Evolvable Features of Lignin Pathway in Wheat under Water Stress

Santosh Kumari
Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi-110012

Recent advances in plant genomics have led to the identification of a vast number of potentially beneficial water-stress-related genes, plus technologies for gene over-expression or silencing. The characterization of key plant physiological mechanisms that restrain plant performance under drought, together with the associated regulatory genes, could therefore facilitate the development by breeders of improved crop varieties showing increased water-use efficiency and drought resistance. Plant growth is the result of daughter-cell production by meristematic cell divisions and subsequent massive expansion of the young cells. Cell expansion is in turn dependent on biophysical changes, which include a regulated loosening of primary cell walls and subsequent yielding to the hydrostatic (turgor) pressure generated by solute and water uptake into the cells. Lignin is a core component of plant cell wall and it is important for water transport, structural integrity, rigidity and pest resistance. Our light microscope and electronmicrographs data on three wheat (hexaploid) cultivars HD 2428 (drought susceptible), HD2285 (thermotolerant) and C306 (drought tolerant) displayed that weak linkage between lignin gene function and metabolic outcome may be advantageous, since it may enhance the tolerance, flexibility and robustness of metabolic regulation.

Key Words: Hexaploid wheat, Water stress, Vascular bundle

Introduction

In annual cropping systems, agricultural fitness is defined as the ability to produce economically harvestable yields of useful and commercially desirable plant products. Agricultural fitness is influenced by many metric factors including germination, pest resistance, survival, growth rate, lodging and yield. For example, glume and caryopses lignin content of the wild relative of sorghum (shattercane) is higher than that of cultivated sorghum [*Sorghum bicolor* (L.) Moench], and lignin content is positively correlated with survival over winter of shattercane seed (Fellows and Roeth, 1992). Agronomically favourable traits are not necessarily those that would promote survival of wild species in at least some environments. For example, crop life-spans are modified to accommodate growing seasons. However, in terms of agronomic fitness, reducing lignin may have positive, neutral, or negative effects. Lignin, a complex phenolic polymer, is integral to plant health, survival, and functions. Lignin provides strength and rigidity to plants and to plant structures such as xylem, which allows for the transport of water; and sclerenchyma and bundle sheath cells, which provide a natural barrier to microorganisms (Bird, 1988). Production of lignin has been demonstrated to be induced in plants in response to mechanical damage or infection by pathogens in numerous species including cucumber, carrot, radish, melon, potato, bean, and grasses.

Variable conclusions regarding the effect of reduced lignin on agricultural fitness are common and most probably derive from interactions of specific genes (which may affect more than just lignin synthesis) and individual plant species, cultivars, and environments. Several studies have attempted to establish a relationship between lodging and lignin content in wheat. However, studies using growth regulators to elicit reduced lodging showed no relationship between lignin content and lodging in wheat (Clark and Fedak, 1977; Knapp *et al.*, 1987). An underlying assumption is that to remain agriculturally fit, plants with reduced lignin would need to remain morphologically similar to commercially desirable unmodified crops. Pincon *et al.* (2001) showed reduced plant size in tobacco transformed with COMT (caffeic acid 3-*O*-methyltransferase) and CCoAOMT (caffeoyl-CoA-*O*-methyltransferase) antisense sequences reducing lignin by 48%. Conversely, Zhong *et al.* (1998) demonstrated significantly reduced lignin (34–59%) in tobacco plants transformed with COMT and CCoAOMT antisense sequences compared with wild-type tobacco, but with normal morphological phenotype. Chabannes *et al.* (2001) reported a synergistic interaction of CAD (cinnamyl alcohol dehydrogenase) and CCR (cinnamyl CoA reductase) antisense transgenes leading to severe reduction in lignin content in transformants that had a normal morphological phenotype under controlled conditions, concluding that "expression of a specific transgene may have different impact depending on the genetic background." Expected responses to significant biochemical or physiological modification of lignin content are reduced vigour and growth.

