

Anatomy of epithemal hydathodes in four monocotyledon plants of economic and academic relevance

Running title: Anatomy of monocot hydathodes

Alain Jauneau ^{1*}, Aude Cerutti ², Marie-Christine Auriac ^{1,2} and Laurent D. Noël ^{2*}

¹ Fédération de Recherche 3450, Université de Toulouse, CNRS, Université Paul Sabatier, Castanet-Tolosan, France

² LIPM, Université de Toulouse, INRAE, CNRS, Université Paul Sabatier, Castanet-Tolosan, France

* Authors for correspondence:

Alain Jauneau; Institut Fédératif de Recherche 3450, Plateforme Imagerie, Pôle de Biotechnologie Végétale, Castanet-Tolosan 31326, France; E-mail: jauneau@lrsv.ups-tlse.fr

Laurent D. Noël; Laboratoire des interactions plantes micro-organismes (LIPM), UMR2594/441 CNRS/INRA, chemin de Borde Rouge, CS52627, F-31326 Castanet-Tolosan Cedex, France; Tel: +33 5 6128 5047; E-mail: laurent.noel@inrae.fr.

AJ and AC contributed equally to this study

21 **Abstract**

22 Hydathode is a plant organ responsible for guttation in vascular plants, i.e. the release of droplets at
 23 leaf margin or surface. Because this organ connects the plant vasculature to the external environment,
 24 it is also a known entry site for some vascular pathogens. In this study, we present a detailed
 25 microscopic examination of monocot hydathodes for three crops (maize, rice and sugarcane) and the
 26 model plant *Brachypodium distachyon*. Our study highlights both similarities and specificities of
 27 those epithemal hydathodes. These observations will serve as a foundation for future studies on the
 28 physiology and the immunity of hydathodes in monocots.

29

30 **Keywords**

31 *Brachypodium*; Epithem; Guttation; Leaf tip; Maize; Rice; Passive hydathode; Sugarcane; Water
 32 pore; Vessels; Xylem.

33

34

35 Introduction

36 Hydathodes are organs found on leaves, sepals and petals of all vascular plants and are responsible
 37 for guttation. This phenomenon is the release of fluids usually observed in conditions where stomata
 38 are closed and humidity high. Guttation is supposed to play an important role in plant physiology to
 39 promote water movement *in planta* in specific conditions [1, 2], to detoxify plant tissues by exporting
 40 excessive salts or molecules [3, 4] and to specifically capture some solutes from xylem sap before
 41 guttation [5]. These hydathodes thus appear as an interface between the plant vasculature and the
 42 outside.

43
 44 Hydathodes can be found at the leaf tip (apical hydathodes), at the surface of the leaf (laminar
 45 hydathodes) and at leaf margin (marginal hydathodes) depending on the plant family [for review, see
 46 6]. Despite this diversity, hydathodes share a conserved anatomy: i) epidermal water pores,
 47 resembling stomata at the surface, ii) a parenchyma called the epithem, composed of small loosely
 48 connected cells and many intercellular spaces and iii) a hypertrophied and branched xylem system
 49 irrigating the epithem [7, 8]. In some plants, the epithem may be physically separated from the
 50 mesophyll by a bundle sheath or a compact layer of cells called tanniferous bundle [7].

51
 52 Hydathodes are also relevant to plant health because they represent natural entry points for several
 53 vascular pathogens in both monocot and dicot plants. Hydathode infection is visible by chlorotic and
 54 necrotic symptoms starting at leaf tips or leaf margins leading to systemic infections as observed in
 55 black rot of Brassicaceae [9], in bacterial blight of aroids caused by *Xanthomonas axonopodis* pv.
 56 *dieffenbachiae* [10, 11], in bacterial canker of tomato caused by *Clavibacter michiganensis* subsp.
 57 *michiganensis* [12] and in bacterial leaf blight of rice caused by *X. oryzae* pv. *oryzae* (*Xoo*) [13-16].
 58 Certain pathogens are thus adapted to colonize the hydathode niche and access plant vasculature.

59

Though hydathodes were first described over a century ago, their anatomy is still poorly described. Most published studies use single microscopic techniques and provide descriptions of either surface or inner organizations so that a global overview of the organ is difficult to capture. Because most of the anatomic studies were performed before the 80s, literature search engines such as Pubmed will not lead you to such publications. Anatomy of arabidopsis hydathodes has only been recently reported [9]. Only scarce descriptions are available for monocot hydathodes, and none in the model plant *Brachypodium distachyon*. In rice (*Oryza sativa*) hydathodes, the large vessel elements are not surrounded by a bundle sheath but included in a lacunar mesophyll facing water pores [17, 18]. In barley (*Hordeum vulgare*), a single hydathode is also found at the leaf tip and water pores are reported to be very close to vascular elements [19]. In wheat (*Triticum aestivum*), an ultrastructural study showed that intercellular space directly connects vessel elements with water pores [17]. Determining or refining the anatomy of hydathodes in those or other monocots is thus a prerequisite to study the physiology and immunity of those organs.

In this study, we report on anatomy of hydathodes in four species of monocots, such as rice, sugarcane, maize and the model plant *Brachypodium distachyon* using a combination of optical and electron microscopy on fresh or fixed tissues. Our study highlights both similarities and specificities of those epithemal hydathodes and provides a comprehensive overview of their anatomy.

Results

SEM observation of leaf tips in four monocot plants reveals the presence of water pores anatomically distinct from stomata

Guttation was observed at leaf tips in maize, rice, *Brachypodium* and sugarcane indicating the presence of apical hydathodes (Fig. 1A, 2A, 3A, 4A). Though guttation at leaf margins can also be observed in sugarcane (Fig. 1A) and maize (data not shown), we did not study marginal hydathodes in the frame of this study. In order to characterize apical hydathodes in these four plants, we first

