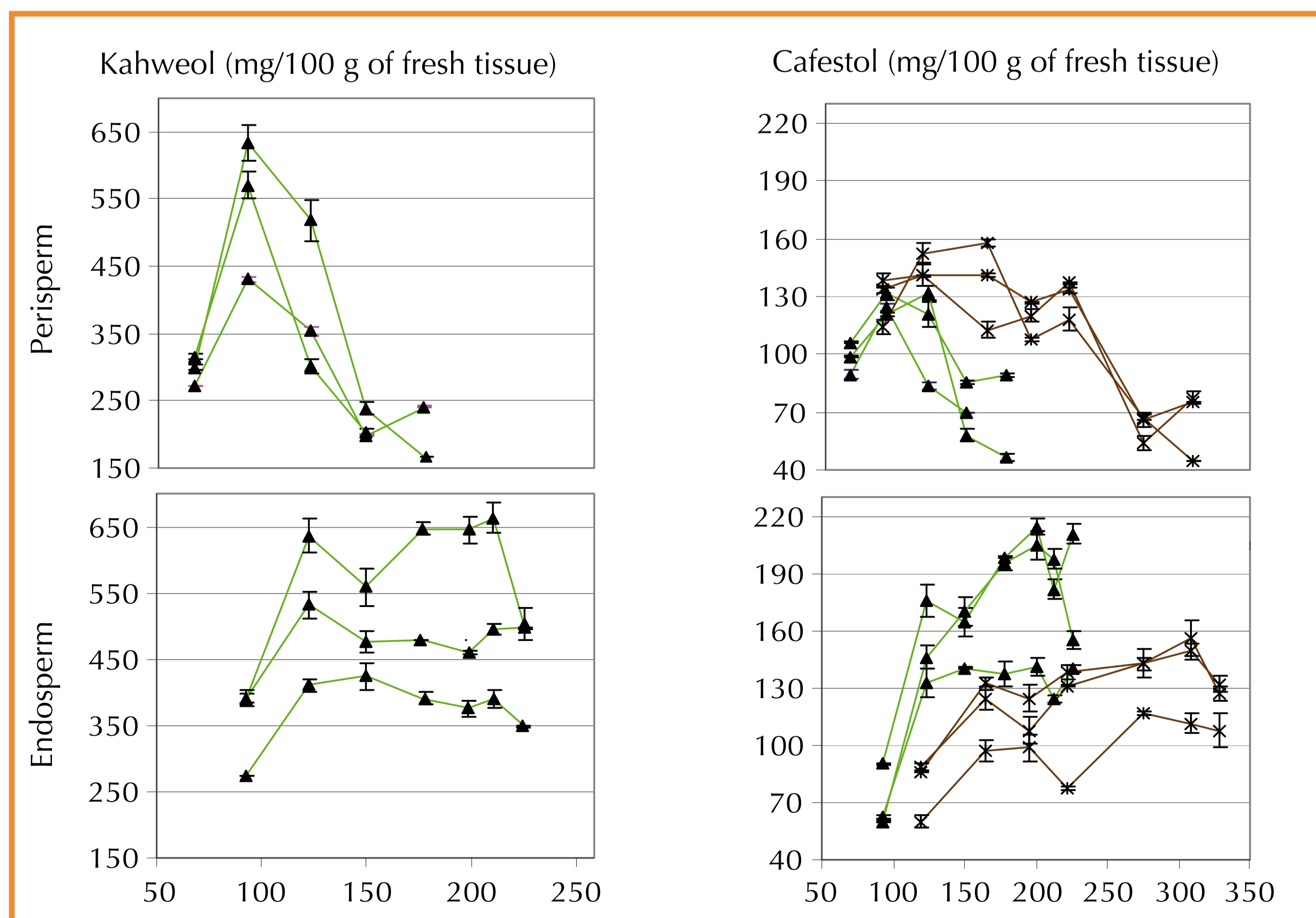


Figure 2. Evolution of kahweol and cafestol concentrations during fruit development (Days After Flowering (DAF) on the X axis) in endosperm and perisperm of *C. arabica* (green lines) and *C. canephora* (brown lines).

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## Genes involved in the cafestol/kahweol biosynthesis

- Information generated by the Genoma Café project (<http://www.lge.ibi.unicamp.br/cafe/>) allowed the full length sequencing of the genes encoding CPS, KO and KS (Fig. 3).
- For CPS and KS, only one isoform was isolated whereas two isoforms (KO\_C1 and KO\_C2) presenting 86% identity at the protein level were identified for KO.

