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Multiple facets of laticifer cells

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Abstract

In the latex-bearing plants, the laticiferous system is the tubing structure that contains the latex and is constituted of living cells (laticifers). While laticifers are present only in a small percentage of the flowering plant species, they represent a type of specialized tissue within the plant where a myriad of metabolites are synthesized, some of them of considerable commercial importance. In this mini-review we synopsise the present knowledge about laticifer cells and discuss about their particular features as well as some evolutionary and ecophysiological cues and the potential exploitation of the knowledge generated around this peculiar type of plant cell. We illustrate some of these questions with the experience in *Euphorbia lathyris* laticifers and latex.

Approximately 10% of flowering plants exude latex upon tissue damage. Latex is a sticky suspension or emulsion of particles in an aqueous fluid containing a wide variety of plant metabolites and this fluid is usually held under pressure in living plant cells, laticifers. The chemical composition of latex is complex and highly variable depending on each plant species: terpenoids (rubber in *Hevea brasiliensis*), cardiac glycosides (in the Apocynales), alkaloids (morphine, codeine, and papaverine in *Papaver somniferum*), phenolic glucosides (in *Cannabis sativa*), sugar (in Asteraceae), large amounts of protein (in *Ficus callosa*) and tannins (*Musa*, Aroideae). Crystals of oxalate and malate may be abundant in latex and starch grains occur in laticifers of some genera of Euphorbiaceae ¹. Laticifers are specialized cells (or rows of cells) that synthesize and accumulate this latex and were first described by De Bary in 1884 ² as ‘laticiferous tubes’ traversing the whole length of the mature parts of the plants as continuous. They have been found in 12,500 plant species of 22 families, including monocots and dicots, and are estimated to exist in more than 20,000 species of 40 families ³. Based on the laticifer anatomy two categories of laticifers were established: the articulated type and the nonarticulated type ². Basically, nonarticulated laticifers (e.g., in *Euphorbia lathyris*) assemble a network that is essentially a single cell permeating the entire plant body while articulated laticifers (e.g., in *Hevea brasiliensis*) are a series of interconnected individual cells. The final tubing system is very similar in both types but the origin is completely different ⁴. Like secretory ducts, cavities and other specialized tissues, both types of laticifers are usually associated with vascular tissues, and they can occur in any organ ⁵. Nonarticulated laticifers arise from a single cell in the embryo, a cell that elongates along with plant development and grows intrusively between other cells via tip growth. The cells undergo karyokinesis without cytokinesis, resulting in multinucleate (coenocytic) cells. In some species, this kind of laticifer develops bifurcations with subsequent elongation of the branches, embedding the plant body with a network of tubes where the laticifer cells develop

by successive cycles of elongation and branching. Articulated laticifers, by contrast, are compound in origin and consists of rows of cells, derived from the surrounding phloem tissues, in which the walls between the individual cells can remain intact, can become perforated or can be completely removed. In the last case, the total perforation of those cell walls results in the laticifer tubing structure with a continuous, multinucleated cytoplasm.

Although knowledge about laticifer cells is being generated, it is still lacking a total or partial model for development and differentiation of laticifers as well as for the latex metabolism. The main aspects studied about these cells are those peculiar features not found in other types of plant cells. The intrusive growth of the nonarticulated laticifer is one of those aspects ⁶, making this cell tens of centimeters long ⁷. Because of intrusive growth, the laticifer elongates between adjacent cells and it is assumed that processes of partial disassembly of the cell wall occur in those cells. In fact, there are clear evidences of differences in wall composition between laticifers and their surrounding cells, as found in *Asclepias speciosa* ⁸. It also has been observed that nonarticulated laticifers have no plasmodesmatal connections with their neighbors ⁸ and do not contain chloroplast ^{7,9}. Large vacuoles occupy a substantial part of the laticifer cells so the exuded latex is likely a mixture of the vacuole content and the laticifer cytoplasm ¹⁰. The nonarticulated laticifers in *Euphorbia lathyris* can be observed with a whole-mount Sudan Black B staining which allows plotting the laticifer system inside the body plant in different tissues, e.g. leaf tissue (Fig. 1A), and the examination of special details, such as the laticifer tip on its way between adjacent cells (Fig. 1B). Moreover, the coenocytic nature of this cell can be confirmed by isolation of laticifer protoplasts (i.e., large fragments of individual laticifer cells) and nuclei DAPI staining (Fig. 1C), showing the characteristic spindle-like form (Fig. 1D) ¹¹. It was proposed that laticifers were sensitive to auxin produced in the apical meristem ¹² and the