observed leaf tips by scanning electron microscopy (SEM). Leaves of rice (Fig. 2B), *Brachypodium* (Fig. 3B, G) and sugarcane (Fig. 4D) present elongated and thin tips compared to maize (Fig. 1A, B). All the leaf tips form a more or less pronounced gutter and are decorated by different appendages such as trichomes and spicules (Fig. 1B, 2B, 3B-C, 4B). Rice leaf tip and blade are covered on both faces by round-shaped spicules (Fig. 2B-D). At a smaller scale, numerous grooves and depressions associated to epidermal cell junctions are observed (Fig. 1C, 2C, 3B-E, 4B-C). On both sides of the leaf tips, numerous pores made of pairs of guard cells can be observed though sometimes with difficulty when located deep in the groove on the adaxial face of the leaf (Fig. 1B-D, 2C-D, 3B-E, 4B-C). In maize, rice and *Brachypodium*, such pores were only observed within 500 μm from the tip where guttation happens and are likely water pores. In sugarcane, the spike epidermis does not present water pores until $\sim 800 \mu\text{m}$ from the tip (Fig. 3B-C). Water pore features were also determined in parallel by the observation of fresh leaf tips mounted in water using confocal microscopy taking advantage of tissue autofluorescence (Fig. 1E-F, 2E, 3F-H, 4D-F). The following criteria discriminate water pores from stomata (S1 Fig.): their location at the tip of the leaf where guttation happens; their irregular distribution on the leaf surface compared to stomata; their insertion below the epidermal layer surface, forming a depression compared to the neighbouring epidermal cells; their thicker guard cells compared to stomata; their opened mouth though some may be occasionally closed or obstructed; the lack or low accumulation of cuticular waxes at the pore and neighbouring cell wall surfaces compared to stomata. Those changes are gradual along the leaf longitudinal axis suggesting a common developmental origin of both cell types influenced by positional or environmental cues.

Fig 1. Anatomic description of maize apical hydathodes by confocal and scanning electron microscopy (SEM). (A) A guttation droplet at the leaf tip. (B-D) The adaxial face of leaf tip was imaged by SEM. Water pores are observed in the gutter. (E-G) Confocal images of fresh adaxial face of the leaf tip. (E) The image is a maximal projection of 50 confocal planes in z dimension (1- μm

112 steps). (F, G) Observations in z axis at the epidermal level (F) and below the epidermal layer (G).
 113 Each overlay image corresponds to the maximal projection of 25-30 confocal planes acquired in z
 114 dimension. (H, J) Transversal sections (1 μm thickness) of fixed tissue at 80-100 μm from the tip
 115 were observed by confocal microscopy. White arrows, dashed arrows and asterisks indicate water
 116 pores, xylem vessels (xv) and large chambers and intercellular spaces, respectively. v, small veins;
 117 ad, adaxial face; ab, abaxial face. (I) Schematic drawing of the hydathode cross section observed in
 118 H. Scale bars: B, E-G: 100 μm ; C: 40 μm ; D, H-J: 30 μm .

119

120 **Fig 2. Anatomic description of rice apical hydathodes by confocal and scanning electron**
 121 **microscopy (SEM).** (A) A guttation droplet at the leaf tip. (B-D) The leaf tip was imaged by SEM.
 122 Water pores observed at the tip (C) and 300 μm from the extremity (D). (E) Confocal images of fresh
 123 tissue at 80-100 μm from the tip. The image is a maximal projection of 140 confocal planes in z
 124 dimension (1- μm steps). (F-G) Transversal sections (1 μm thickness) of fixed tissue at 50 μm from
 125 the tip were observed by confocal microscopy. White arrows and asterisk indicate water pores, and
 126 large chambers and intercellular spaces, respectively. ad, adaxial face; ab, abaxial face; xv, xylem
 127 vessels. (H) Schematic drawing of the hydathode cross section observed in F. Scale bars: B: 100 μm ;
 128 C-H: 30 μm .

129

130 **Fig 3. Anatomic description of *Brachypodium distachyon* apical hydathodes by confocal and**
 131 **scanning electron microscopy (SEM).** (A) A guttation droplet at the leaf tip. (B-E) The leaf tip was
 132 imaged by SEM. Water pores observed on the abaxial (B, D) and adaxial (C, E) faces of the leaf tip.
 133 (F-H) Confocal images of fresh leaf tips on their abaxial (F) and adaxial (G, H) faces. (I, K)
 134 Transversal sections (1 μm thickness) of fixed tissue at 60-70 μm (I) and 200 μm (K) from the tip
 135 were observed by confocal microscopy. White arrows, dashed arrows and asterisk indicate water
 136 pores, xylem vessels (xv) and large chambers and intercellular spaces, respectively. v, small veins;

ad, adaxial face; ab, abaxial face. (J) Schematic drawing of the hydathode cross section observed in I. Scale bars: B, C, F, G: 100 μ m; I, J, K: 50 μ m; H: 20 μ m; D, E: 10 μ m.

Fig 4. Anatomic description of sugarcane apical hydathodes by confocal and scanning electron microscopy (SEM). (A) A guttation droplet at the leaf tip. (B-C) The leaf tip was imaged by SEM on the adaxial face at 200 μ m (B) and 300 μ m (C) from the spike tip. Water pores can be observed. (D-F) Confocal images of fresh leaf tip at 250-300 μ m from the spike tip. Details from water pores (E-F). (G-I) Transversal sections (1 μ m thickness) of fixed tissue at 150-200 μ m (G) and 800 μ m (H, I) from the spike tip were observed by confocal microscopy. White arrows and asterisk indicate water pores and large chambers and intercellular spaces, respectively. ad, adaxial face; ab, abaxial face; xv, xylem vessels. (J) Schematic drawing of a hydathode cross section observed at 800 μ m from the spike. Scale bars: D, J: 100 μ m; B, C: 50 μ m; H, I: 40 μ m; E-G: 30 μ m.

Anatomy of monocot apical hydathodes is characteristic of epithemal hydathodes

The inner organization of hydathodes was first investigated by confocal microscopy on fresh samples using the autofluorescence of cell walls. In maize, chambers and xylem vessels could be visualized below the water pores (Fig. 1F-G). Yet, collecting information on the organization of the rest of the tissue remained challenging likely due to the limited cell wall fluorescence. Transversal thin sections from fixed samples were thus prepared and observed to refine the cytology of inner tissues. In the four monocot species studied, we confirmed the presence of a chamber below each water pore (Fig. 1H-J, 2F-G, 3K, 4H-I). The surrounding tissues are formed by loosely packed parenchyma cells with numerous intercellular spaces which form a continuous network from the water pore chamber to the vascular elements. In contrast to the leaf vasculature, hydathode vasculature is more disorganized and assembled into groups of two and more xylem vessels each which are surrounded neither by a bundle sheath nor by a layer of thickened-wall cells (Fig. 1H-J, 2F-H, 3I-K, 4G-J). Such organization is typical of epithemal hydathodes as defined [6]: epidermal water pores, sub-water pore chambers,

163 loose small-celled parenchyma called epithem tightly and directly connected to an abundant
164 vasculature.
165 Some levels of variation in this organization pattern can be observed (See schematic drawings in Fig.
166 1I, 2H, 3J, 4J). In maize and rice, the epithem occupies with the vasculature the whole inner space of
167 the hydathodes. In rice, the epithem is sometimes so reduced (Fig. 2F-H) so that the connection
168 between the water pores and the vascular elements is sometimes direct (Fig. 2G). In sugarcane and
169 *Brachypodium*, the hydathode tissues are embedded in a parenchyma distinct from the epithem and
170 made of larger and more compact cells with occasional (sugarcane) or systematic (*Brachypodium*)
171 cell wall re-enforcements (Fig. 3I-K, 4G-J). In all instances, the plant vasculature remains easily
172 connected to the outside thanks to the absence of a bundle sheath and the loose organization of the
173 epithem thus allowing a free apoplastic flow of guttation fluid from the xylem vessels to the water
174 pores.

