Histological changes associated with the graft union development in tomato

Carlos Frey (cfred@unileon.es)
Universidad de León Facultad de Ciencias Biologicas y Ambientales https://orcid.org/0000-0002-0369-5536
José Luis Acebes
Universidad de Leon
Antonio Encina
Universidad de Leon
Rafael Álvarez
Universidad de Leon

Research article

Keywords: Callus, graft junction, grafting, histology, tomato

Posted Date: February 3rd, 2020

DOI: https://doi.org/10.21203/rs.2.22507/v1

Version of Record: A version of this preprint was published at Plants on November 3rd, 2020. See the published version at https://doi.org/10.3390/plants9111479.

Abstract

Background. Despite the importance of grafting in agriculture, particularly in horticultural crops such as tomato (*Solanum lycopersicum* L.), the structural changes that occur during the establishment of a graft are little understood. Using histochemical techniques, the present work examines the progression of the structure of the graft junction in tomato plants over time.

Results. At 10 days after grafting, the cell walls of the scion and rootstock in the area of the graft junction were thicker than usual, and undifferentiated cells appeared associated with the pre-existing vascular tissue. New vascular tissue appeared as branches arising from the pre-existing vasculature, as vascular pockets dispersed within the callus, and as the result of the transdifferentiation of parenchyma cells. Areas showing vascular connections between the scion and rootstock were also seen. Adventitious roots appeared on the scion, arising from the pre-existing vasculature. At 20 days, a great deal of vascular tissue was visible, along with large areas showing vascular connection. At 210 days, vestiges of the changes undergone were still visible. However, no adventitious roots persisted.

Conclusions. The area of the graft junction undergoes modifications essential for adequate physiological functioning of grafted plant. The cell walls of the adhesion line change during the process. Pre-existing vasculature plays an important role in the appearance of callus tissue, new vascular cells, and adventitious roots. A long time later the tissues maintain vestiges of graft union development.

Background

The regenerative properties of plants have been harnessed by growers since ancient times to graft together specimens of different varieties or even species (1). A graft is composed of a root-bearing portion (the rootstock) and an aerial portion (the scion) provided by two plants (2). Grafting is now performed worldwide and is of great economic importance (3–6). Grafting may be performed to improve resistance to disease, insects and abiotic stress, to improve yields, to control plant size, to shorten the juvenile period, as a method of propagation, to induce unusual forms of growth, and to provide models for studies of plant physiology (1,2,7,8).

Many complex biochemical and structural process take place during the establishment of a graft, from the response to the initial wound, changes at the edges of the cut, the formation of callus tissue, the differentiation of new vascular tissue, and the connection of the vasculature (2,9,10). All these processes affect the success of a graft; understanding them, their timing, and where they occur, is key in understanding and promoting graft establishment.

Grafting is widely used with tomato (*Solanum lycopersicum* L.) (5) - one of the most economically important crops in the world (3,5). The advantages that grafted plants offer in terms of productivity render the process indispensable to many growers. The literature contains little information on the precise structural development of graft junctions in this or any other crop. Moreover, a better understanding of structural and molecular mechanisms underlying graft formation might help improve the success and

quality of grafting to increase the economic outcome of this technique. Within this frame, the aim of the present work was to record the sequence of histological modifications that occur in the graft junction of tomato homografts, as a model of good scion-rootstock compatibility.

Results

At 5 weeks post-emergence, the non-grafted control plants normally had a green shoot with a relatively large number of trichomes. The tissues making up the stem included a monolayer of epidermal cells, laminar collenchyma, photosynthetic parenchyma, storage parenchyma (cortical), primary phloem, vascular meristematic tissue, primary xylem and storage parenchyma (medullary) (**Figs. 1ab**). The vascular system appeared in a ring with clear differentiation between fascicular and interfascicular areas. Phloem fibres as well as microcrystals were also present.

At 10 days after grafting, a necrotic line appeared where the stem was cut. Later this disappeared as the callus grew (it can attain a large size). The histological organisation of the graft junction was very different to that of the control stems. At 10 days after grafting (**Figs. 1c-f**) the tissues of the scion and rootstock began to join. An adhesion line **(al in Figs. 1c-f)** formed by thickened walls of cells belonging to the rootstock and scion was distinguishable to a varying extent at this stage of grafting. The remains of necrotic tissue and areas of non-adhesion were also visible. The rootstock and scion tissues were often interdigitated. Masses of undifferentiated cells, developing vascular elements, and sometimes vascular connections, were also appreciable. These masses of undifferentiated cells make up most of the callus, and were seen in their largest numbers near pre-existing vascular tissue and in the medullary and cortical parenchyma close to the wound (**Figs. 1c-d**). Vascular cells differentiated during the establishment of the graft (**Figs. 1c-e**): (1) appearing as a branch of the pre-existing vasculature; (2) as forming vascular pockets or groupings dispersed throughout the callus; (3) and as cells transdifferentiating from parenchyma into xylem vessels. Generally, the most distal extreme of the pre-existing vasculature remained inert (**Fig. 1g**), playing no active part in the vascular re-connection occurring during grafting. The response of the scion cells seemed to be greater than that of the rootstock in terms of the differentiation of vascular connections.