Here, the discussion is limited to expression of lignin under sudden imposition of water stress and gradual adaptation of wheat cultivars under high temperature in late sown conditions, and the associated changes in plant morphology, and implications.

Materials and Methods

Wheat cultivars C 306 (water stress tolerant), HD 2285 (heat tolerant during grain filling period) and HD 2428 (water stress susceptible) were planted under normal (15 November) and late sown (15 January) conditions. Sowing was done in earthen pots of 30 x 30 cm size filled with sandy loam soil and farmyard manure in 3:1 under natural environment. Each pot was fertilized corresponding to 120, 90 and 60 kg ha^{-1} of N, P and K, respectively. Four seedlings were maintained in each pot. Plants were watered, as per requirement and stress was imposed by lack of water supply for 4 days. Plant protection measures were taken as required. The water stress treatment was imposed at ear emergence (1 cm length) and at grain filling stage (half grain). Seven days period was given for the revival of the plants from water stress. For the estimation of relative water content (RWC) and all anatomical analysis using light and electron microscope, flag leaf of the mother shoot was selected. Each value of RWC, presented in figure is a mean of three replicates. For yield analysis five ears were selected at the time of harvest from the mother shoot. The flag leaf in January sowed plants develops at high temperature.

Yield components were assessed by analysis of complete factorial randomized design. Standard error was calculated for RWC and shown in graphs. Critical deference was calculated by using statistical package for agriculture (opstate).

Results and Discussion

The cells of plants grown under favorable environmental conditions were plasmolysed and cell volume is reduced due to water loss when subjected to water stress (Plate 1-A). The vascular bundle was lignified during revival from water stress in drought tolerant wheat cultivar (Plate 1-B). Therefore, this provided a clear example of a wall localized enzyme action *in vivo*. While in late sown

