"Without secondary cell walls – there would be no cotton industry. So what do we really know about them?"

Cotton's extraordinary secondary cell walls

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ACROSS the globe, secondary cell walls (SCWs) underpin natural fibre-based industries. In fact, without SCWs there would be no cotton industry. Recent insights from cotton research are unlocking the mysteries of how one plant – cotton – can make remarkably different SCWs, and this wealth of knowledge is being used for producing novel materials. Much of this research has been supported by CSIRO and Cotton Breeding Australia (CBA), a joint venture between Cotton Seed Distributors and CSIRO.

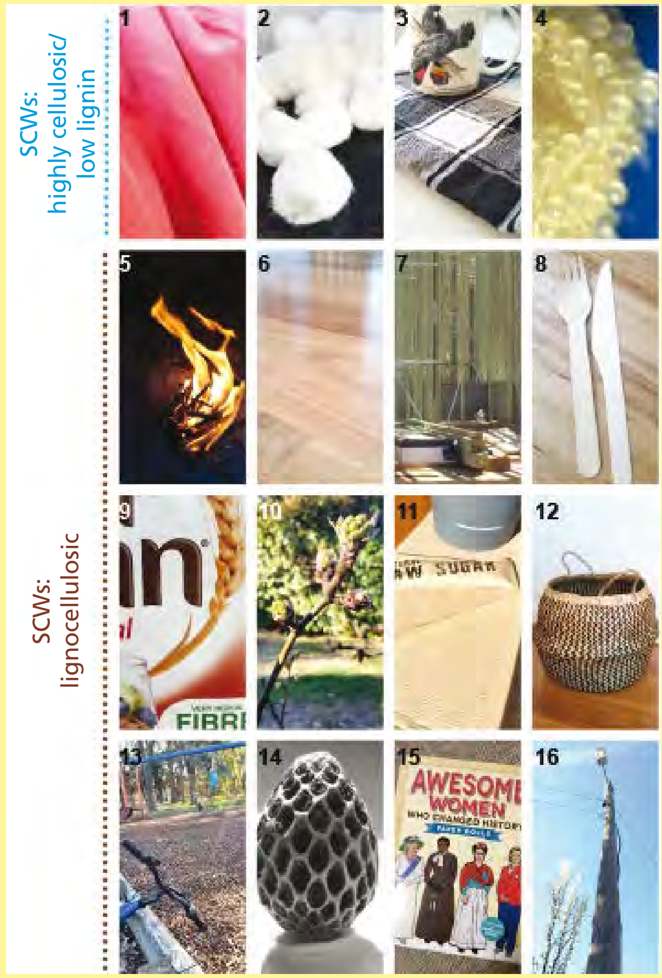
All plant cells are surrounded by a cell wall. For cells that need to change shape or grow, that cell wall is thin and flexible and typically referred to as a primary cell wall. In certain plant cells or conditions, a secondary cell wall is produced and deposited on the inner surface of the primary cell wall, usually as a thick additional layer and often when cell-expansion has ceased. Plant SCWs and their components are closely linked to human activity, and they span many aspects of the plant world.

Dive in to any plant growing on land and you will find SCWs. In fact, life on land as we know it, including all agricultural crops would not exist without SCWs. For the cotton industry, SCWs are almost entirely what the seed fibre is made of – and is harvested and traded as a global fibre commodity.

In nature, SCWs are the reason trees can stand so tall (sometimes up to 100 metres) and how water and nutrients can be piped through the bodies of plants like wheat. They are the hard shells of nuts and seeds, the long tubes that pollen grains make to fertilise and set seeds in plants, and they are also the local wall type that plants can construct as a physical and chemical barrier against fungal attack.

Colleen MacMillan.

FIGURE 1: SCWs are found across a wide spectrum of human activities



These SCWs are renewable and biodegradable. 1.Cotton T-shirt (Sydney City2Surf 2008, and still wearing well) – the textile is made from cotton fibres that are mostly SCWs. 2.Cotton balls are a first-aid necessity when blood/wounds are to be cleaned – the fibres are largely composed of highly-cellulosic SCWs. 3.Cotton tea-towels, from highly cellulosic seed fibre SCWs, are very efficient for drying implements like mugs. 4.The pollen tubes that grow from pollen grains, such this cotton pollen, to fertilise ovules for seed-set are made from specialised SCWs. 5.Fire has fuelled much human survival and evolution – for heat, cooking, and light; the wood-fuel is largely made from lignocellulosic SCWs. 6.The floors of millions of homes are made from SCWs, in the form of wood from many different plants such as eucalypts, pines, and even bamboo. 7.The frames of millions of homes are in essence SCWs – from wood such as pine. 8.Many renewable and biodegradable eating implements are used instead of plastics – such as forks, knives and chopsticks. 9.Food products that are very high in fibre contain key components of SCWs – such as crystalline cellulose. 10.Trees such as this nashi-pear fruit tree – and like all land plants – transport water throughout the plant via SCW-encased cells (xylem). 11.Renewable and biodegradable coffee-cups, stirrers and sugar sachets are made from the components of SCWs. 12.Baskets can be woven from natural fibres made of SCWs. 13.Playground-floors are often made from renewable SCW-based tan bark/wood chips. 14. Seed pods are composed of SCWs. 15.High-end cardboard and paper products are SCW extracts, such as this book of paper-dolls. 16. Street lamps – composed of SCWs in the form of wood trunks – have long provided light for many at night. "#secondarycellwalls" (via Twitter).