175

176 **Loss of pit membrane integrity can be observed in some xylem vessels inside apical hydathodes**

177 In order to better observe the cell wall of xylem vessels within hydathodes, we used transmission
178 electron microscopy (TEM) coupled to PATAg labelling of cell wall polysaccharides (S2 Fig.).
179 Xylem vessel elements were identified by the presence of their lignified secondary cell wall
180 thickenings. Cell wall thickenings were most developed in maize (S2A-B Fig.) compared to rice,
181 sugarcane or *Brachypodium* (S2C-J Fig.). Between these ornamentations, a cell wall ca. ten times
182 thinner than the primary cell wall of the neighbouring epithem parenchyma cells is observed and
183 called pit membrane. PATAg labelling of these pit membranes is heterogeneous and discontinuous
184 (S2D, H, J Fig.) indicative of either a distinct polysaccharidic composition of these domains or the
185 absence of any cell wall barriers. Altogether, these observations indicate that barriers potentially
186 limiting the flow of fluids across the cell wall of the xylem vessels are minimal.

187

188 **Discussion**

Variations on the theme of epithemal hydathodes in monocots

In monocots, guttation is always observed at leaf tips and sometimes at leaf margins such as in maize and sugarcane (Fig. 4) [6]. Apical hydathodes were easy to identify at leaf apex where the vasculature converges. Previous observations of monocots hydathodes by light microscopy and sometimes by scanning and transmission electron microscopy were often partial and limited to rice, barley and wheat [5, 17, 19-23]. Here, a full set of microscopic techniques was used yielding a comprehensive description of both surface and inner anatomy of hydathodes in rice and three additional monocot plants. Our study confirmed some of the observations made in rice and revealed the conservation of several features of epithemal hydathodes in monocots: reduced wax apposition on the epidermis, opened water pores morphologically distinct from stomata, presence of a reduced epithem [17, 19] and dense xylem system. Main differences besides leaf curling were the shape of the leaf tip, the hydathode surface (presence of trichomes or spicules...), the size of hydathodes or the abundance of epithem cells relative to the parenchyma.

A developmental gradient: from water pores to stomata

Mutations affecting stomatal development often similarly affect water pore development [24]. Several markers for stomatal identity or differentiation cannot be used to differentiate water pores for stomata either [9], thus suggesting a common origin of both cell types. Yet, several morphological differences can distinguish water pores from stomata. Water pores are often inserted deeper in the epidermis. Also, the subsidiary cells known to be important for stomatal movement [25] could not be observed around water pores. Yet, the transition from water pores to stomata is not as dramatic in monocots as in dicots [9, 26] and it is thus sometimes difficult to locate the hydathode boundaries in monocots. We could observe a developmental gradient between water pores and stomata along the leaf longitudinal axis with morphological traits of water pores being more pronounced at leaf apex. If the chemical nature of such gradient is unknown, auxin maxima and expression of auxin biosynthetic genes is observed in rice hydathodes [27] similar to dicots [28, 29]. Because auxin was

recently described as a negative regulator of stomatal differentiation [30], it remains to be tested whether auxin accumulating at hydathodes could impact water pore differentiation and be responsible for the observed developmental gradient.

Morphological adaptations of monocot hydathodes driving guttation

Monocots hydathodes exhibit some features that can help support guttation such as the absence of bundle sheath between the xylem vessels and the epithem, the thin cell walls of xylem vessels, the reduced epithem with many lacunas, the pores and the cup shape of the leaf. These features are not specific of monocots and some can be found in dicots [6]. Similar to cauliflower and Arabidopsis [9], reduced epicuticular wax depositions are observed at hydathodes compared to the leaf blade which could help preventing guttation droplets from falling. These surface properties come in addition to leaf shape adaptations such as grooves, trichomes or indentations which should also favour droplet formation and accumulation at hydathodes.

Monocot hydathodes can provide facilitated access to plant vasculature for microbial pathogens

Leaf surface properties such as the cuticle and epicuticular waxes also strongly affect microbial adhesion, behaviour and survival in the phyllosphere [for review, see 31]. For instance, leaf wettability in maize is positively correlated to the charge in epiphytic bacteria [32]. Thus, rain or spray irrigation may concentrate microbes at hydathodes. While hydathode anatomy likely offers little resistance to water fluxes, it also represents a potential breach which could be exploited by pathogens to access plant inner tissues, including the vasculature. For instance, we describe that rice xylem vessels are almost directly accessible once through water pores. Besides, we also observe holes in the pit membrane of xylem vessels in *Brachypodium* or sugarcane giving a facilitated access to the vasculature. Thus, xylem vessels within monocot hydathodes seem a lot more vulnerable to infection compared to dicot hydathodes where the epithem tissue is much more developed. The number of pathogens able to infect monocot hydathodes is also likely underestimated since *X. albilineans*, the

causal agent of leaf scald in sugarcane [33, 34] and *X. translucens*, the causal agent of bacterial leaf streak on a broad host range of cereal crops and grasses [35-38] both cause symptoms starting from leaf tip or the leaf margin.

To conclude, we described apical hydathodes from four monocot species. Besides species-to-species and leaf-to-leaf variations, we recognized anatomical features typical of epithemal hydathodes. The presence of grooves and trichomes and lower wax apposition at apical hydathodes seem adapted to hold guttation droplets at leaf tips. Open water pores provide an almost direct access the vascular elements due to a sometime reduced epithem and a thin primary cell wall of xylem vessels. Our analyses form the basis for further investigations on the physiology and the immunity of hydathodes those monocot plants.

Materials and Methods

Plant material and growth conditions

The following plant species were studied: *Brachypodium distachyon*, rice (*Oryza sativa* var. Kitaake), sugarcane (*Saccharum officinarum* x *Saccharum spontaneum* hybrid, var HOCP04838, Q155 or CAS2) and maize (*Zea mays* var. P1524, Pioneer Dupont). The position of the leaf used for microscopy for *Brachypodium distachyon* and sugarcane was not possible to determine. For maize and rice, hydathodes of the second leaf were observed.