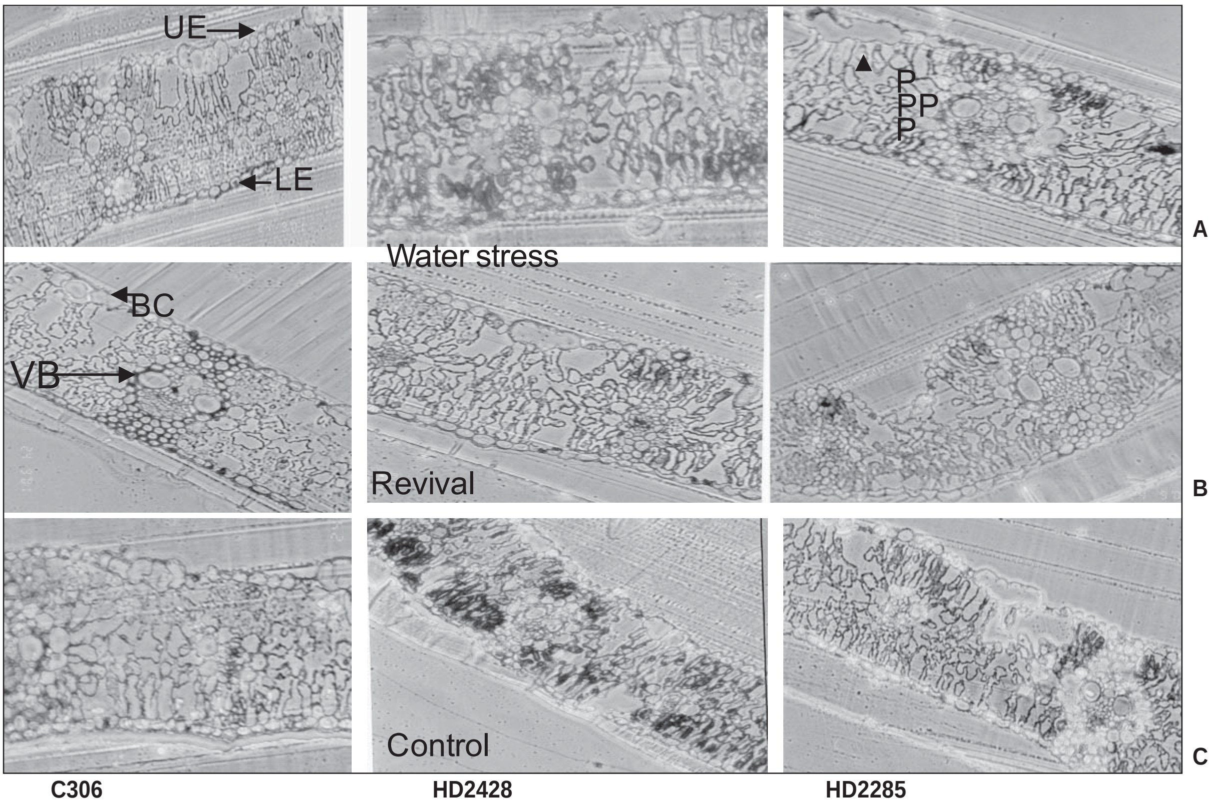


Plate 1: Transverse section of flag leaf of three cultivars of wheat under light microscope (20x) showing lignification of vascular bundle in C306 after revival of water stress. -10X; UE and LE (upper and lower epidermis), PP (palisade parenchyma), BC (bulliform cell) and VB (vascular bundle

plant, cell volume is reduced due to the increased lignifications of cell wall (Plate 2). There was significant reduction in RWC under water stress in all cultivars. Genotype C 306 retained significantly higher RWC when submitted to water stress conditions under all sowing dates. HD 2285 exhibited intermediate value, while HD 2428 showed lowest value under water stress.

Presence of endoplasmic reticulum, ribosomes and Golgi apparatus indicate *de-novo* synthesis and modification of proteins in vascular bundle of C 306 during revival of stress (Plate 3). We have demonstrated that C306 is drought tolerant genotype because it synthesized lignin using reactive oxygen species induced Cu-Zn-SOD antioxidants (proposed to function in the apoplast in lignifications and that in the nucleus protects the cell against fatal mutations caused by O_2 molecules) in the vascular bundle during recovery from water stress.