What makes cotton SCWs so different is that humans have bred a highly unusual SCW – one that is almost pure cellulose (about 94 per cent). Linked to this, humans have also bred one of the longest known plant cells. Furthermore, this is very different to the typical SCWs found in many plant SCWs like those in trees and tree-based products. Those SCWs are lignocellulosic – that is, composed of lignin, cellulose and other molecules.

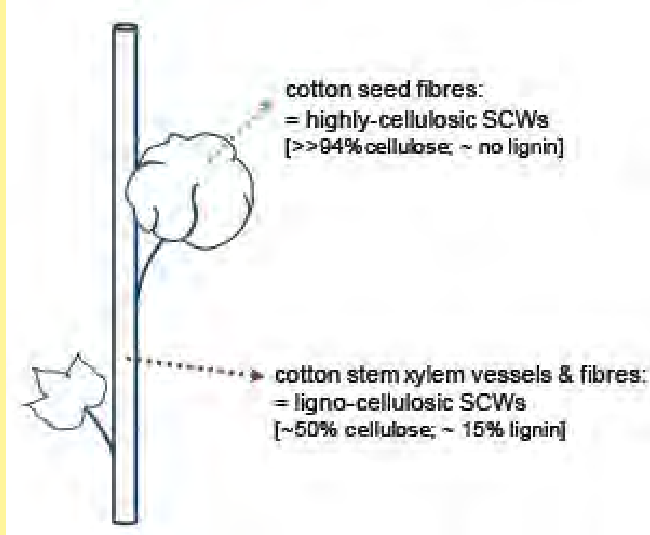
SCWs are important, especially as they are renewable biodegradable materials that differentiate natural fibres such as cotton from the synthetic fibre market. We are so fascinated by SCWs and their close links with humans and in nature that we 'tweet' about '#secondarycellwalls' on Twitter at every opportunity – to share the extraordinary range of places these materials are found/used in. SCWs can be seen in many places once one starts to look (Figure 1).

Highly cellulosic versus lignocellulosic

Some of our favourites are those from cotton fibres which are highly cellulosic, or that lack lignin. These include the durable cotton running T-shirt from the Australian 2008 Sydney City2Surf fun run – it breathes well, wicks moisture well, washes well, and repeatedly so, has excellent sun protection, and nine years on still holds its shape (Figure1.1). During a first-aid crisis, particularly when there is a wound and blood to be cleaned, cotton balls are essential (Figure1.2). Drying of crockery, such as mugs and plates, is most efficient with a cotton tea-towel because synthetics don't capture the moisture well (Figure1.3). Pollen tubes, such as those that develop from cotton pollen grains (in Figure 1.4), make thick secondary cell walls so they can grow to fertilise ovules, and thus produce the next generation of plants. Without these walls, there would be no fertilisation and no seed produced. All these are cotton examples of SCWs that are not lignocellulosic.

There is also an immense array of lignocellulosic SCWs. These include wood which is long-standing as a source of fuel, heat and light for many humans on the planet and throughout human evolution. Also, paper and wood-pulp products – the demand for which is growing as the global middle class increases – are made from SCWs, but the lignin frequently has to be removed for quality and long-term stability purposes.

FIGURE 2: The cotton plant is an exquisite experimental system to investigate how one crop can produce very different SCWs



Until recently, little was known about cotton SCWs and how they form, despite their critical importance to the cotton industry. This was particularly intriguing, given that cotton seed fibre SCWs are very different to typical SCWs found in many other plants. Cotton seed fibre SCWs are largely made of cellulose, whereas SCWs in other plants are generally lignocellulosic. While research in other plants over the last decade has started to shed a light on how lignocellulosic SCWs are made, there has been a significant knowledge gap about the molecular control of cotton seed fibre SCW formation and how these very special walls are made.

The cotton plant turns out to be an elegant experimental system to tease apart the difference between highly-cellulosic and lignocellulosic SCWs. Answering this question gives us direct insights in to how cotton seed fibres make their remarkable SCWs. It also provides new knowledge on how a single plant goes about making very different SCWs – this has not been shown before for any plant.

The simple question was: what genes are expressed differently when the cotton plant makes a highly cellulosic SCW and a lignocellulosic SCW? To answer this question, genes expressed as RNA were extracted from different parts of the plant at different time periods:

- Cotton seed fibres when they were well into producing SCWs (25 DPA – Days Post Anthesis);
- Cotton seed fibres when they transition to SCW synthesis (15 DPA);
- Cotton seed fibres when they only produce primary cell walls (7 DPA);
- From cotton stem xylem during production of lignocellulosic SCWs; and,
- The stem pith when it is producing its thin primary cell walls.