identification and analysis of the *shoot meristemless* (*sml*) mutant in *E. lathyris* has confirmed this fact, since laticifer cell establishment was severely compromised in the cotyledons of this mutant lacking a distinguishable shoot apical meristem¹¹. Other aspects quite reported about laticifer cells are those related to latex metabolism, however, the huge variety of latex metabolites and proteins does not allow a simple scheme and the main focus is mostly put on latex-bearing plants with metabolites of high economic value and/or biological activity⁴. And the role of the latex in the plant metabolism has not been decisively established. None of the latex metabolites have been found to participate further in the metabolism of the plant and, for instance, the latex starch does not function as utilizable carbohydrate in the laticifer cells^{13,14}. The identification of latex- and laticifer-deficient mutants (*pil* mutants) in *E. lathyris* (Fig. 1E) can provide new insights in several aspects of the laticifer cells and the latex metabolism¹¹. For instance, the absence of triterpenes in these mutants (Fig. 1F) corroborated, by solid genetic evidences, that these metabolites are latex-specific. Whole-genome and whole-transcriptome amplification techniques are routinely performed for a lot of plant species but not for latex-bearing plants and only in the few last years some reports have appeared for transcriptomic analysis such as in *Hevea brasiliensis*¹⁵ and *Euphorbia tirucalli*¹⁶ while genome sequencing has been reported in *Hevea brasiliensis*¹⁷, *Cannabis sativa*¹⁸, *Jatropha curcas*¹⁹ and *Carica papaya*²⁰. The knowledge derived from genome, transcriptome and gene expression profiles will help to understand the underlying gene networks in laticifers and latex.

Latex and laticifers have been always associated to plant defense, especially against herbivores, but only few direct evidences can be found in the literature^{21,22,23,24}. The indirect evidences of this role are mainly based in the fact that most of the latex metabolites are toxic for herbivores or have a biological effect on them, as well as the fact that latex is mobilized to

the site of damage and the laticifers are internal secretory structures where plant defense metabolites can be stored²⁵. It has been also hypothesized a form of physical defense of the latex, referred to 'squirting gun', by means of which the pressurized flow of latex after tissue damage provides a physical barrier to an herbivore²⁶. Agrawal and Konno²⁷ have reviewed the concept of latex as a coordinated plant defense system presenting the evidences of latex as a trait that reduces herbivory or the preference or performance of herbivores. There are some studies dealing with latex activity against phytopathogenic microorganisms and it is well established that pathogenesis-related (PR) proteins are commonly found in latex including proteases, chitinases, proteinase inhibitors, glucanases, and oxidative enzymes²⁷. Perhaps the most prominent example is hevein, the major latex protein from *H. brasiliensis*, which exhibits antifungal activity upon several phytopathogens²⁸. It has been also reported that laticifer proteins from *Calotropis procera* and *Carica candamarcensis* inhibit in vitro growth of several necrotrophic and hemibiotrophic fungi although latex proteins from *Euphorbia tirucalli* and *Plumeria rubra* did not exert this inhibition²⁹.

At this point, an unavoidable question sprouts about the evolutionary origin of latex and laticifers, and this question comes from different aspects of laticifer research. When defining laticifers on their anatomy, it is generally presumed that articulated and nonarticulated laticifers evolved independently and represent polyphyletic origins within vascular plants¹. Furthermore, the fact that latex is restricted to a small number of plant families, between many of which there is no evidence of close taxonomic relationship, suggests that the capacity to produce latex has been evolved more than once³⁰. The chemical composition of latex within a given laticiferous family also varies and even, within a genus, there are usually species with and without laticifers⁴. In the ecological context, it has been hypothesized that latex is a highly convergent trait, providing plants with a defense system that