Scanning electron microscopy (SEM)

Leaf samples were fixed under vacuum for 30 min with 2.5% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) containing 0.1% triton X-100 and at atmospheric pressure for 1h in the same solution without Triton X-100. Samples were dehydrated in a series of aqueous solutions of increasing ethanol concentrations (25, 50, 70, 95, 100%, 1 h each) and then critical-point dried with

liquid CO₂. Samples were attached with double-sided tape to metal stubs grounded with conductive silver paint and sputter-coated with platinum. Images were acquired with a scanning electron microscope (Quanta 250 FEG FEI) at 5kV with a working distance of 1 cm.

Optical and transmission electron microscopy

Preparation of hydathode samples for both optical and transmission electron microscopy were previously detailed [9, 39]. To observe fresh samples, leaf tips (1.5 cm in length) were mounted in water on a glass slide and covered with a coverslip. Images were acquired with a laser scanning confocal microscope (LSCM, Leica SP2 AOBS, Mannheim, Germany). To perform hydathode sections, leaf tips were fixed under vacuum for 30 min with 2.5% glutaraldehyde in 0.2 mM sodium cacodylate buffer (pH 7.2) containing 0.1% triton X-100 and then at the atmospheric pressure for 1h in the same solution without triton X-100. The samples were then rinsed in the same cacodylate buffer, dehydrated in a series of aqueous solutions of increasing ethanol concentrations and infiltrated step-wise in LR White resin. They were finally polymerized for 24h at 60°C. From embedded material, thin (1 µm in thickness) or ultra-thin (80–90 nm in thickness) sections were prepared using an UltraCut E ultramicrotome equipped with a diamond knife (Reichert-Leica, Germany). Transversal thin sections were used to acquire images with LSCM. All confocal images are the overlay of blue (410-470 nm), green (500-580 nm) and red (650-750 nm) channels used to depict the cell walls and the chlorophyll autofluorescence after excitation using a 405-nm diode laser. For transmission electron microscopy (TEM), ultra-thin sections were collected on gold grids and submitted to the periodic acid-thiocarbohydrazide-silver proteinate reaction (PATAg). PATAg staining of polysaccharides was used to enhance contrast and observe xylem ornamentations and pit membranes. Images were acquired using a Hitachi-HT-7700 (Japan) transmission electron microscope operating at 80 kV.

Acknowledgements

292 We are grateful to Jean-Heinrich Daugrois, Monique Royer (CIRAD, Montpellier, France) and Pam
 293 Ronald (UC Davis, CA) for contributing fixed sugarcane material and rice seeds, respectively. We
 294 wish to thank Jean-Marc Routaboul (INRA Toulouse) for critically reading the manuscript. Electron
 295 microscopy was performed on the CMEAB (Centre de Microscopie Electronique Appliquée à la
 296 Biologie) core facility with the expert assistance of Isabelle Fourquaux et Bruno Payre (Université
 297 Toulouse III Paul Sabatier, Toulouse, France). AJ and LDN are supported by the NEPHRON project
 298 (ANR-18-CE20-0020-01). This work was supported by a PhD grant from the French Ministry of
 299 National Education and Research to AC. LIPM is part of the TULIP LabEx (ANR-10-LABX-41;
 300 ANR-11-IDEX-0002-02).

301

302 **Author Contributions**

303 AJ, AC and LDN designed the experiments; AJ, AC and MCA performed the experiments; All
 304 authors analysed the results; AJ and AC designed the figures. AJ, AC and LDN wrote the manuscript.
 305 All authors approved the final version of the manuscript.

306 **References**

307

- 308 1. Pedersen O. Long-Distance Water Transport in Aquatic Plants. Plant Physiol.
 309 1993;103(4):1369-75. Epub 1993/12/01. doi: 10.1104/pp.103.4.1369. PubMed PMID: 12232030;
 310 PubMed Central PMCID: PMCPMC159128.
- 311 2. Chen YC, Lin TC, Martin CE. Effects of guttation prevention on photosynthesis and
 312 transpiration in leaves of *Alchemilla mollis*. Photosynthetica. 2015;52(3):371-6. doi: 10.1007/s11099-
 313 014-0040-y.
- 314 3. Sutton T, Baumann U, Hayes J, Collins NC, Shi BJ, Schnurbusch T, et al. Boron-toxicity
 315 tolerance in barley arising from efflux transporter amplification. Science. 2007;318(5855):1446-9.
 316 Epub 2007/12/01. doi: 10.1126/science.1146853. PubMed PMID: 18048688.

- 317 4. Shapira OR, Israeli Y, Shani U, Schwartz A. Salt stress aggravates boron toxicity symptoms
318 in banana leaves by impairing guttation. *Plant Cell Environ.* 2013;36(2):275-87. Epub 2012/07/07.
319 doi: 10.1111/j.1365-3040.2012.02572.x. PubMed PMID: 22765264.
- 320 5. Nagai M, Ohnishi M, Uehara T, Yamagami M, Miura E, Kamakura M, et al. Ion gradients in
321 xylem exudate and guttation fluid related to tissue ion levels along primary leaves of barley. *Plant*
322 *Cell Environ.* 2013;36(10):1826-37. Epub 2013/03/08. doi: 10.1111/pce.12090. PubMed PMID:
323 23464633.
- 324 6. Cerutti A, Jauneau A, Laufs P, Leonhardt N, Schattat MH, Berthome R, et al. Mangroves in
325 the Leaves: Anatomy, Physiology, and Immunity of Epithelial Hydathodes. *Annu Rev Phytopathol.*
326 2019;57:91-116. Epub 2019/05/19. doi: 10.1146/annurev-phyto-082718-100228. PubMed PMID:
327 31100996.
- 328 7. Perrin A. Contribution à l'étude de l'organisation et du fonctionnement des hydathodes:
329 recherches anatomiques ultrastructurales et physiologiques: Université Claude Bernard - Lyon; 1972.
- 330 8. Haberlandt G. *Physiological Plant Anatomy*: Macmillan and Co.; 1914 1914.
- 331 9. Cerutti A, Jauneau A, Auriac MC, Lauber E, Martinez Y, Chiarenza S, et al. Immunity at
332 Cauliflower Hydathodes Controls Systemic Infection by *Xanthomonas campestris* pv *campestris*.
333 *Plant Physiol.* 2017;174(2):700-16. Epub 2017/02/12. doi: 10.1104/pp.16.01852. PubMed PMID:
334 28184011; PubMed Central PMCID: PMC5462019.
- 335 10. Robene-Soustrade I, Laurent P, Gagnevin L, Jouen E, Pruvost O. Specific detection of
336 *Xanthomonas axonopodis* pv. *dieffenbachiae* in anthurium (*Anthurium andreanum*) tissues by nested
337 PCR. *Appl Environ Microbiol.* 2006;72(2):1072-8. Epub 2006/02/08. doi: 10.1128/AEM.72.2.1072-
338 1078.2006. PubMed PMID: 16461651; PubMed Central PMCID: PMC1392979.
- 339 11. Toves PJ. Enhancement of biological control of anthurium blight caused by *Xanthomonas*
340 *axonopodis* pv. *dieffenbachiae*. 2008.

