Abstract

Sorghum bicolor L., essentially a major forage crop, has been considered as a potential source of carbohydrate components for the production of bio-fuel. However, presence of high lignin content in sorghum stem often appears as an impediment in chemical extractability of desired carbohydrate components for industrial use. To resolve the problem, the present study was aimed to develop an improved variety of *S.bicolor* with reduced lignin through hpRNA induced RNA interference of 4 Coumarate: CoA ligase (4CL) gene involved in lignin biosynthetic pathway. A 4CL gene of 1.713 kb encoding 571 amino acids was identified in *S.bicolor* and characterized by the presence of conserved peptide motifs and cysteine residues responsible for its substrate binding and catalytic activity as identified in other plant species. Hereinafter, the presently isolated gene was referred as *Sb*4CL. Southern hybridization demonstrated the *Sb*4CL gene was present as single copy in sorghum genome. Introduction of hpRNA inducing gene silencing cassette of the *Sb*4CL linked with hygromycin resistant gene through *Agrobacterium* mediated transformation in shoot tips of *S. bicolor* resulted in production of two independent transgenics sorghum lines as revealed by PCR amplification of hygromycin resistance gene and southern hybridization. The endogenous *Sb*4CL gene was found to down-regulate in varying quantum as revealed by northern hybridization and enzyme activity in the independent transgenics. A direct correlation between down-regulation of *Sb*4CL and lignification in sorghum stem tissue could be assigned as variation of the lignin content was observed between the transgenic lines with a highest reduction of 17.5% compared to untransformed plants. Additionally, decreased lignin content in the transgenic lines was found to be compensated by increase in total carbohydrate, especially with respect to the presence of more amount of soluble sugar (~26.56 mg/g of tissue) compared to untransformed plants. Thus, the present study suggested that the suppression of *Sb*4CL gene is an efficient means to develop improved sorghum variety in terms of lignin content. Furthermore, the transgenic lines could be of better use as the favourable alteration of lignin content and enhanced carbohydrate content may facilitate its industrial utilization towards economic gain.

Key words: *Sorghum bicolor*, Lignin, hpRNA, RNAi, 4CL, Bio-fuel, transgenic, regenerative callus, Southern hybridization, Northern hybridization, Enzymatic activity.