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CLONING OF KENAF (*HIBISCUS CANNABINUS* L.) LIGNIN AND CELLULOSE BIOSYNTHESIS GENES

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Kenaf (*Hibiscus cannabinus*) is an annual herbaceous plant, native to tropical regions of Asia and Africa. It shows has a rapid growth and erect stem, and it is grown for obtaining cellulose and lignin. Kenaf's stem is formed mainly of two parts: a cortical external part (called "bark") that gives long fibres (2-6 mm) accounting up to 35-40% of the total stem weight; a woody inside part (called "core") that furnishes light and absorbent wood. The core shows short fibres (0.6 mm) and represents 60-65% of the total stem weight.

Nowadays, kenaf is having an increasing interest as industrial crop due to high quality cellulose for paper production. Furthermore, the core and the bark are amenable to be used in bio-composite materials, mainly for automotive and house building industries. Kenaf-based bio-composite materials are highly biodegradable and, hence, their uses are suggested to reduce the environmental pollution and wastes. In recent years, the enforced EU policy deals with the reduction of woody plants as source of cellulose, replacing them with annual herbaceous plants; therefore, kenaf could be one of the best candidates as source of cellulose.

In order to modify the quantity and the quality of kenaf fibres for specific industrial uses by using up-to-date breeding strategies and techniques, there is a need to enlighten kenaf cellulose and lignin biosynthetic pathways. Recently, we have carried out some investigation on kenaf cellulose and lignin biosynthetic pathways, in a frame of a collaboration with the AgriKenaf Volturno, an industry interested in to improve this species in our country. As first step in our breeding programme, we are going to clone and sequence as many genes as possible involved into cellulose and lignin biosynthesis. We have focused our interest on the following eleven genes: (a) lignin biosynthesis genes: CAD, cinnamyl alcohol dehydrogenase; CCR, cinnamoyl-CoA oxidoreductase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate:CoA ligase; (b) cellulose synthase gene family: from CeSA1 to CeSA7.

Two cultivars have been used as source of DNA: Everglades 41, a historical variety for commercial kenaf growers; Dowling, a cultivar with higher bast fibres percentage. Plants were grown in a growth chamber, under controlled conditions. In order to design primers for PCR of the above-listed genes, we did a bio-informatics approach; primers were designed on highly conserved coding sequences of these genes already isolated and sequenced from several monocots and dycots species. PCR analysis showed some amplified fragments. Regarding genes related with lignin biosynthesis, C4H and CAD primers gave some amplification products. Regarding genes related with cellulose biosynthesis, some amplification products were obtained from PCR reactions with primers designed to amplify CeSA4, CeSA5 and CeSA7 genes.

One of the CAD amplified fragments has been sequenced in our laboratory, and the resultant sequence appeared to be homologous to the sequence of the *Nicotiana tabacum* CAD14. We are now sequencing the other amplified fragment and modifying the other primers that did not give any amplification results.