- 341 12. Carlton WM, Braun EJ, Gleason ML. Ingress of *Clavibacter michiganensis* subsp.
342 *michiganensis* into Tomato Leaves Through Hydathodes. *Phytopathology*. 1998;88(6):525-9. Epub
343 2008/10/24. doi: 10.1094/PHYTO.1998.88.6.525. PubMed PMID: 18944904.
- 344 13. Feng T, Kuo T. Bacterial leaf blight of rice plant. VI. Chemotactic responses of *Xanthomonas*
345 *oryzae* to water droplets exudated from water pores on the leaf of rice plants. *Bot Bull Acad Sin*.
346 1975;16:126-36.
- 347 14. Nino-Liu DO, Ronald PC, Bogdanove AJ. *Xanthomonas oryzae* pathovars: model pathogens
348 of a model crop. *Mol Plant Pathol*. 2006;7(5):303-24. Epub 2006/09/01. doi: 10.1111/j.1364-
349 3703.2006.00344.x. PubMed PMID: 20507449.
- 350 15. Buttner D, Bonas U. Regulation and secretion of *Xanthomonas* virulence factors. *FEMS*
351 *Microbiol Rev*. 2010;34(2):107-33. Epub 2009/11/21. doi: 10.1111/j.1574-6976.2009.00192.x.
352 PubMed PMID: 19925633.
- 353 16. Zhang H, Wang S. Rice versus *Xanthomonas oryzae* pv. *oryzae*: a unique pathosystem. *Curr*
354 *Opin Plant Biol*. 2013;16(2):188-95. Epub 2013/03/08. doi: 10.1016/j.pbi.2013.02.008. PubMed
355 PMID: 23466254.
- 356 17. Maeda E, Maeda K. Ultrastructural studies of leaf hydathodes. II. Rice (*Oryza sativa*) leaf
357 tips. *Japanese journal of crop science*. 1988;57(4):733-42. doi: 10.1626/jcs.57.733.
- 358 18. Kawata S-i, Yamazaki K. On the Barrel-Shaped Vessel Elements in Rice Leaves. *Japanese*
359 *journal of crop science*. 1963;31(4):332-6. doi: 10.1626/jcs.31.332.
- 360 19. Dieffenbach H, Kramer D, Lüttge U. Release of Guttation Fluid from Passive Hydathodes of
361 Intact Barley Plants. I. Structural and Cytological Aspects. *Annals of Botany*. 1980;45(4):397-401.
362 doi: 10.1093/oxfordjournals.aob.a085837.
- 363 20. Pedersen O, Bolt Jorgensen L, Sand-Jensen K. Through-flow of water in leaves of a
364 submerged plant is influenced by the apical opening. *Planta (Germany)*. 1997;202:43-50.

- 365 21. Tapparo A, Giorio C, Marzaro M, Marton D, Solda L, Girolami V. Rapid analysis of
366 neonicotinoid insecticides in guttation drops of corn seedlings obtained from coated seeds. J Environ
367 Monit. 2011;13(6):1564-8. Epub 2011/04/22. doi: 10.1039/c1em10085h. PubMed PMID: 21509402.
- 368 22. Bailey KJ, Leegood RC. Nitrogen recycling from the xylem in rice leaves: dependence upon
369 metabolism and associated changes in xylem hydraulics. J Exp Bot. 2016;67(9):2901-11. Epub
370 2016/04/08. doi: 10.1093/jxb/erw132. PubMed PMID: 27053722; PubMed Central PMCID:
371 PMCPMC4861031.
- 372 23. Hossain MB, Matsuyama N, Kawasaki M. Hydathode morphology and role of guttation in
373 excreting sodium at different concentrations of sodium chloride in eddo. Plant Production Science.
374 2016;19(4):528-39. doi: 10.1080/1343943x.2016.1210990.
- 375 24. Chater CCC, Caine RS, Fleming AJ, Gray JE. Origins and Evolution of Stomatal
376 Development. Plant Physiol. 2017;174(2):624-38. Epub 2017/03/31. doi: 10.1104/pp.17.00183.
377 PubMed PMID: 28356502; PubMed Central PMCID: PMCPMC5462063.
- 378 25. Peterson KM, Rychel AL, Torii KU. Out of the mouths of plants: the molecular basis of the
379 evolution and diversity of stomatal development. Plant Cell. 2010;22(2):296-306. Epub 2010/02/25.
380 doi: 10.1105/tpc.109.072777. PubMed PMID: 20179138; PubMed Central PMCID:
381 PMCPMC2845417.
- 382 26. Chen C-C, Chen Y-R. Study on the laminar hydathodes of *Ficus formosana* (Moraceae) V.:
383 Divergent evolution between stomata and water pores. Taiwan. 2019;64(2):149-62. doi:
384 10.6165/tai.2019.64.149
- 385 27. Yamamoto Y, Kamiya N, Morinaka Y, Matsuoka M, Sazuka T. Auxin biosynthesis by the
386 *YUCCA* genes in rice. Plant Physiol. 2007;143(3):1362-71. Epub 2007/01/16. doi:
387 10.1104/pp.106.091561. PubMed PMID: 17220367; PubMed Central PMCID: PMCPMC1820910.
- 388 28. Muller-Moule P, Nozue K, Pytlak ML, Palmer CM, Covington MF, Wallace AD, et al.
389 *YUCCA* auxin biosynthetic genes are required for Arabidopsis shade avoidance. PeerJ. 2016;4:e2574.

