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Cotton Shirt as Strong as Steel?

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The views expressed in this column are his own and not that of Cotton Association of India)

Can you imagine a normal light weight shirt that may act as a durable bullet proof vest? Unbelievable, but true, spider silk proteins expressed within the lumen of cotton fibre produce flexible light weight fibre with properties of steel. Spider silk is 5 times stronger than steel and a pencil thick spider silk strand can stop a Boeing 707 in flight. Can you imagine that cotton fibre can be genetically modified to contain proteins of silk from the silkworm or spinach or the wild plant Calotropis? Such genetically modified fibres have superior fibre traits of high strength, durability, climate friendly and favourable thermal properties. This is not futuristic research. There are several patents and scientific papers in recent times with such outstanding accomplishments made by either altering cotton fibre regulatory genes or by expressing proteins from any external sources inside the cotton fibre to enhance its value.

For more than a century, cotton breeders across the world have been working on improving fibre quality through classical plant breeding approaches. The main subject areas of focus have been fibre quality parameters such as length, strength, fineness

and maturity. Over the past two decades, significant progress was made in unravelling the molecular aspects of fibre development and identification of genes and tissue specific promoters that regulate length, strength and fineness. The new knowledge of fibre molecular biology along with genetics has strengthened cotton breeding approaches. The step by step process of cotton fibre development has been tracked to identify the genes involved in determining the specific properties of the cotton fibre. Currently efforts are being made by cotton breeders and biotechnologists to explore possible modifications in key parts of the biochemical processes which could lead to improvements in cotton fibre quality.

Fibre quality and yield can be improved by either a) Regulating genes to modify cell wall synthesis, elongation and deposition of cellulose or b) Genetic engineering to express specific proteins in the fibre cell lumen to add strength or special properties. This article describes the recent research progress made in both approaches to increase cotton fibre strength, length and thermal properties.

Cotton fibre is a single elongated cell derived from the ovule epidermis. The formation of cotton fibre happens in four well-defined sequential steps of initiation, elongation, secondary cell wall synthesis and maturation. In the process of fibre development, plant hormones (auxin, gibberellins, cytokinins and ethylene) play very important roles at various stages of growth. Auxin and cytokinin determine fibre initiation and number of fibres. Several studies showed that targeted regulation of

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auxin and cytokinin hormones in specific parts of the flowers and ovules can improve quality and yield. Interestingly, the number of fibres per ovule increased significantly even with external application of Indole acetic acid (Auxin) on squares and flowers of cotton (Seagull et al., 2004). In 2011, Professor Zhang and his group reported an increase of more than 15% increase in lint yield by expressing the auxin gene *iaaM* in cotton fibres. Cellulose synthase genes, *ces* that control secondary wall formation of cotton fibre were also explored to improve fibre quality. Several attempts were made to increase fibre quality and yield by regulating fibre specific genes of cotton through over-expression or gene silencing.

Recent reports published during 2011-2013 showed that fibre strength and yield could be improved through genetic manipulation of cotton fibre regulating genes. A few candidate genes such as Sucrose phosphate synthase *susA1* genes, extension, *Myb*, aquaporin, expansin, annexin were tried with fibre specific promoters using low fibre length and strength genotypes to improve fibre strength. Professor Jiang's group showed in 2012, that over-expression of a sucrose synthase gene *GhSusA1* increased plant biomass and improved cotton fibre yield and quality. Transgenic cotton over-expressing a profilin gene *GhPFN2* that codes for actin bundling protein resulted in higher fibre bundle strength (Bao et al. 2011; Hinchliffe et al., 2011; Haigler et al., 2012).

Over the past decade, attempts were made to increase fibre length through genetic engineering. Lee et al., (2010) developed transgenic cotton plants to over-express a native gene *GhXTH1* that produced 15–20% longer fibre. Xiao et al. (2010) reported that an enzyme Gibberellin 20-oxidase promotes initiation and elongation of cotton fibers by regulating gibberellin synthesis. Wang et al., (2009) developed transgenic cotton with increase in fibre length (+5.6%) and thicker secondary walls by regulating the expression of actin-depolymerizing genes *GhADF1*. Recently, Chen et al. (2012) reported that down-regulated expression of abscisic acid (ABA) and ethylene signalling pathway genes and high-level and long-term expression of positive regulators including auxin and cell wall enzyme genes for fibre cell elongation at the fibre developmental transition stage may account for superior fibre qualities. Jin Qu et al. (2013) from University of Singapore achieved increased fibre length by silencing an endogenous gene 'Wrinkled1' in cotton.