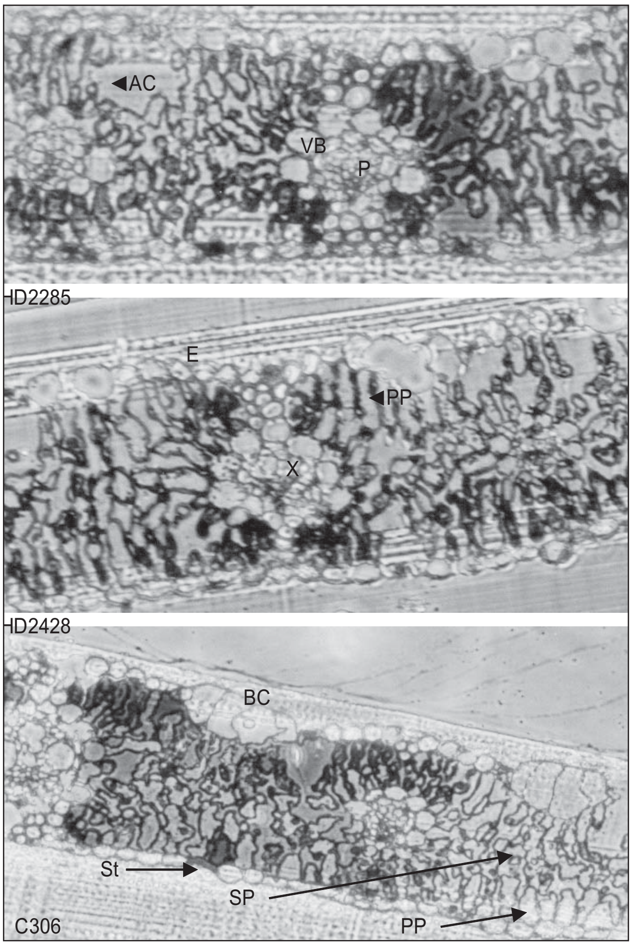
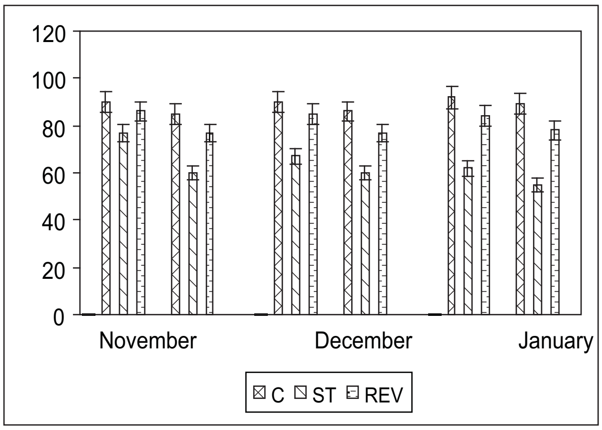
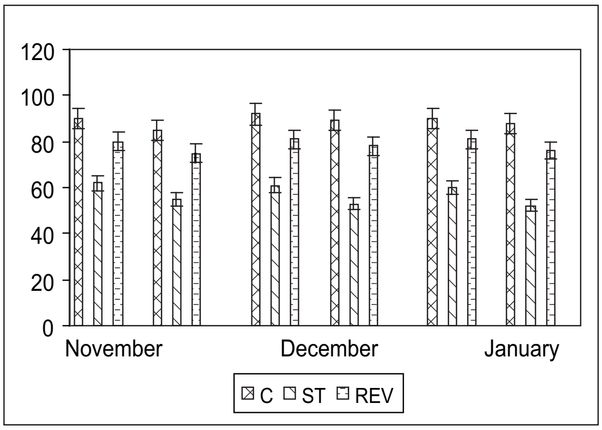


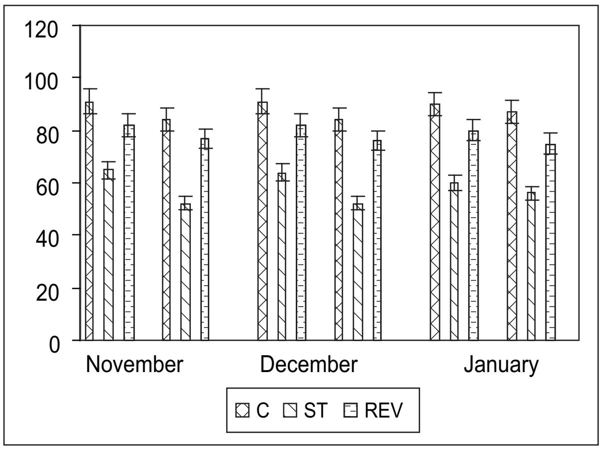
Plate 2: Transverse section of flag leaf of three cultivars of three cultivars sown in January under light microscope (20x) showing reduced cell size and lignification of all cells. E (Epidermal cells); X (Xylem); B (Bulliform cells); VB (Vascular bundle) and P (Phloem); PP (Palisade parenchyma); SP (Spongy parenchyma); St (Stoma)-10X



C 306



HD 2285



HD 2428

Fig. 1: Relative water contents (%) of flag leaf in C306, HD 2428 and HD 2285 at ear emergence and grain filling stages under water stress at different dates of sowing