- 390 Epub 2016/10/21. doi: 10.7717/peerj.2574. PubMed PMID: 27761349; PubMed Central PMCID:
391 PMCPMC5068344.
- 392 29. Bilsborough GD, Runions A, Barkoulas M, Jenkins HW, Hasson A, Galinha C, et al. Model
393 for the regulation of *Arabidopsis thaliana* leaf margin development. Proc Natl Acad Sci U S A.
394 2011;108(8):3424-9. Epub 2011/02/09. doi: 10.1073/pnas.1015162108. PubMed PMID: 21300866;
395 PubMed Central PMCID: PMCPMC3044365.
- 396 30. Balcerowicz M, Hoecker U. Auxin--a novel regulator of stomata differentiation. Trends Plant
397 Sci. 2014;19(12):747-9. Epub 2014/12/03. doi: 10.1016/j.tplants.2014.10.006. PubMed PMID:
398 25458848.
- 399 31. Mechaber WL, Marshall DB, Mechaber RA, Jobe RT, Chew FS. Mapping leaf surface
400 landscapes. Proc Natl Acad Sci U S A. 1996;93(10):4600-3. Epub 1996/05/14. doi:
401 10.1073/pnas.93.10.4600. PubMed PMID: 11607676; PubMed Central PMCID: PMCPMC39323.
- 402 32. Beattie GA, Marcell LM. Comparative dynamics of adherent and nonadherent bacterial
403 populations on maize leaves. Phytopathology. 2002;92(9):1015-23. Epub 2008/10/24. doi:
404 10.1094/PHYTO.2002.92.9.1015. PubMed PMID: 18944027.
- 405 33. Pieretti I, Royer M, Barbe V, Carrere S, Koebnik R, Couloux A, et al. Genomic insights into
406 strategies used by *Xanthomonas albilineans* with its reduced artillery to spread within sugarcane
407 xylem vessels. BMC Genomics. 2012;13:658. Epub 2012/11/23. doi: 10.1186/1471-2164-13-658.
408 PubMed PMID: 23171051; PubMed Central PMCID: PMCPMC3542200.
- 409 34. Pieretti I, Pesic A, Petras D, Royer M, Sussmuth RD, Cociancich S. What makes
410 *Xanthomonas albilineans* unique amongst xanthomonads? Front Plant Sci. 2015;6:289. Epub
411 2015/05/13. doi: 10.3389/fpls.2015.00289. PubMed PMID: 25964795; PubMed Central PMCID:
412 PMCPMC4408752.
- 413 35. Vauterin L, Hoste B, Kersters K, Swings J. Reclassification of *Xanthomonas*. Int J Syst
414 Bacteriol. 1995;45(3):472-89.

- 415 36. Vauterin L, Rademaker J, Swings J. Synopsis on the taxonomy of the genus *Xanthomonas*.
 416 Phytopathology. 2000;90(7):677-82. Epub 2008/10/24. doi: 10.1094/PHYTO.2000.90.7.677.
 417 PubMed PMID: 18944485.
- 418 37. Bragard C, Singer E, Alizadeh A, Vauterin L, Maraite H, Swings J. *Xanthomonas translucens*
 419 from Small Grains: Diversity and Phytopathological Relevance. Phytopathology. 1997;87(11):1111-
 420 7. Epub 2008/10/24. doi: 10.1094/PHYTO.1997.87.11.1111. PubMed PMID: 18945007.
- 421 38. Duveiller E, Fucikovsky L, Rudolph K. The Bacterial Diseases of Wheat: Concepts and
 422 Methods of Disease Managements. CIMMYT ed. Mexico1997.
- 423 39. Cerutti A, Auriac M-C, Noël LD, Jauneau A. Histochemical Preparations to Depict the
 424 Structure of Cauliflower Leaf Hydathodes. Bio-protocol. 2017;7(20):e2452.

425

426 **Supporting information captions**

427 **S1 Fig.** Observation of leaf stomata from maize (A-B), rice (C-D), *Brachypodium* (E-F) and
 428 sugarcane (G) by scanning electron microscopy reveal high levels of surface waxes.

429

430 **S2 Fig.** Observation of pit membranes integrity in hydathodes of maize (A-B), rice (C-D),
 431 *Brachypodium* (E-F) and sugarcane (G) by transmission electron microscopy.