At 20 days after grafting (**Figs. 2a-d**), the adhesion line was almost imperceptible. The thickened cell walls were now only as thick as those of other cells, and no necrotic layer remained. Some areas of non-adhesion persisted. Hyperplasia and hypertrophy was observed at this stage in the graft junction (**Fig. 2a**). The callus in the junction area contained many differentiated vascular cells - more than any other type. Vascular connections were complete. The outer callus tissue of the scion formed a 'skirt' containing vascular tissue surrounded by parenchyma (**Fig. 2d**).

At 210 days after grafting (**Fig. 2e-g**), the graft junction looked similar to that seen at 20 days. However, the vascular connections were better defined, and the tissues were arranged in a more orderly fashion. Vestiges of the process of union between the scion and rootstock were visible in transverse sections.

At 10 and 20 days after grafting, adventitious roots appeared on the scion (**Figs. 3a-d**), but these were gone by 210 days. They were only seen in that part of the scion close to the graft junction, arising from the meristematic tissue of the pre-existing vasculature and growing between callogenic tissue. The cells of these adventitious roots contained many amyloplasts.

Discussion

The distribution of the tissues in the scion and rootstock is essential in the correct establishment of the graft, in particular the vascular meristematic tissue (2,11). The stems of tomato have the typical structure of dicotyledonous, mesophytic plants, with a vascular cylinder that appears discontinuous in the early stages of life, but which later becomes continuous with the interfascicular regions (12,13). This distribution allows for grafting, unlike the dispersed vascular pattern of monocotyledonous plants (2,10,14).

The cells walls at the line of contact between the scion and rootstock commonly appeared thickened. These structures are thought to play a key role in graft establishment, taking part in the recognition and adhesion of the two sides of the graft (15–21). The increase in thickness is related to the deposition of wall polysaccharides by the protoplasts of nearby cells and the compacting of necrotic remains from the cut (20). The later thinning of these walls is accompanied by the restitution of the symplastic pathway owing to the formation of plasmodesmata (15,18,22) and the clearing of cell wastes (7,23).

The tissues close to the cut also undergo profound changes (2,10). In tomato, the callus is large, but this would not appear to have a bearing on the success of the graft; in other dicotyledonous species the callus can be very small (in some cases almost imperceptible), yet grafting is successful (23,24).

The callus is generated by the de-differentiation of cells close to the wound (10,25,26), but it is hard to say which types are most involved. In the present work, the location and appearance of the cells and tissues point to the callus is formed largely from live cells associated with the vascular tissue. This might be related to the preferential expression of the transcription factor *WIND1* by the vascular meristematic tissue (26). *WIND1* is involved in de-differentiation and callus formation via the activation of a signalling pathway in which cytokinins participate (26,27,28). Auxins also have the capacity to induce callus formation (25), although in graft establishment their induction of vascular differentiation is likely more important (29,30–34). These hormones, which travel in a basipetal direction (i.e., downward from the tip of the scion), move through the stem, transported by PIN-FORMED (PIN) transport proteins (35). These transporters can alter their position in the cell surface towards wounds (36,37). Their accumulation in the scion vasculature, provoked by the cut, would seem to induce nearby cells to become meristematic. The differentiation of new vasculature occurs in different directions, away from the pre-existing vascular tissues, and these new branches may connect with pockets of vasculature forming in the callus. As reported in other studies (15,22,38), vascular connections between the rootstock and scion are clearly visible at around 10 days after grafting. The connection of the scion and rootstock tissues leaves a permanent mark at the graft junction, especially in the vascular tissue.

The formation of adventitious roots by the scion is a response to the stress caused by the wound (39,40). The process is directly related to the blockage of the basipetal movement of auxins at the cut and their accumulation in that area too (41,42). The production of these roots is not helpful in graft establishment, indeed, rooting could prevent the graft being successful (42). However, it could be useful in nurse-rooting, a method of propagation that requires rooting occur (2,41). Sala *et al*. (40) identified two types of adventitious root, produced at different times after grafting: one with and one with no parenchyma envelope.

Conclusion

The early stage of graft establishment in tomato is marked by thickened cell walls at the graft junction. The pre-existing vasculature plays an important role producing callus tissue and new vascular cells. By 10 days post grafting, some areas of xylem and phloem of the scion and rootstock are connected and functional. The scion vasculature is also the source of adventitious roots. Long after the graft is established, vestiges of the process of joining remain apparent. Understanding how grafts become established could throw light on how the process could be improved, and why grafts sometimes fail.

