Callus Induction and Plant Regeneration of *Beta* Germplasm

Y. Shimamoto, H. Hayakawa, J. Abe, H. Nakashima and T. Mikami

Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan

ABSTRACT

Callus and leaf disc cultures of different sugarbeet germplasm, including various sugarbeet *(Beta vulgaris)* lines, some accessions of *B. maritima* and *B. macrocarpa*, were initiated from aseptically germinated seeds. Plant regeneration through organogenesis was obtained either on MS or B5 medium containing BAP and NAA. Shoot formation from the basal tissues of leaf disc which were trimmed from shoots and infested with *Agrobacterium tumefaciens* was obtained on MS medium containing BA and some antibiotics. Sugarbeet germplasms within the Beta section differed in their abilities to form callus, regenerate plants, and to produce transgenic plants.

Additional Key Words: *Sugarbeet, transformation, Agrobacterium, leaf disc*

Transformation of herbicide, pest, etc. resistant germplasm, from various sources, to the sugarbeet genome has been attempted by several workers. A successful transformation requires reliable tissue culture techniques. In sugarbeet, gene transfers have not yet been realized due to limited progress in regeneration of functional plants.

A few genotypes are known to regenerate readily. The identification of specific germplasms which are capable of excellent rates of plantlet regeneration in tissue culture is the principal step in transgenic approaches to cultivar improvement. This paper reports the evaluation of diverse sugarbeet germplasm in the section Beta for *in vitro* morphogenic potential.

RESULTS AND DISCUSSION

The response of 21 sugarbeet genotypes to tissue culture was surveyed on MS medium containing 2 μM and 5 μM BAP. Callus growth was observed in the shoot region of the germinated seed after incubation for 20 to 30 days on callus initiation medium. Further incubation promoted continued growth of callus around the shoot region. *B. maritima* accession SP673000-0 and sugarbeet line NK182 showed high callusing ability 30 days after inoculation. Most of the calli obtained were fast growing, i.e., they doubled in volume in 10 to 14 days. The callus was pale yellow to white with a loose friable texture. A few cultures produced a yellow to light tan callus which was firm and often proliferated poorly. These two types frequently adjoined each other in cultures originating from a single explant. Vigorous red callus formation was observed in the red pigmented sugarbeet line NK150.

Friable calli were selected for plant regeneration. Firm calli were discarded because they grew slowly, frequently gave abundant rhizogenesis, and gradually developed necrotic sectors. Friable calli were subcultured from 2 to 3 months in the callus initiation medium and transferred to MS medium with 5μM BAP for shoot organogenesis. Green spots began to appear 7 to 20 days after culture initiation. Shoots and occasionally roots arose from the green primordia. Genotypes differed in their ability to regenerate shoots. Regeneration was achieved for eight of the 21 sugarbeet germplasms. Sugarbeet line T2n-24-115-24 had the highest rate, with 71% of callus cultures forming shoots 40 days after culture initiation. Histological studies showed that organogenic callus is characterized by regions of meristematic cells located near the callus surface. Whole plants were obtained when roots were induced with MS medium supplemented with 2 μM NAA from shoots 14 days after cultures were

transferred. Several regenerants exhibited somaclonal variation, expressed as reduced plant vigor, leaf malformation and variegation.

Co-cultivation of shoot base tissues of nine sugarbeet germplasms with *Agrobacterium tumefaciens* was carried out on MS medium with 0.1mg/l IBA and 0.25mg/l BA, after trimming the shoot from the leaf disc. Newly growing shoots were induced from the basal tissues in 3 to 10 days on shoot selection medium containing the antibiotics. No shoots were obtained from the shoot base tissues which had not received the co-cultivation process. A *B. maritima* accession from France had the highest rate of regeneration as well as a high rate of shoot formation on the leaf disc.

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