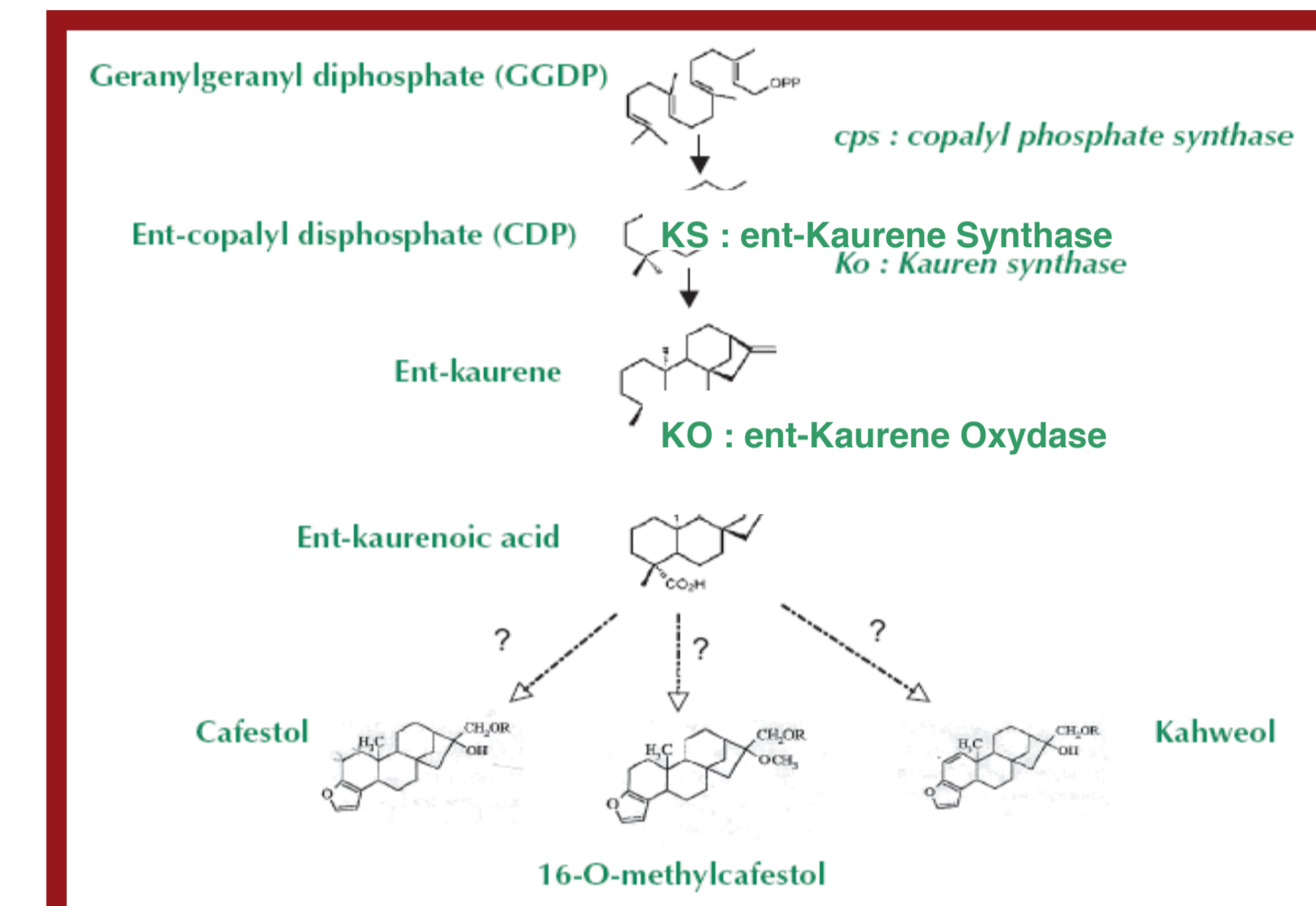


Figure 3. Biosynthesis pathway of cafestol and kahweol.

## Expression patterns of CPS, KO and KS during fruit development

- Expression of the genes were analyzed in perisperm (green bars) and endosperm (brown bars) along fruit development in one plant of *C. arabica* cv Iapar59 by qPCR (Fig. 4). Among the 5 housekeeping genes tested (Actin2, GAPDH1, Cyclohylin1, ADP1, Cathepsin1), Actin2 and Cathepsin1 were selected for the normalization of the data according to the results obtained using GeNorm (Vandesompele *et al.*, 2002). Qbase (<http://medgen.ugent.be/qbase>) was used to analyze expression evolution:
  - CPS is highly differentially regulated between tissues and presents an overexpression in the first stage of perisperm development.
  - KS expression was stable between tissues and between stages.
  - The two KO isoforms exhibit slightly different patterns of expression.