Abbreviations

al: adhesion line; *ar:* adventitious root; *C:* callus; *CE:* callus expansion; *co:* collenchyma; *cr:* microcrystals; *e:* epidermis; *ev:* enveloping parenchyma; *f:* fibre; *FAA:* formalin–acetic acid–alcohol; *na:* non-adhesion; *pa:* parenchyma; *ph:* phloem; *PV:* pre-existing vascular tissue; *rm:* root meristem; *RS:* rootstock; *SC:* scion; *sp:* storage parenchyma; *t:* transdifferentiating cells; *uc:* undifferentiated cells; *vb:* vascular branch; *VC:* vascular connection; *vmc:* vascular meristematic cells; *x:* xylem.

Methods

Plants and growth conditions

Cherry tomato (Minibel-Cocktail) seeds (Mascarell Semillas SL) were sown individually in 200 ml plastic pots containing 170±10 ml of peat, placed on plastic trays. Watering with complete Hoagland nutritive solution, directly into the trays, was performed twice per week; all the pots had a hole in the bottom to maintain the peat nearly at their field capacity. The plants were grown in a growth chamber maintained at 23±1ºC and with a 16 h light period.

Grafting method

Grafting was undertaken when the plants stems were 4-5 mm thick (after 5 weeks of growth). Homografts, scions and rootstocks from different plants, were performed. Non-grafted plants were used as controls.

Scions and rootstocks were obtained by making a transverse cut below the cotyledon leaves. Cut faces were then joined, and the junctions wrapped in Parafilm® and protected using Toogoo® silicon clips. The grafted plants were kept for 7 days in a humid atmosphere, which was afterwards gradually ventilated.

Histological techniques

Segments containing the graft junction were taken from successfully grafted plants at 10, 20 and 210 days after grafting (n=5 per graft type and time point). All samples were fixed in formalin–acetic acid–alcohol (FAA) (24-48 h), before placing in increases ethanol series, isoamyl acetate, and finally embedded in paraffin wax. A rotary microtome was then used to make 12 µm sections which were stained with either safranin and fast green, haematoxylin and eosin, lugol, phloroglucinol, or sirofluor (a fluorescent marker). After mounting (see **Table 1** for these and other details), slides were observed under bright field, polarized and epifluorescence conditions using a Nikon E600 microscope.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All of the data that was generated or analysed during this study are included in this published article.

Competing interest

The authors declare that they have no competing interests.

Funding

CF is financially supported by the Spanish Ministry of Science, Innovation and Universities (FPU18/04934).

Authors´ contributions

JLA and AE conceived, designed and supervised the research, RA designed and supervised the sample handling and microscopy techniques, CF conducted the experiments, analysed the data and wrote the manuscript, JLA, RA and AE contributed to analyse the data and supervised the manuscript. All of the authors read and approved the manuscript.

Acknowledgements

Authors acknowledges the work of Adrian Burton in the translation of this manuscript.

References

1. Mudge K, Janick J, Scofield S, Goldschmidt EE. A history of grafting. In: Janick J, editor. Horticultural Reviews. West Lafayette: Wiley-Blackwell; 2009. p. 437–93.
2. Hartmann HT, Kester DE, Davies FT, Geneve RL. Hartmann & Kester's plant propagation principles and practices. 9th ed. Harlow: Pearson; 2018
3. Lee JM, Kubota C, Tsao SJ, Bie Z, Echevarria PH, Morra L, *et al*. Current status of vegetable grafting: diffusion, grafting techniques, automation. Sci Hortic. 2010;127(2):93–105.
4. Kyriacou MC, Rouphael Y, Colla G, Zrenner R, Schwarz D. Vegetable grafting: the implications of a growing agronomic imperative for vegetable fruit quality and nutritive value. Front Plant Sci. 2017; 8(741).
5. Singh H, Kumar P, Chaudhari S, Edelstein M. Tomato grafting: a global perspective. HortScience. 2017; 52(10):1328–36.
6. Tirupathamma TL, Ramana CV, Naidu LN, Sasikala K. Vegetable grafting: a multiple crop improvement methodology. Curr J Appl Sci Technol. 2019; 33(3):1–10.
7. Warschefsky EJ, Klein LL, Frank MH, Chitwood DH, Londo JP, von Wettberg EJB, *et al*. Rootstocks: diversity, domestication, and impacts on shoot phenotypes. Trends Plant Sci. 2016; 21(5):418–37.
8. Tsutsui H, Notaguchi M. The use of grafting to study systemic signaling in plants. Plant Cell Physiol. 2017; 58(8):1291–301.
9. Martínez-Ballesta MC, Alcaraz-López C, Muries B, Mota-Cadenas C, Carvajal M. Physiological aspects of rootstock–scion interactions. Sci Hortic. 2010; 127(2):112–8.
10. Melnyk CW. Plant grafting: insights into tissue regeneration. Regeneration. 2017; 4(1):3–14.
11. Garner RJ, Bradley S. The grafter´s handbook. 6th ed. London: Mitchell Beazly; 2013.
12. Crang R, Lyons-Sobaski S, Wise R. Plant anatomy: a concept-based approach to the structure of seed plants. New York: Springer; 2018.
13. Schweingruber FH, Börner A. The plant stem: a microscopic aspect. New York: Springer; 2018.
14. Melnyk CW, Meyerowitz EM. Plant grafting. Curr Biol. 2015; 25(5):R183–8.
15. Jeffree CE, Yeoman MM. Development of intercellular connections between opposing cells in a graft union. New Phytol. 1983; 93(4): 491–509.
16. Miller H, Barnett JR. The structure and composition of bead-like projections on sitka spruce callus cells formed during grafting and in culture. Ann Bot. 1993; 72(5):441–8.
17. Yeoman M. Cellular reconigtion systems in grafting. In: Cellular interactions. New York: Springer; 1993. p. 441–8.
18. Pina A, Errea P, Martens HJ. Graft union formation and cell-to-cell communication via plasmodesmata in compatible and incompatible stem unions of *Prunus* spp. Sci Hortic. 2012;