All the extracted RNA molecules were sequenced – for each tissue-type about 50 million individual sequences were obtained, and then bioinformatics was used to identify and quantify the genes expressed in each tissue. Also extracted were the cell walls for these tissues to measure their molecular composition, including cellulose quantity and lignins. These analyses included state-of-the-art NMR (nuclear magnetic resonance) and cell wall GC-MS (gas-chromatography mass-spectrometry) techniques.

A wealth of new insights

The simple experimental question of how cotton produces very different SCWs has yielded a wealth of new insights, some surprises, and a massive and valuable data-set of gene expression patterns. The cotton seed fibre is an unusual 'cellulose factory' – the results point to extremely high expression of specific cellulose biosynthetic genes, but also some genes that are likely critical in how this unique SCW is made. (MacMillan and Birke et al. (2017) BMC Genomics 18:539 – *https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-017-3902-4*)

In contrast, the cotton stem lignocellulosic SCWs produce modest amounts of these cellulose-related gene-products. Some of the genes have never been known to have a function in SCW formation. There was a distinct reduction in lignin biosynthetic gene expression in seed fibres, which is consistent with a SCW that is highly cellulosic and lacks lignin. Furthermore we discovered a significant collection of cotton gene-regulators (transcription factors) that have not been reported before in controlling the different cotton SCWs.

Some surprising regulators were the SND1-type, which we found to be expressed earlier than other transcription factors in seed fibres at the transition to SCW synthesis. This difference in timing was unknown for plant SCWs until now.

Another significant insight was the reduction in a regulator that in other plants is known to control lignin synthesis directly. By comparing the stem and seed fibre SCW genes, we discovered that a class of lignin regulators called MYB42/85 was severely lacking in the seed fibres during SCW synthesis, whereas in the lignocellulosic stems, expression was abundant. This points to a mechanism by which cotton can make lignin in one type of SCW, but not another.

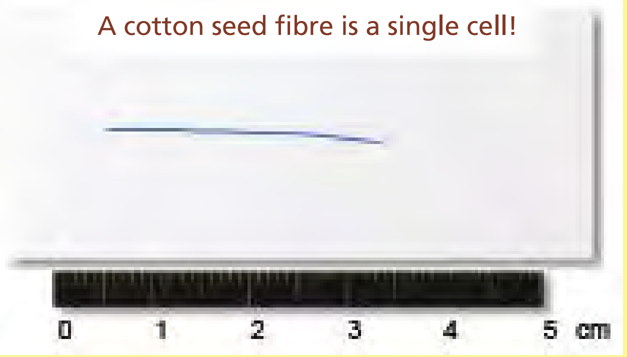
The cotton plant is also proving to be a fascinating source of new insights into lignin synthesis and deposition. When we used state-of-the-art NMR with our collaborators in the US Wisconsin Energy Institute, we discovered some very unusual lignin profiles. Remarkably, unlike many other plants, the pith of the cotton stem is lignified.

The cotton plant produces highly unusual lignins and there is virtually no typical lignin in the seed fibre SCWs. Married together with the genes we've discovered, we can now gain insights in to the potential gene regulation of different types of lignins, or lack thereof.

LONGEST KNOWN PLANT CELL

Humans have bred one of the longest plant cells known. The cotton seed fibre is a single cell and is another extraordinary feat of agricultural breeding by humans, in addition to the SCW being highly cellulosic. Usually if one wants to see an individual plant cell, the only way to do so is using a microscope, because the average plant cell is about 10–50 microns long, that is 1/100 – 1/20 of a millimetre. But the cotton seed fibre cells are more than 3 cm long – over 3000 mm! This incredible length for a single plant cell, linked with the abundance of cellulose which is a high tensile strength material, produces a natural fibre that is strong, renewable, biodegradable, and incredibly diverse in its uses by humans.

FIGURE 3: Humans have bred one of the longest known plant cells known



Using this information

"So what?" you might ask. What can all this new knowledge and insight be used for? For the first time we have answers to how one organism – a commercially significant crop in fact – produces SCWs that are completely different. Knowing which genes regulate and contribute to how the highly cellulosic SCW of cotton seed fibres is made enables us to manipulate the cell walls and cell wall components to change the strength and elongation properties of these walls.

The improved knowledge of how different SCWs are made also makes a contribution to our understanding of fundamental plant biology. For example, take the discovery of unique proteins that until now were not know to be involved with the formation of highly cellulosic SCWs. These molecules have specific sequences that tell us how they get trafficked within the seed fibre to get to the SCW, and then how they sit within the SCW as it is formed. Another example is the group of regulatory genes for switching lignin synthesis on, or off, and these depend on what SCW type is being made in the cotton plant.

This new knowledge is now being used in CSIRO-funded SCW research to identify previously undiscovered genes for different SCW pathways; in next-generation synthetic biology (a CSIRO Future Science Platform); and, in CBA-funded fibre quality research. One aim of the new research is to make renewable, biodegradable cotton fibres that have properties that make them competitive with synthetic fibres.