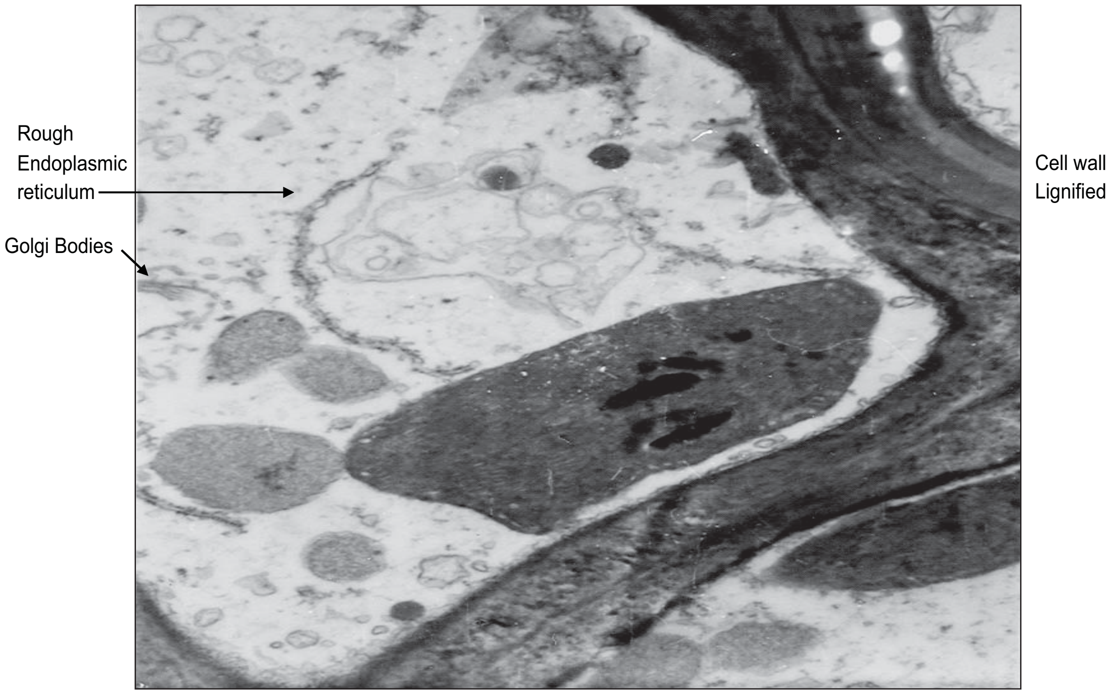


Plate 3: Golgi bodies and rough endoplasmic reticulum appear in vascular parenchyma cell during rehydration of tissue in response to water stress