143:144–50.

19. Cookson SJ, Clemente Moreno MJ, Hevin C, Nyamba Mendome LZ, Delrot S, Trossat-Magnin C, *et al*. Graft union formation in grapevine induces transcriptional changes related to cell wall modification, wounding, hormone signalling, and secondary metabolism. J Exp Bot. 2013; 64(10):2997–3008.
20. Sala K, Karcz J, Rypie A, Kurczy EU. Unmethyl-esterified homogalacturonan and extensins seal *Arabidopsis* graft union. BMC Plant Biol. 2019; 19(151).
21. Pitaksaringkarn W, Matsuoka K, Asahina M, Miura K, Sage-ono K, Ono M. XTH20 and XTH19 regulated by ANAC071 under auxin flow are involved in cell proliferation in incised *Arabidopsis* inflorescence stems. Plant J. 2014; 80(4):604–14.
22. Fan J, Yang R, Li X, Zhao W, Zhao F, Wang S. The processes of graft union formation in tomato. Hortic Environ Biotechnol. 2015; 56(5):569–74.
23. Yin H, Yan B, Sun J, Jia P, Zhang Z, Yan X, et al. Graft-union development: a delicate process that involves cell-cell communication between scion and stock for local auxin accumulation. J Exp Bot. 2012; 63(11):4219–32.
24. Melnyk CW, Schuster C, Leyser O, Meyerowitz EM. A developmental framework for graft formation and vascular reconnection in *Arabidopsis thaliana*. Curr Biol. 2015; 25(10):1306–18.
25. Asahina M, Azuma K, Pitaksaringkarn W, Yamazaki T, Mitsuda N, Ohme-Takagi M, *et al*. Spatially selective hormonal control of RAP2.6L and ANAC071 transcription factors involved in tissue reunion in *Arabidopsis*. Proc Natl Acad Sci USA. 2011; 108(38):16128–32.
26. Iwase A, Mitsuda N, Koyama T, Hiratsu K, Kojima M, Arai T, *et al*. The AP2 / ERF transcription factor WIND1 controls cell dedifferentiation in *Arabidopsis*. Curr Biol. 2011; 21(6):508–14.
27. Immanen J, Nieminen K, Smolander O-P, Kojima M, Alonso Serra J, Koskinen P, *et al*. Cytokinin and auxin display distinct but interconnected distribution and signaling profiles to stimulate cambial activity. Curr Biol. 2016; 26(15):1990–7.
28. Ikeuchi M, Iwase A, Rymen B, Lambolez A, Kojima M, Takebayashi Y, *et al*. Wounding triggers callus formation via dynamic hormonal and transcriptional changes. Plant Physiol. 2017; 175(3):1158–74.
29. Nanda AK, Melnyk CW. The role of plant hormones during grafting. J Plant Res. 2018; 131(1):49–58.
30. Sachs T. The role of the root in the induction of xylem differentiation in peas. Ann Bot. 1968; 32(2):391-9.
31. Sachs T. The control of the patterned differentiation of vascular tissues. Adv Bot Res.1981; 9: 151-262.
32. Aloni R. Role of auxin and sucrose in the differentiation of sieve and tracheary elements in plant tissue cultures. Planta. 1980; 150(3):255–63.
33. Donner TJ, Sherr I, Scarpella E. Regulation of preprocambial cell state acquisition by auxin signaling in *Arabidopsis* leaves. Development. 2009; 136(19):3235–46.
34. Biedroń M, Banasiak A. Auxin-mediated regulation of vascular patterning in *Arabidopsis thaliana* leaves. Plant Cell Rep. 2018; 37(9):1215–29.