The earliest attempts of genetic transformation of fibre using genes from external sources, were pioneered by Professor John Maliyakal and his colleagues. In 1996, they produced genetically modified (GM) cotton by introducing poly hydroxyl butyrate, PHB genes isolated from bacteria *Alcaligenes*

eutrophus. The transgenic fibres expressed PHB proteins inside the fibre lumen to exhibit better insulating characteristics with higher heat capacity, lower thermal conductivity, slower cooling, and take up more heat than natural cotton fibre. However the changes in thermal properties were relatively small, as expected from the small amounts of PHB in fibres (0.34% fibre weight). Attempts are being made to increase the PHB synthesis several fold for product applications. PHB proteins were also expressed in linseed (flax fibre) through genetic engineering technologies by Professor Wroebel's group in 2004, to improve thermal insulation.

Over the past 10 years, a few potential candidate genes from various biological sources have been explored for fibre quality improvement. Some of the interesting and useful sources are, SPS gene from spinach, *acsA* and *acsB* genes of bacteria *Acetobacter xylinum*, the spider silk gene, spidroin and genes governing the expression of Fibroin (H- Fib, L- Fib, P25), Sericin (Ser1 and Ser 2) and Seroin in the silk worm *Bombyx mori*.

Silk from silkworms is composed of two proteins, fibroin and sericin and is 5-10 times more extensible than cellulose with superior thermal properties. Fibroin is a natural keratin protein with high tensile strength and soft texture. Expression of fibroin gene in cotton plants resulted in significant changes in fibre quality traits (LI FeiFei et al 2009). Insect silk proteins were also produced in wool producing mammals through genetic engineering Patent no: 2001-218289/22 Karatzaz and Huang (China).

Spider webs made of the thin silk appear extremely delicate. But, interestingly, spider silk is one of the strongest natural substances available in nature. Spider silk is at least 5 times stronger than steel, twice as elastic as nylon, water proof, stretchable and exhibit extreme heat stability. Spider silk is so strong that a pencil thick silk strand can stop a Boeing 707 in flight. Spider silk is being used commercially in spacecraft, artificial ligaments, biodegradable fishing lines, super strong surgery thread for ocular, neurological, cosmetic surgeries, and for light bullet proof fabric. Expression of spider proteins as leaf protein and seed protein has been achieved in plant vacuoles and endoplasmic reticulum of potato and tobacco. Spidroins were expressed up to 2 % of the total soluble protein in tobacco leaves and potato tubers. Barr et al. (2004) produced spider silk proteins in genetically modified *Arabidopsis* leaves and seeds. In 2010, Randy Lewis and his group at the University of Wyoming isolated spider silk protein gene from the spider species, golden orb weaver, *Nephila clavipes* to produce the spider silk proteins in the milk of genetically engineered goats, that can be inter-bred and perpetuated as genetically modified (GM) goats. The silk is extracted from milk

and extruded through fine nozzles to reproduce threads of spider silk commercially called 'Bio-steel'. In 2012, Teule and co-workers from the University of Wyoming developed genetically modified silk worms engineered to produce a spider silk protein 'Spindroin-1' from *Nephila clavipes*. The silk fibres produced by the silk worms is commercially called "Big Red" monster silk. The fibre comprises of composite chimeric silkworm/spider silk proteins that are tougher than the parental silkworm silk fibres and as tough as native dragline spider silk fibres. Such bio-silks can be used commercially in the textile, pharmaceutical, aeronautical, spacecraft and satellite related industries. Wang, Li and Niu (2002) obtained a patent -CN1380418:2:20.11.2002 on 'cotton fibrocyte expression vector plasmid of spider silk gene'.

Shehzad et al. (2013) from University of Punjab, Pakistan isolated an expansin gene CpEXPA3 from the wild plant *Calotropis procera* and introduced it into local cotton (*Gossypium hirsutum* variety NIAB-846) through genetic engineering. Data from

three years of field performance of the transformed cotton plants indicate that fibre strength was significantly improved as compared to a control. Five years ago in 2007, Professor Haigler's group isolated 'sucrose phosphate synthase' sps gene from spinach and introduced it into cotton to improve fibre quality especially under stress. The quality of the genetically engineered fibre was of a premium range even when grown under stressful cool night conditions.

Thus it is clear that there can be unlimited opportunities for the creation of 'wonder-cotton-fibres' either through modification of fibre-regulatory genes within cotton itself, or by over-expressing some external proteins in fibre lumen. Such achievements are likely to get consolidated through strong research efforts in the near immediate future not just for the creation of wonder-cotton-fibres, but also for wonder-cotton-fabric with unimaginable superior qualities such as strength and durability. 'Cotton shirt as strong as steel', will not be just a slogan, but something that all of us may live to use.