Effects of Genotype, Subculture Interval and Growth Regulators on Shoot Regeneration from Serially-Subcultured Hormone-Autonomous Sugarbeet (*Beta vulgaris* L) Callus.

William P. Doley and Joseph W. Saunders*

SUMMARY

Many genotypes of sugarbeet initiate hormone-autonomous callus when leaf disks are incubated on Murashige and Skoog (MS) medium with 1.0 mg/L N^6-benzyladenine (BA) as the sole growth regulator (B1 medium). When this callus is serially subcultured on B1 medium, shoot regeneration frequency rapidly declines. We investigated the effects of genotype, subculture interval, BA concentration and 2,3,5-triiodobenzoic acid (TIBA) on shoot regeneration from serially-subcultured calli up to 18 wk old. Calli of three genotypes were initiated from leaf disks on B1 medium and subcultured to various MS based media after 3 wk growth. Competence was assessed by shoot regeneration frequency and shoot number per callus on maintenance media as well as after subculture to challenge media. When calli were subcultured every 3 wk on B1, genotypes differed significantly in rate of decline in shoot regeneration. After 15 wk on B1, more than half of EL 45/2-108 calli were still regenerating shoots, while regeneration by calli of REL-1 and FC 607-O-20 was approaching zero. Although EL 45/2-108 had excellent long term regeneration, the extreme vitrification of the regenerant shoots coupled with a very high shoot to callus ratio make this genotype a poor choice for applications involving serially-subcultured callus. Subculture interval did not effect subsequent shoot regeneration frequency, but calli subcultured more frequently were lighter in color and appeared less senescent. Regeneration frequency from calli maintained on B1 was increased after subculture to MS + 3 mg/L BA. The frequency of calli regenerating shoots and the number of shoots per callus were both significantly enhanced by repeatedly doubling the BA concentration at each subculture or by maintenance on B1 + 1 mg/L TIBA. Calli of REL-1 were generally more responsive than calli of FC 607-O-20 to maintenance on TIBA. Increases in regeneration frequency were greater when concentrations of both BA and TIBA were higher in the challenge medium relative to the maintenance medium. Calli maintained in a non-regenerating state on hormone-free medium were induced to regenerate by transfer to challenge medium containing 3 mg/L BA. Manipulation of shoot regeneration with BA and TIBA appears to be compatible with a regeneration model involving auxin/cytokinin ratio.

* American Crystal Sugar Company, P.O. Box 1227, Moorhead, MN 56561-1227 (formerly Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824); and USDA-ARS, Sugarbeet, Bean & Cereal Research, Department of Crop & Soil Sciences, Michigan State University, East Lansing, MI 48824-1325, respectively.