35. Křeček P, Skůpa P, Libus J, Naramoto S, Tejos R, Friml J, *et al*. The PIN-FORMED (PIN) protein family of auxin transporters. Genome Biol. 2009; 10(12):1–11.
36. Sauer M, Balla J, Luschnig C, Wisniewska J, Reinöhl V, Friml J, *et al*. Canalization of auxin flow by Aux/IAA-ARF-dependent feedback regulation of PIN polarity. Genes Dev. 2006; 20(20):2902–11.
37. Mazur E, Benková E, Friml J. Vascular cambium regeneration and vessel formation in wounded inflorescence stems of *Arabidopsis*. Sci Rep. 2016; 6(1):33754.
38. Lindsay DW, Yeoman MM, Brown R. An analysis of the development of the graft union in *Lycopersicon esculentum*. Ann Bot. 1974; 38(3):639–46.
39. Bellini C, Pacurar DI, Perrone I. Adventitious roots and lateral roots: similarities and differences. Annu Rev Plant Biol. 2014; 65(1):639–66.
40. Sala K, Malarz K, Barlow PW, Kurczyńska EU. Distribution of some pectic and arabinogalactan protein epitopes during *Solanum lycopersicum* (L.) adventitious root development. BMC Plant Biol. 2017; 17(25).
41. Castro M, Darrouy N, Iturrieta R. Franqueamiento: a new vegetative propagation technique for loquat. Acta Hortic. 2007; 750(51):325–30.
42. Meyer LJ, Kennelly MM, Pliakoni ED, Rivard CL. Leaf removal reduces scion adventitious root formation and plant growth of grafted tomato. Sci Hortic. 2017; 214:147–57.

Tables

Table 1. Preparation of slides and microscope observations.

Staining	Mounting	Microscopy technique	Target
Safranin-Fast Green	Entellan	Bright field	General staining
Haematoxylin-Eosin	Entellan	Bright field	General staining
Lugol	No	Bright field	Starch
Sirofluor	No	Epifluorescence	Callose
Phloroglucinol	No	Bright field	Lignin
No	Entellan	Epifluorescence	Autofluorescence
No	Entellan	Polarization	Birefringent structures

Table 2. Histology of the graft junction in *Solanum lycopersicum* at 10, 20 and 210 days after grafting.

Days after grafting	10	20	210
Thickened cell walls	+++	+	+
Size of the callus	+	+ + +	-
Necrotic remains	+	-	-
Areas of non-adhesion	+	+	+
Undifferentiated cells	+ +	+	-
Xylem cells	+	+ + +	+ + +
Vascular connections	±	+	+

(+) presence; (-) absence; relative amounts indicated by number of symbol repetitions (subjective description).