improves the fitness of the plant in the presence of herbivores, so it should have evolved independently several times ²⁷. Additionally, latex has also been reported in conifers (e.g., *Gnetum* spp.), in pteridophytes (e.g., ferns) and even in the fungal kingdom (e.g., *Lactarius* spp). For Lange, two hypotheses (or a combination of both) are consistent with these observations: (a) laticifers existed in the last common ancestor of laticiferous clades but were lost in some species (divergent evolution), or (b) laticifers emerged multiple times in independent lineages (convergent evolution) ⁵. Kliebenstein postulates that if laticifers represent independent evolutions there is an implicit assumption of independent genes for each event and suggests that there must be a central module of genes providing the potential to repeatedly evolve laticifers, based on the fact that nearly every major plant group has species with laticifers ³¹. The question could be extensive to other specialized tissues and plant secondary metabolites, not only laticifers and latex. Phloem associated S-cells, accumulating glucosinolates, are similar in structure and function to the nonarticulated laticifers and both types of cells may share a common evolutionary origin. Hence common gene regulatory models could explain the vast majority of plant secondary metabolite specialized tissues ³¹. Going further in the evolution of laticifers, the question arises about what was present before, the biosynthetic capacity to produce certain metabolites or the convenient storage site for novel compounds, i.e., what came before, the metabolism or the compartment.

Thus laticifer cells, as a type of specialized cells, represent a natural biofactory for the production of several types of compounds and chemicals. In this sense the main example is the case of the natural rubber, an isoprene polymer produced in the laticifer cells of *Hevea brasiliensis* (rubber tree) which is the only commercial source of this product. Another example is the photosynthetic production of hydrocarbons in the laticifer cells of the genus

Euphorbia as a source for renewable fuels. This idea, the hydrocarbon plants, was proposed and studied in detail by Nobel Prize Melvin Calvin more than 30 years ago^{32,33} and in that moment several projects explored deeply this possibility as an alternative to petroleum³⁴. In our group, an experimental work has been developed for testing the suitability of *E. lathyris* as an energy crop with a further vision on a genetic breeding program directed to improve hydrocarbon yields and biomass. Additionally, the study of the biochemical, molecular and cellular basis of laticifer differentiation and latex formation can set up the basis to increase the content of energy-rich terpenes in this crop. The latex triterpenes account for the 6% of the dry weight of the biomass and generation of high yield new varieties are in progress in our lab that more than double this hydrocarbon content. The biocrude obtained after biomass extraction can be processed by cracking, as if crude oil, and it is compatible for blending purposes with fossil fuels (Fig. 2A-C). Additionally, in this plant other raw materials can be used with energetic purposes (Fig. 2D-F) making this plant a 'zero-waste' energy crop.

In summary, laticifer cells represent a specialized cell with a complex metabolism and an unusual cellular arrangement and dynamics. We still have a long road to discover all about the astonishing biology beyond laticifer cells, their secret life, their function in the plant, their evolutionary origin, and the multiple possibilities that the latex-bearing plants can afford.

References

1. Esau K. Plant Anatomy. Wiley, New York, 1965.
2. De Bary A. Comparative Anatomy of the Vegetative Organs of the Phanerogams and Ferns. Oxford, Clarendon Press. 1884
3. Lewinsohn TM. The geographical distribution of plant latex. Chemoecology 1991; 2:64-68. doi: 10.1007/BF01240668.
4. Hagel JM, Yeung EC, Facchini PJ. Got milk? The secret life of laticifers. Trends in Plant Science 2008; 13:631–639. doi: 10.1016/j.tplants.2008.09.005.
5. Lange BM. The Evolution of Plant Secretory Structures and Emergence of Terpenoid Chemical Diversity. Annu Rev Plant Biol 2015; 66:139–59. doi: 10.1146/annurev-arplant-043014-114639.
6. Lev-Yadun S. Intrusive growth - the plant analog of dendrite and axon growth in animals. New Phytologist 2001; 150:508-512. doi: 10.1046/j.1469-8137.2001.00143.x.
7. Mahlberg PG. Laticifers: an historical perspective. Botanical Review 1993; 59:1–23.
8. Serpe MD, Muir AJ, Driouich A. Immunolocalization of β -D-glucans, pectins, and arabinogalactan-proteins during intrusive growth and elongation of nonarticulated laticifers in *Asclepias speciosa* Torr. Planta 2002; 215:357-310. doi: 10.1007/s00425-002-0756-y.
9. Sacchetti G, Ballero M, Serafini M, et al. Laticifer tissue distribution and alkaloid location in *Vinca sardoa* (Stearn) Pign. (Apocynaceae), an endemic plant of Sardinia (Italy). Phytol (Annales Rei Botanicae) 1999; 39:265-275.
10. Konno K. Plant latex and other exudates as plant defense system: Roles of various defense chemicals and proteins contained therein. Phytochemistry 2011; 72:1510-1530. doi: 10.1016/j.phytochem.2011.02.016.

