Abstract

Increasing population and rapid industrialization creates shortage of fossil fuel. Bioethanol is an important alternative to meet that need. Lignocellulosic biomass is a promising source for the production of bioethanol. Among many other plants, sweet pearl millet (*Pennisetum glaucum*, 2n=14) has been demonstrated as one of the rich lignocellulosic biomass sources. But utilization of its cellulose to the maximum extent is hindered due to presence of high amount of lignin in its cell wall. So aim of this present study is to develop an improved variety of *P. glaucum* with reduced lignin content through genetic manipulation by transgenic approach. Efficient regeneration and transformation system is a prerequisite for getting maximum number of transformants. In this study firstly we developed the regeneration and transformation protocol with comparable regeneration efficiency (90%) and a better transformation efficiency (10%) in comparison to previous reports (7%). The regeneration was carried out through somatic embryogenesis using shoot apical meristems as explants. Transgenic plants were developed *viaAgrobacterium* mediated transformation. Total lignin content was reduced through RNA interference (RNAi) of 4-coumarate:coenzyme A ligase (*4cl1*) genes, a key regulatory enzyme, that controls the synthesis of different lignin monomers. Three stable transgenic plants were obtained when RNAi gene cassette of *4cl1* was introduced through *Agrobacterium* mediated transformation system. Endogenous enzyme activity was reduced in all the three transgenic plants compared to untransformed control. In T_2 generation, maximum reduction of total lignin content observed was 26.11% with respect to untransformed control. Saccharification efficiency of pretreated biomass was increased up to 18.25%. All the transgenic plants showed lodging characteristic without any significant changes of dry biomass. The present study suggested that the three transgenic lines with reduced endogenous *4cl1* activity and increased saccharification efficiency could be the better source of bioethanol production.

Keywords: *4cl1*, Sweet pearl millet, Biofuel, Lignin, Transgenic plant