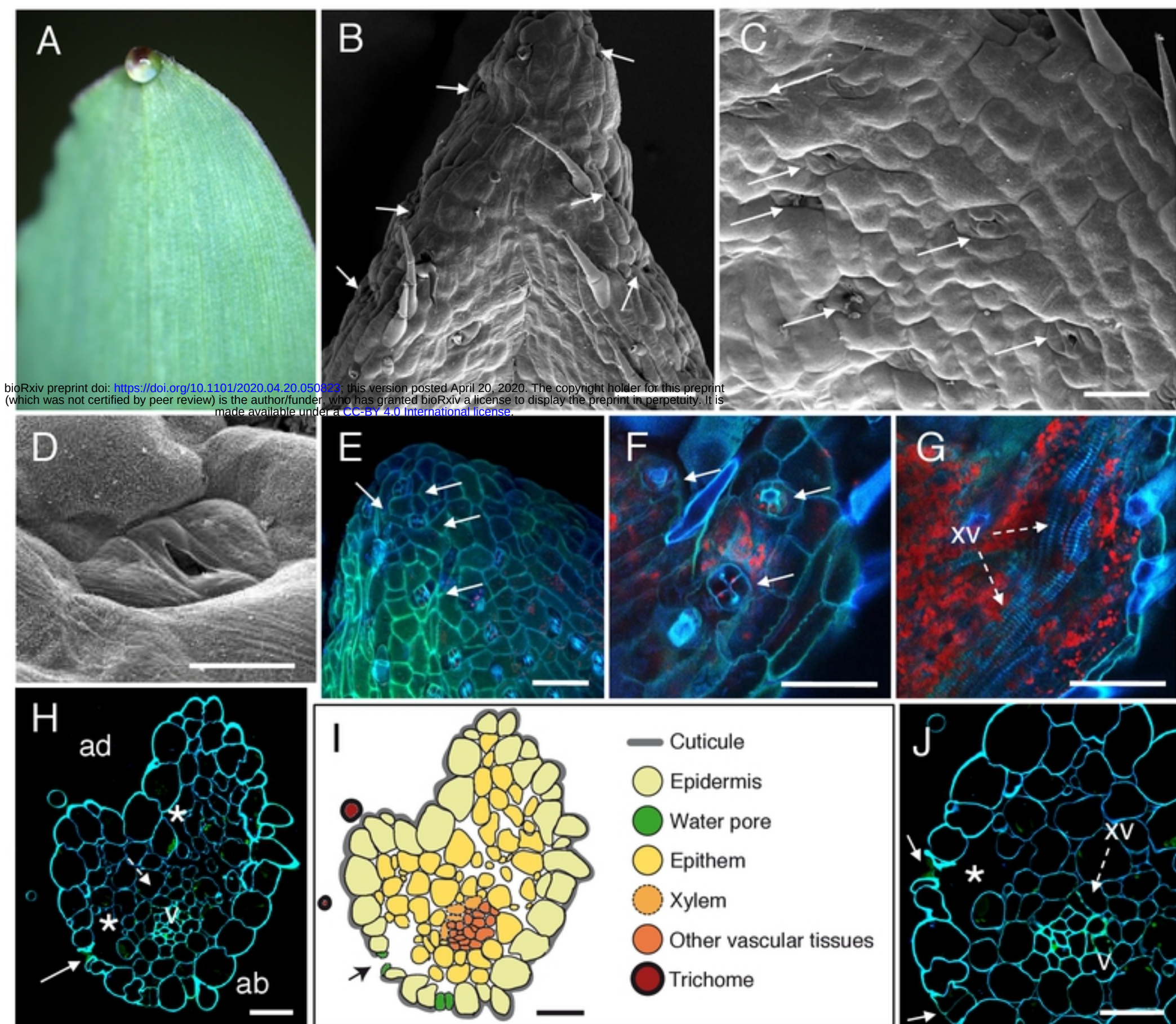


Figure 1
Jauneau et al.

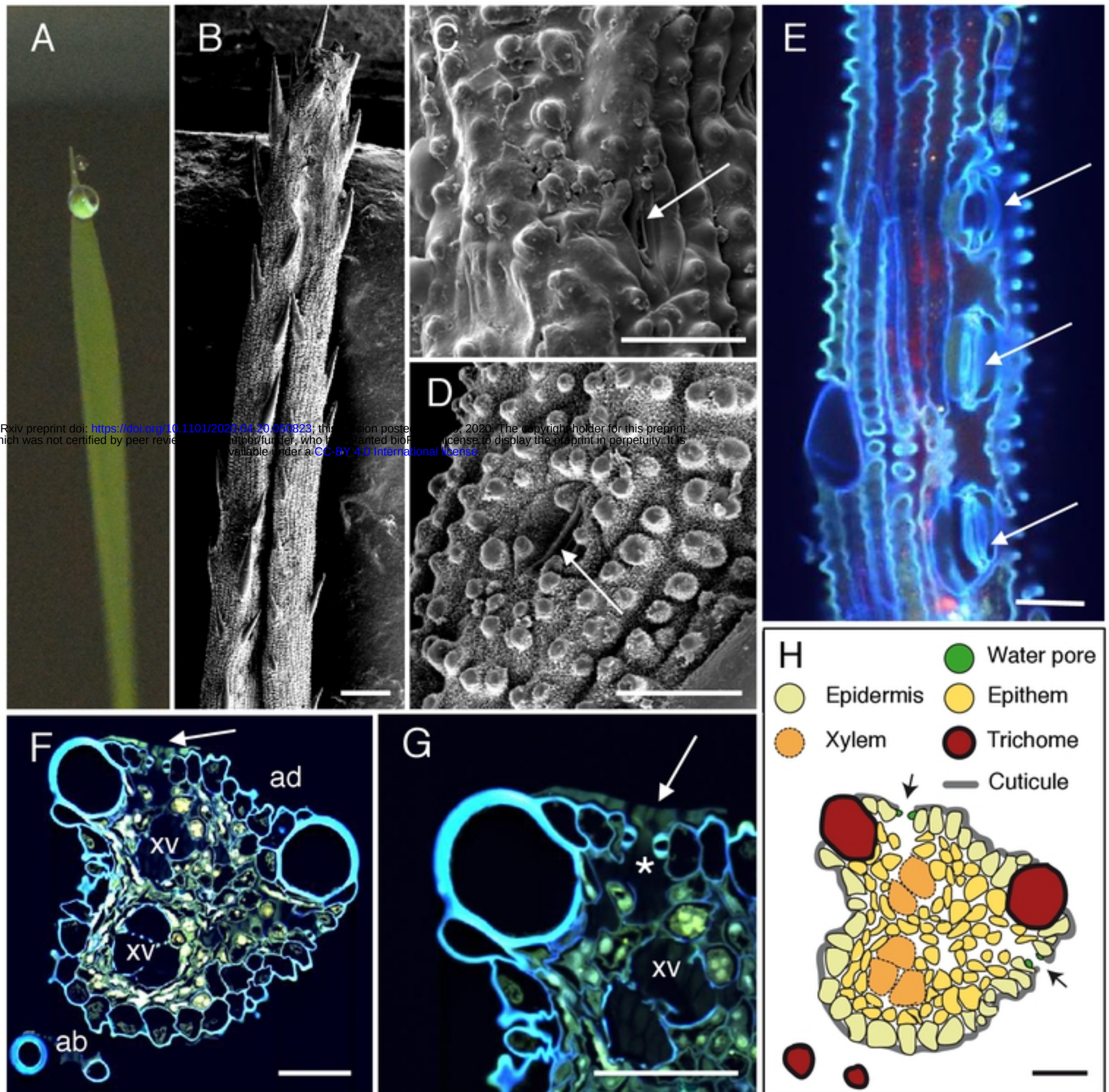


Figure 2
Jauneau et al.