11. Castelblanque L, Balaguer B, Martí C, Rodríguez JJ, Orozco M, Vera P. Novel Insights into the Organization of Laticifer Cells: A Cell Comprising a Unified Whole System. *Plant Physiol.* 2016; 172:1032-1044. doi: 10.1104/pp.16.00954.
12. Schaffstein G. Untersuchungen an ungegliederten Milchröhren. *Botanisches Centralblatt* 1932; 49: 197-220.
13. Biesboer DD, Mahlberg, PG. Accumulation of non-utilizable starch in laticifers of *Euphorbia heterophylla* and *E. myrsinites*. *Planta*, 1978; 143:5-10. doi: 10.1007/BF00389045.
14. Nissen SJ, Foley ME. No Latex Starch Utilization in *Euphorbia esula* L. *Plant Physiology* 1986; 81:696-698.
15. Xia Z, Xu H, Zhai J, et al. RNA-Seq analysis and de novo transcriptome assembly of *Hevea brasiliensis*. *Plant Mol Biol* 2011; 77:299. doi: 10.1007/s11103-011-9811-z.
16. Kitajima A, Miura K, Aoki W, et al. Transcriptome and proteome analyses provide insight into laticifer's defense of *Euphorbia tirucalli* against pests. *Plant Physiology and Biochemistry* 2016; 108:434-446. doi: 10.1016/j.plaphy.2016.08.008.
17. Rahman AYA, Usharraj AO, Misra BB, et al. Draft genome sequence of the rubber tree *Hevea brasiliensis*. *BMC Genomics* 2013; 14:75. doi: 10.1186/1471-2164-14-75.
18. Van Bakel H, Stout JM, Cote AG, et al. The draft genome and transcriptome of *Cannabis sativa*. *Genome Biology* 2011; 12:R102. doi: 10.1186/gb-2011-12-10-r102.
19. Sato S, Hirakawa H, Isobe S, et al. Sequence Analysis of the Genome of an Oil-Bearing Tree, *Jatropha curcas* L. *DNA Research: An International Journal for Rapid Publication of Reports on Genes and Genomes* 2011; 18:65-76. doi: 10.1093/dnares/dsq030.
20. Ming R, Hou S, Feng Y, et al. The draft genome of the transgenic tropical fruit tree papaya (*Carica papaya* Linnaeus). *Nature* 2008; 452:991-996. doi: 10.1038/nature06856.