Figures

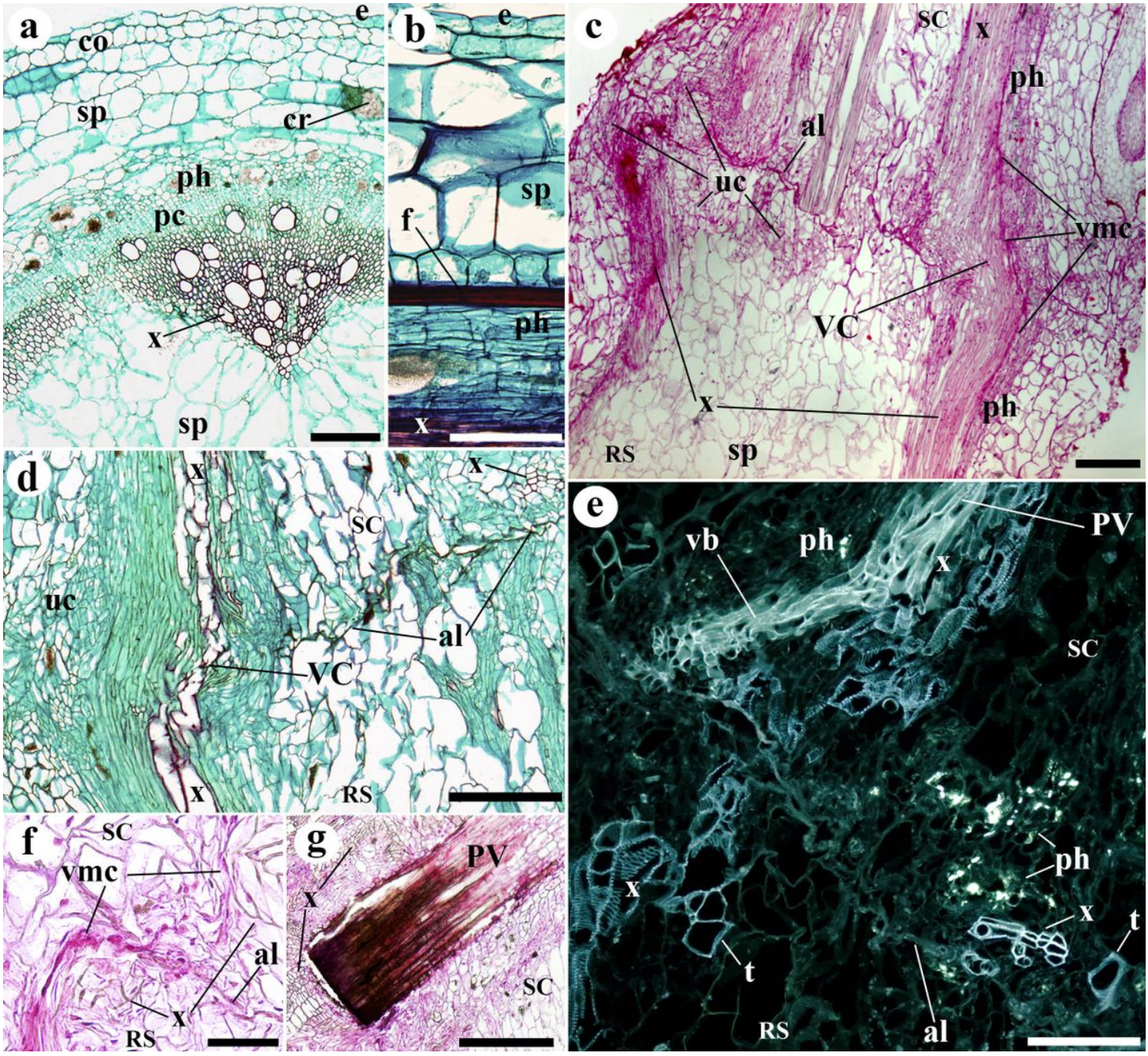

Figure 1

a Transverse section of a stem of Solanum lycopersicum showing the distribution of the tissues during primary growth. b Longitudinal section of a stem; note the distribution of tissues from the exterior to interior (up-down). c Longitudinal section of the graft junction area at 10 days after grafting; note the adhesion line (al), the new vascular connections (VC), and the areas with undifferentiated cells (uc). d Longitudinal section of the graft junction area at 10 days after grafting; note the vascular connections (VC) and adhesion line (al). Close to the adhesion line, in the scion, note the groups of xylem cells (x). e Longitudinal section of the graft junction area at 10 days after grafting; note the transdifferentiated parenchyma cells (t) in the scion and rootstock, the phloem (ph, yellow points), the vascular branch (vb) arising from the pre-existing vasculature (PV), and the large number of neo-differentiated cells in both

scion and rootstock. f Longitudinal section of the graft junction area at 10 days after grafting; note the vascular connection between the scion and rootstock, and how the vascular meristematic cells make contact across the adhesion line (al). g Pre-existing vascular tissue (PV) at 10 days after grafting; note how the most distal end is now composed of dead cells only. a, b, d Safranin-fast green; c, f, g Haematoxylin-Eosin; e Sirofluor. a-d, f, g Bright field view. e Epifluorescence microscopy. al adhesion line, cr microcrystals, co collenchyma, e epidermis, f fibre, PV pre-existing vascular tissue, vmc vascular meristematic cells, ph phloem, RS rootstock, SC scion, sp storage parenchyma, t transdifferentiating cells, uc undifferentiated cells, vb vascular branch, VC vascular connection, x xylem. Scale bars: a, d, e = 150 μm; b, g = 100 μm; c = 300 μm; f = 200 μm.

