

Microscopy observations of the coffee graft junction: a compatibility study on grafted plants

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RATIONALE

The use of improved rootstock material for grafting is a quick and good alternative to increase plant health and resilience to climate change. The graft success is generally characterized by normal growth, a strong attachment and a vascular reconnection (1). This work aimed to study the anatomical aspect of graft junction of Robusta scion, grafted on different coffee species as rootstocks. The samples derived from research activity of the EU funded project BOLERO that mainly aims to develop resilient rootstock varieties for coffee crop and to assess the influence of grafting on coffee roots' microbiome.

Grafting type (wedge or cleft grafting, fig.2): **Robusta** (*Coffea canephora*) as **scion**, and **different coffee species as rootstocks**, along with controls (Robusta non grafted and grafted on itself).

The motives are:

- Robusta (R)
- R/R
- R/Arabica (R/A)
- R/Liberica (R/L)
- R/Congensis (R/C)
- R/Stenophylla (R/S)

A portion of the graft junction was dehydrated in ethanol and embedded in Technovit resin, cut by a microtome in longitudinal sections (8 µ) and stained in TBO (fig.2a).

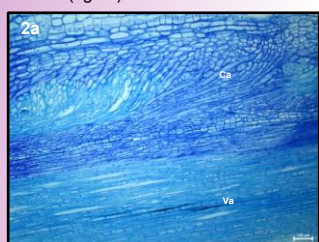


Fig.2a Longitudinal sections of a graft junction portion. Va: vascular tissue cells; Ca: callus cells; bar: 100 µm.



Fig.2 Cleft grafting. Bar: 1 mm.

MATERIAL & METHODS

The graft junction of the stem were cut and put in 50% ethanol in 2ml tubes (4°C), six samples per each graft junction type were prepared for microscopy observations (fig.1). Samples were sent at the end of July 2024 in illycaffé, Trieste.

The middle segment was chosen for transversal sections (fig.2b): prepared by hand, observed by an optical microscope in fluorescence (filter A, UV-blue) or in bright field. Some sections were stained in TBO solution (pH 4.4, acetate buffer for metachromatic stain)

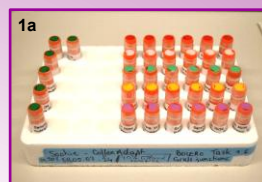


Fig.1a,b. Samples sent to illycaffé and an example of a graft junction sample.

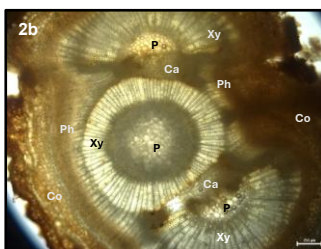
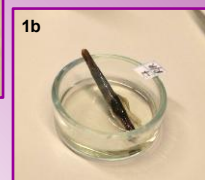


Fig.2b Transversal section of graft junction. bar: 250 µm.

DESCRIPTIONS OF GRAFT JUNCTION TISSUES

All the grafted samples in transversal section could be described with the following anatomical tissues:

In the middle the scion portion (s) and laterally two rootstock portions (rs).

From outside to inside:

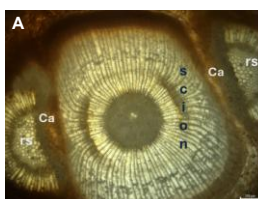
- Co: cortex + endodermis
- Ph: secondary phloem
- Xy: secondary xylem of rs
- Ca: callus
- Xy: secondary xylem of s
- P: pith (parenchymatic cells in the middle)

RESULTS

Grafted samples observed are composed of the scion portion in the middle and the two rootstock portions laterally (V-Shape, fig.2,3). All the graft junctions have a promising aspect from the anatomical point of view, considering the following features observed that contribute to grafting success: the presence of callus at the grafted union along the perivascular cells, the partial or total reconnection of vascular systems, the absence of necrotic layer (fig.3). Vascular reconnection was longitudinally observed in the grafted R/R and R/A samples. Some samples had the junction point still visible (ex. R/L). Some point of discontinuity was still observed in R/S (fig.3 I,J).

CONCLUSION & PERSPECTIVES

All the grafted junctions observed seems to have compatibility from the anatomical point of view, except for R/S that has point of discontinuity in the tissues between rootstock and scion. The following features contribute to grafting success: the presence of callus, the continuity of vascular system in some areas (not completed), the necrotic layer not present, the presence of vascular cambium between the grafted parts. Plants were sampled after 9 weeks: the major part of the coffee grafted-samples were in the last stage of graft formation described by Melnyk 2016 (2): callus was present, observing its cell modification in vascular cells. Vascular cambium was present. Callus thickness (distance between rootstock and scion) was similar in each species, indicating a similar growth rate of grafting junction. Further investigations will be necessary in a long-term period to discover the continuity of the compatibility observed.



R/R graft junction (control)

A: Presence of callus (parenchymatic cells that modified in vascular tissue, Ca) in both areas of contact s+rs. B: discontinuity of endodermis, replace by the callus (white arrows)



R/A graft junction

C: Presence of callus, continuity in phloem, vascular tissue (xylem) and cortex. D: black segment indicate the portion of vascular tissue that grows after the grafting, in continuity with other vascular cells.



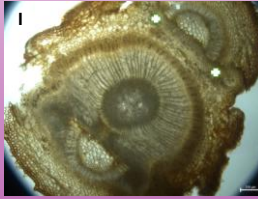
R/L graft junction

E, F: Presence of callus(Ca), continuity in phloem, vascular tissue and cortex only in some areas; vascular cambium (VaC)



R/C graft junction

G, H: Presence of callus(Ca), continuity in phloem (Ph), vascular tissue partially and cortex (Co) in some areas



R/S graft junction

I: Presence of callus, continuity in phloem (only with one rs portion), and cortex in some areas. Points of discontinuity (white cross). J: necrotic cells (red arrows)

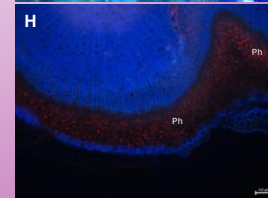
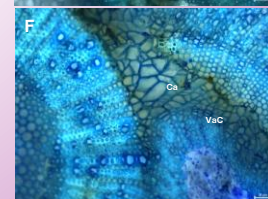
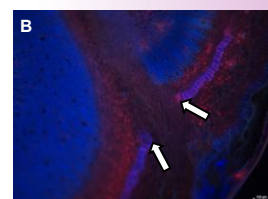


Fig.3 Transversal sections of all type of the graft junctions. A,B: R/Robusta; C,D: R/Arabica; E,F: R/Liberica; G,H: R/Congensis; I,J: R/Stenophylla, bars A,C,E,G,I: 250 µm; bars B,D,H,J: 100 µm; F: 50 µm.