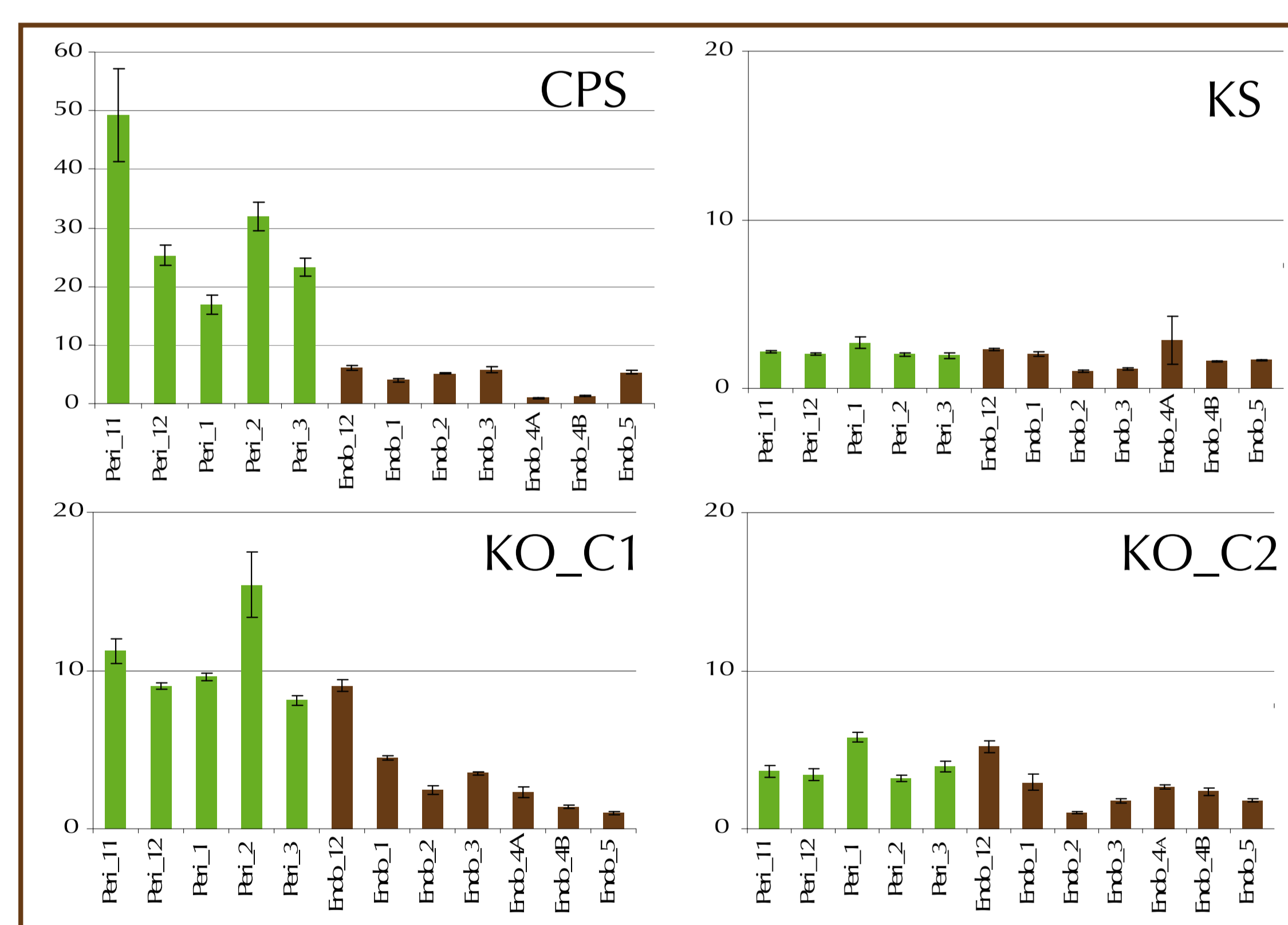


Figure 4. Evolution of gene expression during fruit development. Peri = Perisperm, Endo = Endosperm; the numbers correspond to the month of collection.

## Conclusion

Inter and intraspecific variability were detected for diterpenes contents. The patterns of accumulation during fruit development were defined suggesting transfers between the perisperm and the endosperm. Finally, CPS seems to be one of the key enzymes controlling diterpenes accumulation.

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## References

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