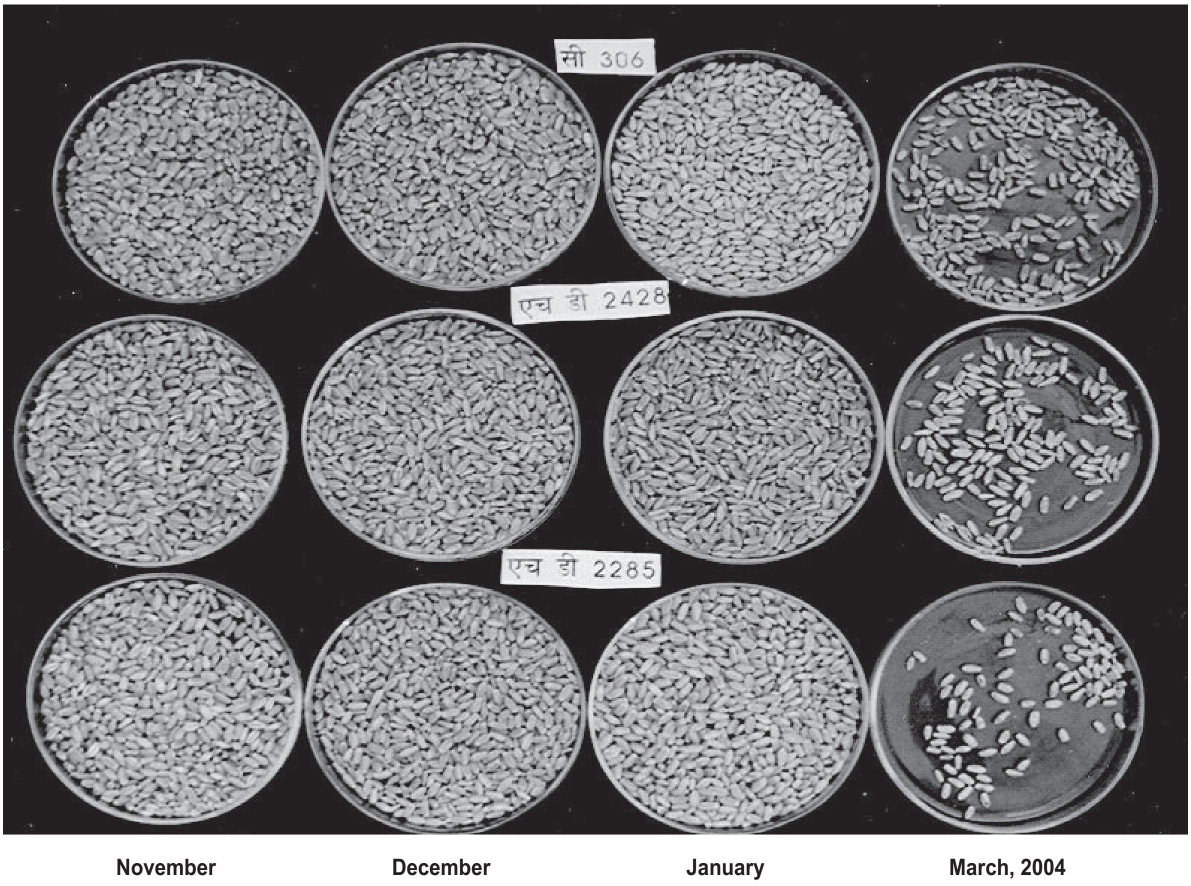


Plate 4: Number and size of seeds in C306, HD2428 and HD2285 cultivars per plant soen at different dates

The cultivars can resist cavitations (breakage of water column) by lignification of vascular bundle under water stress conditions. The size of the xylem vessels were reduced due to secondary growth (lignin formation); reducing the efficiency of water flow in wheat at high temperatures. Furthermore, lignin is water insoluble and thus immobile; therefore, lignin must be made where it is found. In fact, lignin is initially formed in the middle lamella and primary cell wall (PCW) of cells such as xylem vessel elements and phloem fibers (Wardrop, (1971). The initial site of lignifications may thus be approximately 10 micron distant (the thickness of the secondary wall) from the nearest living protoplasm. Gene inactivation, in the ref8 Arabidopsis thaliana mutant, with a point mutation in the CYP98A3 gene, was associated with reduced cell expansion, altered sugar composition of cell walls, and decreased contents in crystalline cellulose and pectins (Nawroz *et al*., 2006).

Cell sizes were reduced because of the increased rate of growth or rapid cell division of smaller size and different cell wall composition (indicated by vascular bundles and epidermal cells) under higher temperature. Secondary metabolism utilizes more energy in terms of synthesis of lignin. Therefore, photosynthets liberated by internode reduction are used in production of thick leaf (multiple layers of mesophyll tissue) and cell wall thickenings. Inhibition of cell expansion results in a slowing of leaf expansion early in the development of water deficits. Finally, it was expressed in reduced internode length, plant height, leaf area and duration of developmental phases in wheat under higher temperature in late sown conditions. Reduced cell expansion as a primary response to water deficit serves to reduce plant water use but lead to reduce plant productivity.