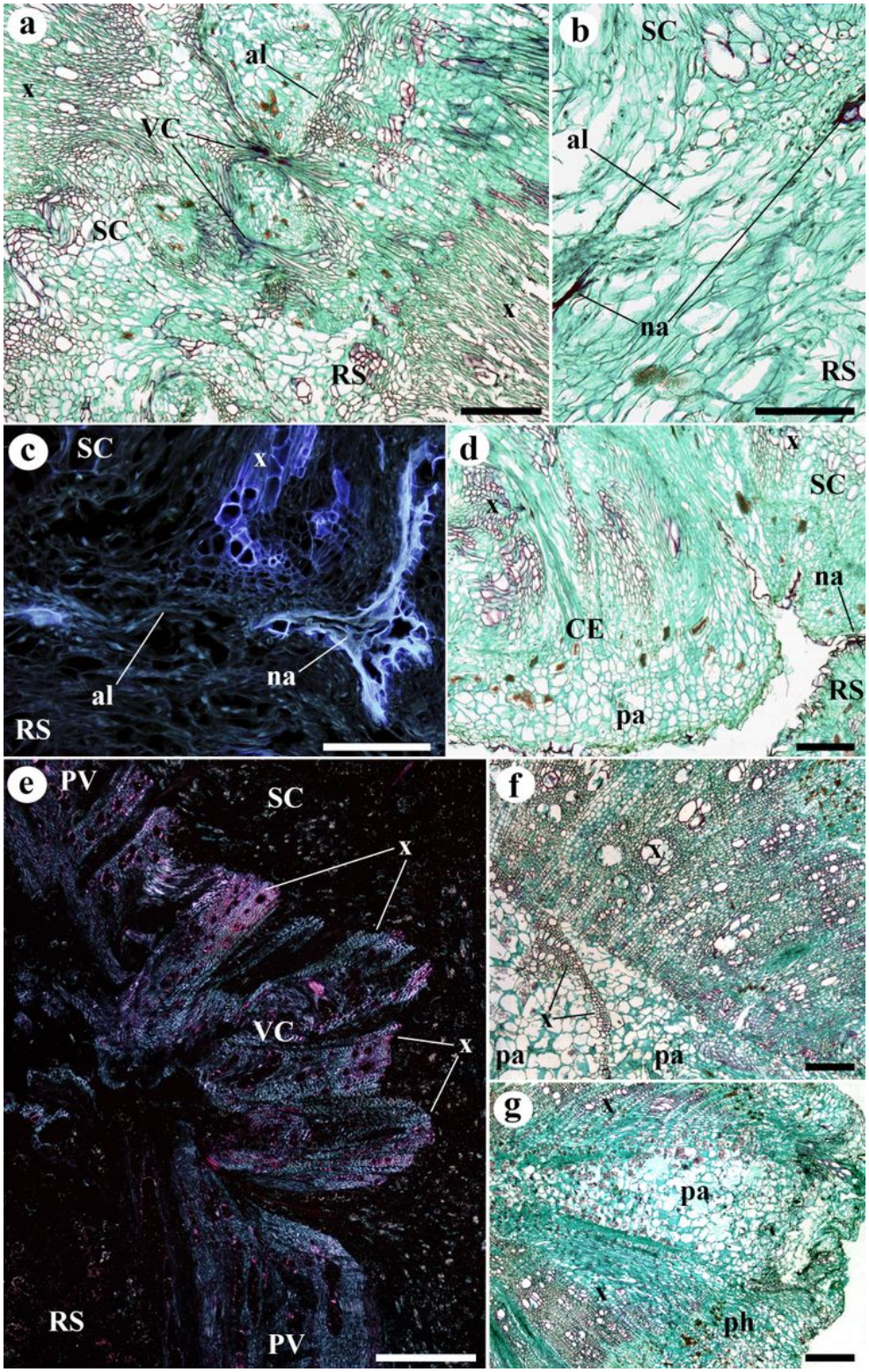

Figure 2

a Longitudinal section of the graft junction area at 20 days after grafting. Note the large amount of vascular tissue, mostly xylem (x), and the vascular connections (VC). The adhesion line (al) is no longer so clearly visible. b, c Longitudinal section of the adhesion zone at 20 days after grafting. The adhesion line (al) is hard to distinguish, but areas of non-adhesion (na) are clearly visible. d Longitudinal section at 20 days after grafting of the expansion of the callus (CE); note the vascular tissue (x) and the

parenchyma (pa) towards the exterior. e Longitudinal section of the graft junction at 20 days after grafting. Note the vascular connection (VC) now clearly formed. Note too that the xylem (x) in the junction is different to that seen in the pre-existing vasculature (PV). f Transverse section of the graft junction area at 210 days after grafting; note the left-over disconnected xylem (x) by parenchyma (pa) tissue. g Transverse section of the graft junction area at 210 days after grafting, showing the discontinuity of the vascular ring. a, b, d, f, g Safranin-fast green. a, b, d, f, g Bright field view. c, e Epifluorescence microscopy. al adhesion line, CE callus expansion, na non-adhesion, pa parenchyma, ph phloem, PV pre-existing vascular tissue, RS rootstock, SC scion, VC vascular connection, x xylem. Scale bars a, c, d, f, g = 200 μm; b = 100 μm; e = 300 μm.

