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BRIEF COMMUNICATION

Detection of Ri T-DNA in Transformed Oilseed Rape Regenerated from Hairy Roots

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Abstract. A fertile plant with altered phenotype of oilseed rape (*Brassica napus*) spring cultivar HM-81 was regenerated from hairy root tumors incited by *Agrobacterium rhizogenes* 15834. Southern blot hybridization analysis revealed several copies of T_L and T_R-DNA of Ri plasmid.

Infection of wounded dicotyledonous plants with *Agrobacterium rhizogenes* can cause prolific root formation, known as hairy root disease (Riker *et al.* 1930). As in the case of *A. tumefaciens*, the newly developing tissue is characterised by the acquisition of a bacterial DNA fragment from a large root inducing (or Ri-) plasmid (White *et al.* 1982). The transferred DNA or T-DNA is stably integrated into plant chromosomes and carries different sets of eucaryotic genes responsible for hairy root development by endogenously altering plant hormone related processes and for opine production (Birot *et al.* 1986).

Application of Ti and Ri plasmids as vectors for gene transfer to plants for a crop improvement requires the regeneration of whole transformed plants from crown gall and hairy root tumors. In some species transformed plants containing Ti or Ri T-DNA have been regenerated (Turgeon *et al.* 1976, Wullems *et al.* 1981, Pavingerová *et al.* 1983, Nečásek *et al.* 1988). Also transformation and regeneration of one of the major crops, winter cultivars of oilseed rape have been reported (Ooms *et al.* 1985, Guerche *et al.* 1987, Pua *et al.* 1987).

Here we give a direct evidence for the transformation of fertile oilseed rape of spring cultivar HM-81 regenerated from hairy root tumors incited by *A. rhizogenes*.

The response of 51 oilseed rape cultivars to the infection with *A. tumefaciens* and *A. rhizogenes* was studied (Dusbábková *et al.* 1988). A whole plant was

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regenerated from hairy root tumors after transformation of *Brassica napus* cv. HM-81 by *A. rhizogenes* 15834. This transformed plant HM-81-JZ was fertile and had a changed phenotype (crinkled leaves, abundant roots that showed reduced geotropism, shorter internodes) but did not produce agropine or manopine (Dusbábková *et al.* 1988). Similarly changed phenotypes in other plants transformed with T-DNA of a similar origin have already been described (Ooms *et al.* 1985).

Direct proof that HM-81-JZ was indeed transformed and contained Ri T-DNA was obtained by the Southern analysis. High molecular plant DNA was obtained according to the method of Taylor and Powell (1982) and digested with restriction endonuclease Eco RI. Southern blot and hybridization were prepared following standard procedures as described by Maniatis *et al.* (1982).

Agropine type Ri T-DNA consists of two independent parts, T_L and T_R-DNA (White *et al.* 1985). Both of them were separately detected. T_L-DNA was detected by hybridization with ^{32}P-labelled plasmid pNW44 (Huffman *et al.* 1984). The structure and organization of T_L-DNA in HM-81-JZ are shown in Fig. 1. All internal T_L-DNA Eco RI fragments (15, 36, 37 and 40) were present. From the number of border fragments and intensity of hybridization we concluded that HM-81-JZ contained several copies of full length T_L-DNA at two different integration sites in the plant genome.

After detection of T_L-DNA the filter was washed to remove the old probe and rehybridized with ^{32}P-labelled T_R-DNA fragments in plasmid pNW56 (Huffman *et al.* 1984). No internal fragments could be detected after digestion by Eco RI because of only one target site for this restriction enzyme. The visible fragments correspond to border fragments of T_R-DNA and indicate the number of insertion sites in plant genome (Fig. 1). At least two copies of Ri T_R-DNA were present in regenerated plant HM-81-JZ.

Phenotypic alteration of plant HM-81-JZ after transformation by *A. rhizogenes* corresponds to the presence of T_L and T_R-DNA integrated into plant genome. Morphological effect of Ri T-DNA can be used as evidence for genetic transformation. The expression of Ri T-DNA genes is compatible with the production of viable and fertile oilseed rape plants and thus genetic manipulation by using Ri plasmid and derived vectors is possible. Evaluation of our regeneration system and comparison with the system published by Guerche *et al.* (1987), as well as analysis of HM-81-JZ progeny are in progress.

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Fig. 1 at the end of the issue.

BOOK REVIEW

ARCHER, R. R.: GROWTH STRESSES AND STRAINS IN TREES. — Springer Verlag, Berlin—Heidelberg—New York—London—Paris—Tokyo 1987. 240 pp., 115 figs. Hardcover DM 98.—

The third book of the Springer series on wood science has been devoted to mechanical stresses and strains which develop in cambial layers of the tree stem, during its growth.

The growth stresses in trees present some special problems, which are of interest to a wide range of specialists. For example, tree physiologists are interested in questions, such as the relation of mechanical stress to stem tapering and the role of reaction wood and gravity forces in determining the form of tree crown. Relations between thinning and wood quality are interesting to foresters. Wood properties are correlated with growth stress levels which are, in turn, significantly changed by thinning. And finally, wood technologists are interested in the relation of residual growth stress gradients in green logs to the dimensional quality of sawn timber.

The book is divided into six topics and five appendices. In my opinion, for tree physiologists are interesting and useful the first three chapters. In these parts all information about "physiologically attractive topics" of growth stresses are involved (An introduction to growth stresses; On the measurement and interpretation of surface growth strains; Mechanisms for the generation of surface strains). In these chapters the experimental results are interpreted by means of comparatively simple models of mechanical actions.

The next chapters are devoted to technically more detailed models of wood, which is a mechanically complex material. A level of mathematical description of the problems under study is adequate to a wide range of biologists and wood mechanics. The book provides a solid basis for understanding growth stresses and strains in trees. A combination of biological and mechanical approaches to the problems studied is of great use.

Archer's book can be recommended to tree physiologists, foresters and wood technologists.

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