Reduction in 100-seed weight (grain weight and size) of wheat could have been by reduction in the endosperm cell expansion indicating involvement of abscisic acid accumulated in water deficit thus limiting the maximal storage capacity of the kernels by water stress at ear emergence stage. Narrow xylem vessels formation due to reduced cell size under the influence of abscisic acid, and early disconnection of grain could be another reason for smaller grain size under water stress particularly in late sowed crops (Plate 4). Our study with fresh tissue further showed that grain yield was reduced not only due to reduced leaf area and spike size but also by infertility of spikes and inability of pollen tube to reach the egg cell or ovule due to the thickness of ovary wall in lignified tissue (observation under microscope). Several factors could be involved in driving lignin biosynthesis evolution, including environmental adaptation and development of new structures and greater plant stature. We cannot exclude any one of them. Drought tolerant cultivar synthesizes lignin around vascular bundle to avoid cavitations during rehydration under normal planting. While all the three contrasting cultivars lignifed whole leaf tissue to survive under drought conditions created by high temperature in January sowed plants. The strong interactions among lignin reducing genes, the genetic background in which they are placed, and the environment in which the resulting plant lines will ultimately determine the agricultural fitness of reduced lignin cultivars and hybrids. Taken as a whole, it appears that reducing lignin content of crop plants can negatively impact their agricultural fitness. However, when evaluating individual events of reduced lignin, effect on agricultural fitness may be neutral or even positive. An example of such a positive reaction to reduced lignin content is provided in a study by Hu *et al.* (1999) that showed enhanced leaf, root, and stem growth in transformed poplar (*Populus tremuloides* Michx.) with a 45% reduction in lignin. It is essential that reduction in lignin content, whether resulting from mutations in lignin biosynthesis genes, from selection for traits associated with reduced lignin, or from transformation, be evaluated in diverse genetic backgrounds and gene combination, and in diverse environments to discover optimal combinations and a true measure of value to, and fitness in agricultural systems.

References

Bird PM (1988) The role of lignification in plant diseases. *In:* RS Singh *et al.* (eds.) *Experimental and Conceptual Plant Phytopathology*. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India, p 523-536.

Clark RV and G Fedak (1977) Effects of chlormequat on plant height, disease development, and chemical constituents of cultivars of barley, oats, and wheat. *Can. J. Plant Sci.* **57**: 31-36.

Chabannes M, A Barakate, C Lapierre, JM Marita, J Ralph, M Pean, S Danoun, C Halpin, J Grima-Pettenati and AM Boudet (2001) Strong decrease in lignin content without significant alteration of plant development is induced by simultaneous down-regulation of cinnamoyl CoA reductase (CCR) and cinnamyl alcohol dehydrogenase (CAD) in tobacco plants. *Plant J.* **28**: 257-270.

Fellows GM and FW Roeth (1992) Factors influencing shattercane (*Sorghum bicolor*) seed survival. *Weed Sci.* **40**: 434-440.

Hu W-J, SA Harding, J Lung, JL Popko, J Ralph, DD Stokke, CJ Tsao and VL Chiang (1999) Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees. *Nat. Biotechnol.* **17**: 808-812.

Knapp JS, CL Harms and JJ Volenac (1987) Growth regulator effects on wheat culm nonstructural and structural carbohydrates and lignin. *Crop Sci.* **27**: 1201-120.

Nawroz AB Pollet, J Ehlting, K Larsen, C Asnaghi, S Ronseau, C Proux, M Erhardt, V Seltzer, R Jean-Pierre, P Ullmann, P Markus, C Lapierre and D Werck-Reichhart (2006) A coumaroyl-ester-3-hydroxylase insertion mutant reveals the existence of nonredundant meta-hydroxylation pathways and essential roles for phenolic precursors in cell expansion and plant growth. *Plant Physiol.* **140:** 30-48.

Pincon G, S Maury, L Hoffman, P Geoffroy, C Lapierre, B Pollet and M Legrand (2001) Repression of O-methyltransferase genes in tansgenic tobacco affects lignin synthesis and plant growth. *Phytochem.* **57**: 1167-117.

Wardrop AB (1971) Lignins, occurrence and formation in plants, In: KV Sarkanen and CH Ludwig (Eds), Chichestar; Wiley, pp.19-14.

Zhong R, WH Morrison, J Negrel and ZH Ye (1998) Dual methylation pathway in lignin biosynthesis. *Plant Cell* **10:** 2003-2046.