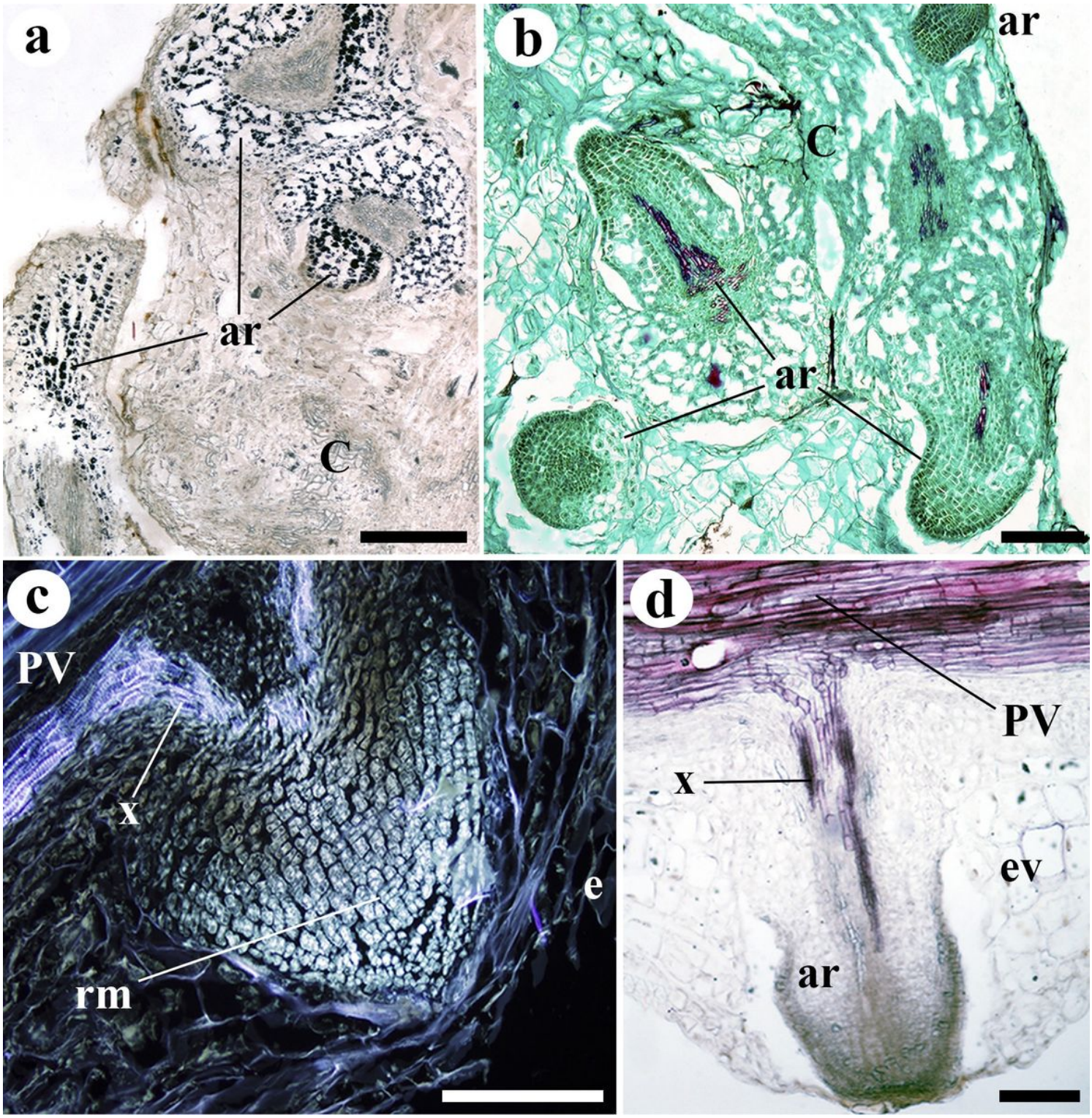

Figure 3

Adventitious roots arising from the scion at 20 days after grafting. a Longitudinal section of an adventitious root (ar); note the many amyloplasts (black dots). b Adventitious roots (ar) arising from the callus (C) of the scion near the graft junction; note how the top adventitious root breaks through the epidermis. c Longitudinal section of an adventitious root primordium, showing how it arises from the pre-existing vasculature (PV); the root meristem (rm) is also distinguishable. Note how the epidermis is

perforated (e). d Longitudinal section of an adventitious root (ar) primordium, showing how the root vascular tissue arises from the pre-existing vasculature (PV). Note the enveloping parenchyma around the structure of the root (ev). a Lugol; b Safranin-fast green; d Phloroglucinol. a, b, d Bright field view. c Epifluorescence microscopy. ar adventitious root, C callus, e, epidermis, ev enveloping parenchyma, PV pre-existing vascular tissue, rm root meristem, x xylem. Scale bars: a = 300 μm; b = 200 μm; c, d = 100 μm.