MOLECULAR AND CELLULAR EVENTS DURING ADVENTITIOUS ROOT INITIATION IN LOBLOLLY PINE CUTTINGS

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One of the principal obstacles to using rooted cuttings to implement clonal forestry with the southern pines is maturation. As the age of the donor plant increases, the rooting ability of its cuttings decreases. To better understand the causes of maturation, we are studying adventitious root formation in stem cuttings of loblolly pine. Our overall approach is to determine: (1) the molecular events that are necessary for root initiation and (2) how the root initiation pathway differs in cuttings from juvenile and mature plants. Endogenous or exogenous auxin is necessary, but not sufficient, to stimulate root meristem initiation. We have examined auxin transport and metabolism in hypocotyl cuttings which root at high frequencies and rapidly and in epicotyl cuttings which root at much lower frequencies and more slowly. Our data indicate that auxin transport and metabolism do not differ substantially between hypocotyl and epicotyl cuttings. Moreover, in hypocotyl cuttings, the presence of auxin is not limited to the cells which divide to form root meristems. Thus, the ability of a cutting to respond by forming roots is not just dependent on the availability of auxin, but by some other determinant of cellular competence.

One potential competence determinant is the ability of cells to respond to the presence of auxin by initiating gene expression. A class of auxin-responsive genes has been cloned from annual plants that are rapidly transcribed after auxin treatment. We have cloned genes from loblolly pine with sequence similarities to the auxin-induced genes from pea, soybean, mung bean and *Arabidopsis.* Preliminary experiments indicate at least one of the genes is induced by auxin in hypocotyl cuttings. Further studies will test the association between the expression of these genes and competence for root initiation.

Another critical step in the root initiation process is cell division. Studies from many organisms indicate that the cell cycle is under control of gene products such as cyclin-dependent kinases (cdks). We have cloned two cdks from loblolly pine which have sequence similarities with cdk1 and cdk2. Preliminary data indicate that one of these genes is expressed equally in hypocotyl and epicotyl cuttings. The other appears to be induced only in the hypocotyl cuttings after auxin treatment. Future experiments will test the localization of expression of the latter gene in root-forming cells and the expression over time in stem cuttings from mature donor plants. These experiments, plus those examining the role of auxin-binding proteins, should provide insight into the mechanisms of adventitious root initiation and its limitation by maturation.