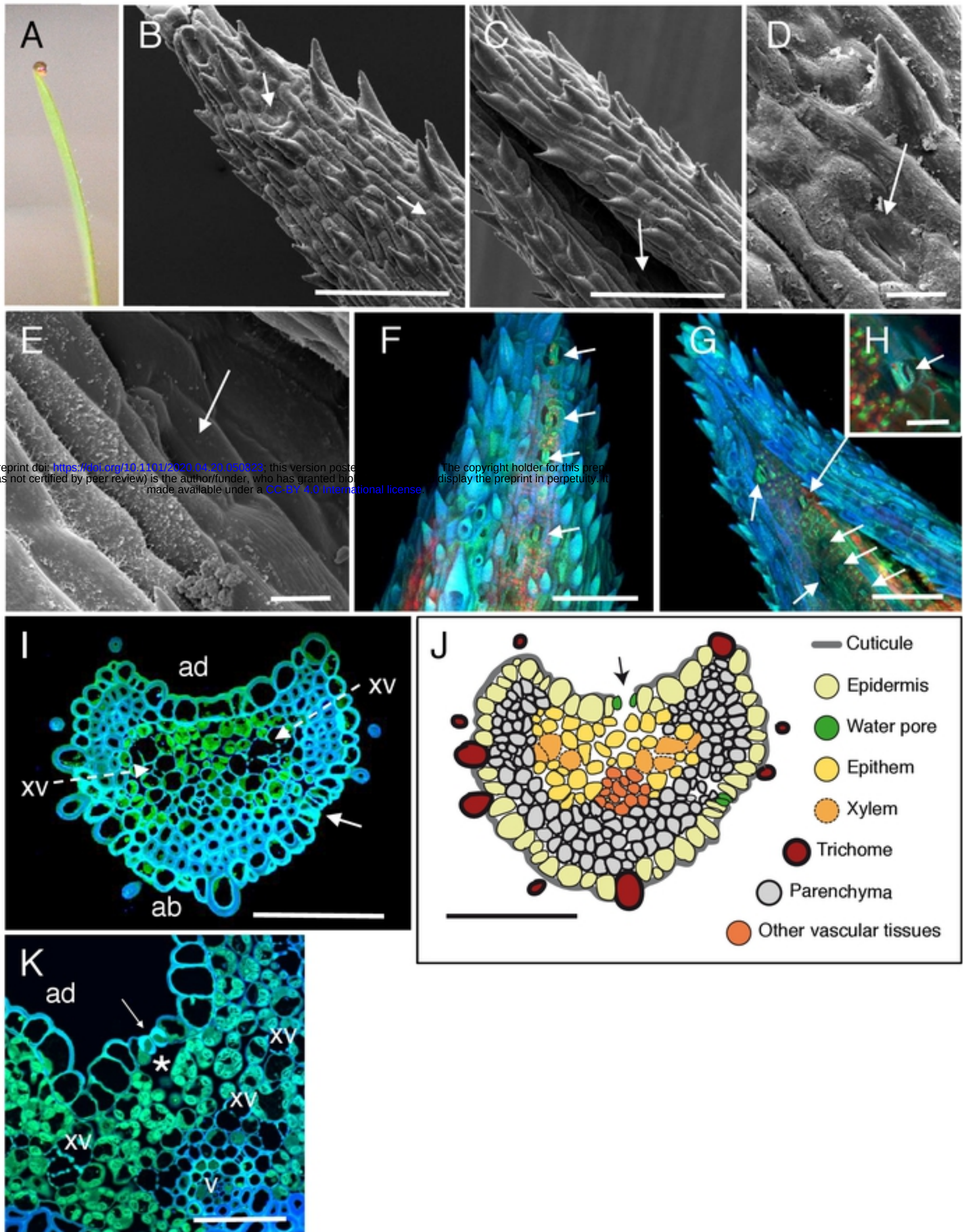


Figure 3
Jauneau et al.

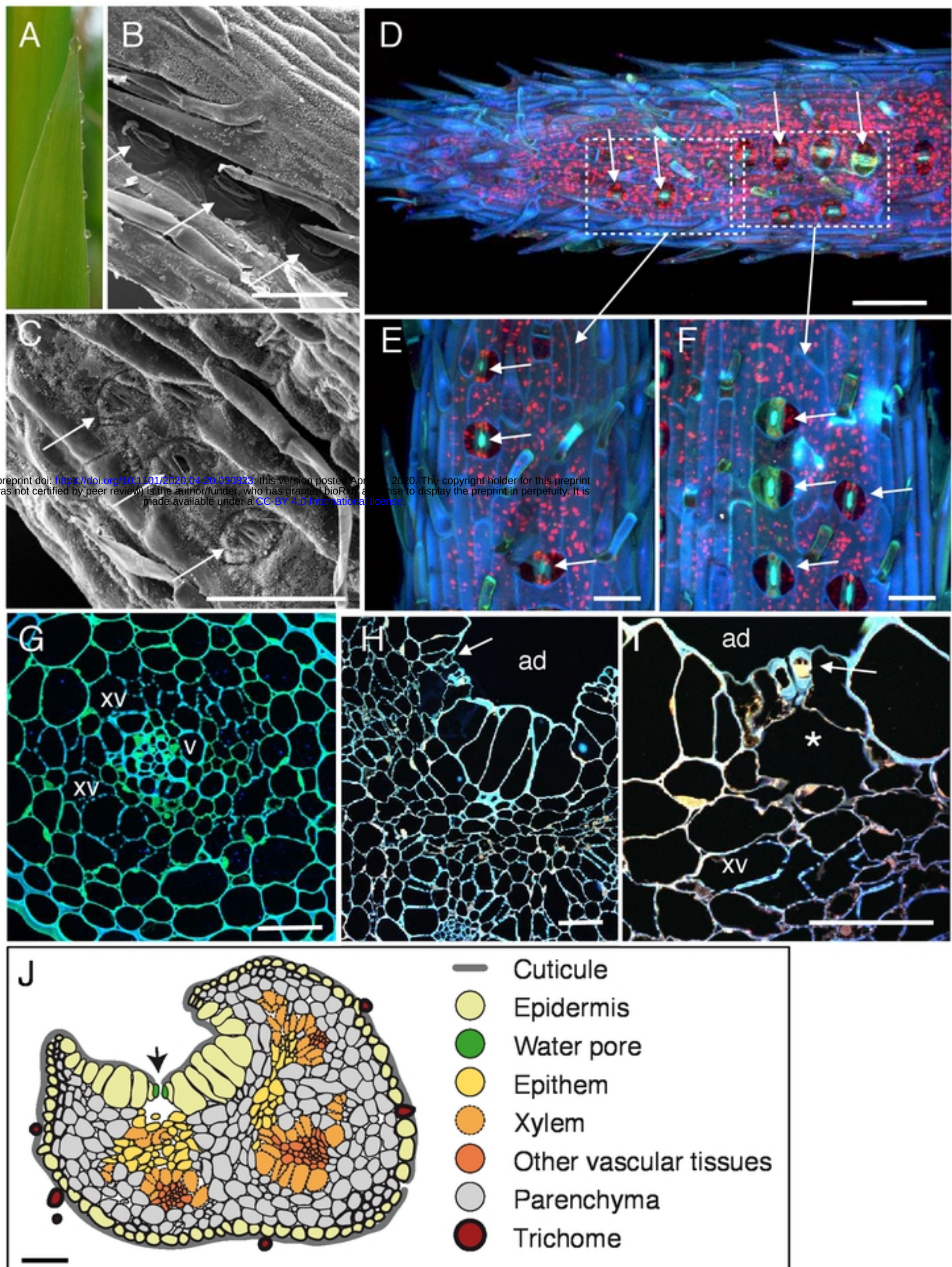


Figure 4
Jauneau et al.