21. Agrawal AA. Natural selection on common milkweed (*Asclepias syriaca*) by a community of specialized insect herbivores. *Evol. Ecol. Res.* 2005; 7:651–667.
22. Dussourd DE, Eisner T. Vein-cutting behavior: Insect counterploy to the latex defense of plants. *Science* 1987; 237: 898–901.
23. Konno K, Hirayama C, Nakamura M, et al. Papain protects papaya trees from herbivorous insects: role of cysteine protease in latex. *Plant J.* 2004; 37:370–378. doi: 10.1111/j.1365-313X.2004.01968.x.
24. Huber M, Epping J, Gronover CS, et al. A Latex Metabolite Benefits Plant Fitness under Root Herbivore Attack. *PLoS Biology* 2016; 14:e1002332. doi: 10.1371/journal.pbio.1002332.
25. Pickard WF. Laticifers and secretory ducts: two other tube systems in plants. *New Phytologist* 2008; 177:877-888. doi: 10.1111/j.1469-8137.2007.02323.x.
26. Becerra JX, Venable DL. Rapid-terpene-bath and “squirt-gun” defense in *Bursera schlechtendalii* and the counterploy of chrysomelid beetles. *Biotropica* 1990; 22:320–323. doi: 10.2307/2388545.
27. Agrawal AA, Konno K. Latex: a model for understanding mechanisms, ecology, and evolution of plant defense against herbivory. *Annu. Rev. Ecol. Evol. Syst.* 2009; 40: 311–331. doi: 10.1146/annurev.ecolsys.110308.120307.
28. Van Parijs J, Broekaert WF, Goldstein IJ, Peumans WJ. Hevein: an antifungal protein from rubber-tree (*Hevea brasiliensis*) latex. *Planta* 1991; 183:258–264. doi: 10.1007/BF00197797.
29. Souza DP, Freitas CDT, Pereira DA, et al. Laticifer proteins play a defensive role against hemibiotrophic and necrotrophic phytopathogens. *Planta* 2011; 234:183-193. doi: 10.1007/s00425-011-1392-1.

30. Metcalfe CR. Distribution of latex in the plant kingdom. *Economic Botany* 1967; 21:115-127.
31. Kliebenstein DJ. Making new molecules - evolution of structures for novel metabolites in plants. *Current Opinion in Plant Biology* 2013; 16:112-117. doi: 10.1016/j.pbi.2012.12.004.
32. Nielsen PE, Nishimura H, Otvos JW, Calvin M. Plant Crops as a Source of Fuel and Hydrocarbon-Like Materials. *Science* 1977; 198:942-944.
33. Calvin M, Nemethy EK, Redenbaugh K, Otvos JW. Plants as a direct source of fuel. *Experientia* 1982; 38: 18-22.
34. Kalita D. Hydrocarbon plant - New source of energy for future. *Renewable and Sustainable Energy Reviews* 2008; 12:455-471. doi: 10.1016/j.rser.2006.07.008.

Figure Legends

Figure 1. Laticifers in *E. lathyris* and latex-defective mutants (*pil* mutants). (A) Whole-mount Sudan Black B staining of leaf tissue showing the nonarticulated laticiferous system. (B) Whole-mount Sudan Black B staining of a laticifer tip. (C) Isolation of laticifers fragments and nuclei DAPI staining. (D) Detail of a laticifer fragment showing the spindle-like form of the nucleus. (E) Latex oozing in wild-type plants and absence of latex in *pil* mutants. (F) GC-MS analysis of latex triterpenes leaf tissue of wt plants and *pil* mutants. Compounds are: 1, internal standard; 2, lanosterol; 3, β -sitosterol; 4, butyrospermol; 5, cycloartenol; 6, 24-methylene-cycloartanol. (G) Structures of the major triterpenes found in the latex of *E. lathyris*.

Figure 2. The latex bearing-plant *E. lathyris* as a hydrocarbon plant. (A) Triterpenes contained in the latex are suitable for cracking and yield a biocrude that can be used blended with gasoline. (B) The biocrude can be extracted from the biomass with a classical solvent extraction (right top) or using supercritical fluid technique (right bottom). (C) Laticifers cell, containing hydrocarbons, resembling oil refinery pipes. (D) Not only the latex triterpenes can be used with energetic purposes but also other parts of the plant like the high content carbohydrates, the high-oleic oil seed, the lignocellulosic residue and the seed cake residue. (E) The exploitation of all raw materials and energy-carriers results in high energetic yields. (F) From lab-to-field approach directed towards obtaining better agronomic performance and enhanced bioenergy potential.

