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Genetic engineering of lignin biosynthesis to improve bioethanol production from Leucaena leucocephala

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Lignin hinders the optimum utilization of cellulosic biomass in bioethanol production. Reduction of lignin could be achieved through down-regulation of monolignol biosynthetic pathway genes. cDNA clones of cinnamoyl CoA reductase (LlCCR) and cinnamyl alcohol dehydrogenase (LlCAD) were isolated from Leucaena leucocephala, a leguminous pulpwood tree exclusively used for paper production in India. Sense and antisense constructs of LlCCR and LlCAD under CaMV 35S constitutive promoter were introduced in tobacco to up and down-regulate CCR and CAD genes. All down-regulated lines displayed stunted growth and development with significantly reduced lignin content. Contrarily, up-regulated lines exhibited robust growth, development and significant increase in lignin content. These observations support a role for CCR and CAD down-regulation in improving wood properties of L. leucocephala for bioethanol production. Such attempts under constitutive promoters are deleterious to the plant due to pleotrophic effect. A 795 bp CCR promoter and 1882 bp CAD promoters were isolated from L. leucocephala by genome walking approach and sequence analysis revealed the presence of cis-elements like AC boxes, XYLAT, WRKY and MBS. Promoters were fused to β-glucuronidase (GUS) reporter gene in pBI101 and introduced in tobacco. Histochemical observations indicated expression of GUS in the vascular tissues of leaf, stem and root of transgenic tobacco. Down-regulating CCR and CAD genes under such vascular tissue specific-promoter could be useful for reducing the lignin content only in vascular tissues of L. leucocephala, overcoming the risks associated with reduction of lignin content across the whole plant.

Biography

Singam Prashant has completed his Ph.D in 2010 at the age of 31 years from Department of Genetics, Osmania University, Hyderabad. He was awarded Dr. D. S. Kothari Postdoctoral Fellowship from University Grants Commission, New Delhi in 2011, pursuing postdoctoral studies at Center for Biotechnology, Jawaharlal Nehru Technological University Hyderabad, Hyderabad. He has published 8 papers in reputed national (2) and International (6) journals.

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