Construct Design for Downregulation of Caffeoyl-Coenzyme A *O*-Methyltransferase and Cinnamate-4-Hydroxylase in *Acacia* Hybrid

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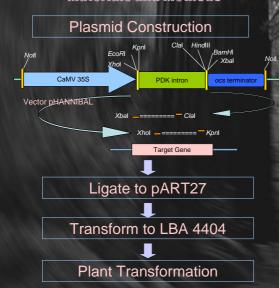


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Introduction

Lignin has significant commercial value and modified-lignin transgenics can provide improved raw materials for pulp and paper industry and agricultural uses. Downregulation of cinnamate-4-hydroxylase (C4H) from *Medicago sativa* in tobacco resulted in significant reduction in lignin content with apparent decreases in their S (syringyl)/G (guaiacyl) ratio (Sewalt et al., 1997). Antisense of caffeoyl-coenzyme A *O*-methyltransferase (CCoAOMT) was transformed to *Populus tremula x Populus alba plants*, and the results showed reduction of 68% in CCoAOMT activity and 45% in lignin content, relative to the controls (Zhong et al., 2000). Most of the lignin downregulation studies use sense or antisense constructs which usually result in only a modest proportion of silenced individuals. Recent studies have demonstrated the use of constructs encoding self-complementary hairpin' RNA (hpRNA) to efficiently silence genes (Wesley et al., 2001). In this study, downregulation of lignin genes is attempted using both antisense and intron-spliced hpRNA constructs.

Materials and Methods



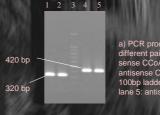






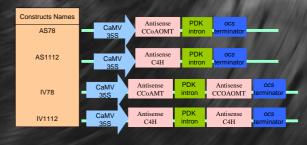


Pasults and Discussion



a) PCR products produced by different pairs of primers. Lane 1: sense CCoAOMT, lane 2: antisense CCoAOMT, lane 3: 100bp ladder, lane 4: sense C4H, lane 5: antisense C4H.

b) PCR fragments were digested with selected restriction enzymes and ligated to vector pHANNIBAL. Four different constructs are shown as follow:



c) Hairpin structure predicted to form in IV 78 and IV1112 mRNA.

Acknowledgements

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