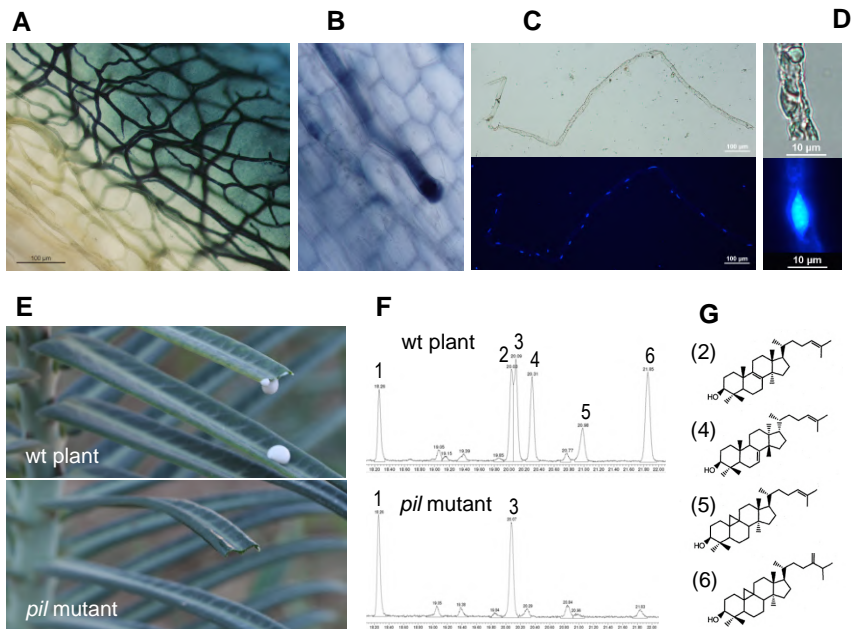


Figure 1.

Laticifers in *E. lathyris* plants and latex-defective mutants (*pil* mutants).

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- (B) Whole-mount Sudan Black B staining of a laticifer tip
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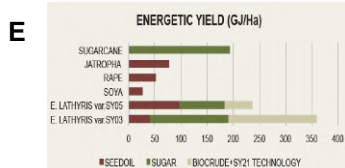
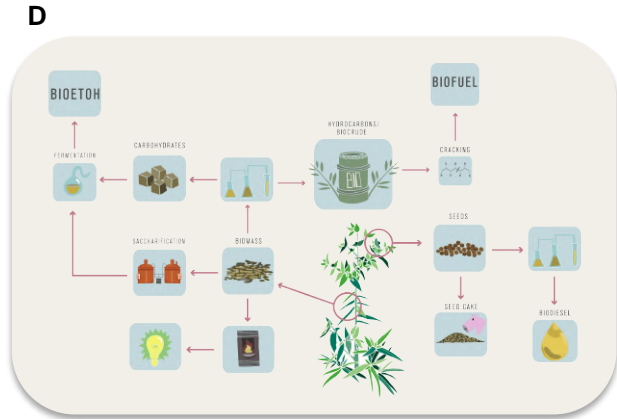
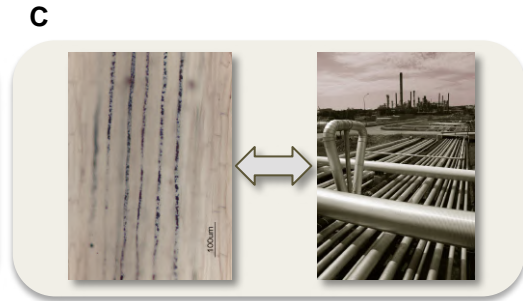
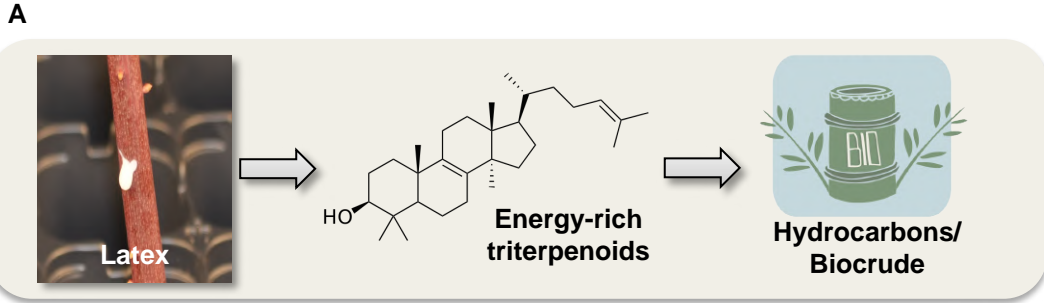


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