

OWENS, LOWELL D.*, JOHN C. INGERSOLL and THOMAS M. HEUTTE, USDA, Agricultural Research Service, Plant Molecular Biology Laboratory, Building 006, Beltsville, MD 20705. - Genetic engineering studies in sugarbeet: Promoter analysis in transiently transformed suspension cells and degradation of antibacterial polypeptides in leaf intercellular fluids.

The rational design of gene constructs for introduction into sugarbeet necessitated development of a transient assay for assessing promoter activity in sugarbeet cells. Inducible promoters from tobacco osmotin and PR-S genes and a potato proteinase inhibitor 2 (PIN2) gene were fused to the β -glucuronidase (*uidA*) coding region and compared with a construct carrying the constitutive 35S promoter from cauliflower mosaic virus. An optimized protocol consisted of preincubating suspension cells 4 h on medium supplemented with equal proportions of sorbitol and mannitol (250 mM total) prior to bombarding with DNA-coated microparticles. At 24 h the osmotin promoter displayed activity 2.5 times that of the 35S promoter. Activities of the PR-S and PIN2 promoters were intermediate. To investigate degradation of the secreted polypeptide products of engineered genes, antibacterial cecropins were incubated with leaf intercellular fluid (ICF) from various crops. Modified cecropin MB39 had a half-life of 5.8 h in sugarbeet ICF, while that for authentic cecropin B was 4.6 h. This influence of structure on stability was observed in ICFs from other crops as well.