Vegetative Propagation of Sugarbeet from Floral Ramets

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ABSTRACT

Three genetically divergent genotypes that possessed good phenotypic traits were used to study morphogenetic potential for the growth of excised axillary buds. The effects on regeneration potential of explant age (ontogenetic phase) and origin within the inflorescence were examined. Genotypes differed significantly in the induction of axillary buds. Ontogenetic phases earlier than the end of floral development produced more buds than full flower. In all three genotypes, the inflorescence tip produced the greatest number of buds. Best micropropagation was achieved on a medium containing 2.2 μM benzyladenine, best rooting on an auxin-free medium. Acclimated plants were genetically stable, displaying no changes in chromosome number in somatic cells. This study confirmed that *in vitro* vegetative propagation of sugarbeets from floral explants is feasible. The genotypes multiplied from various explants were identical to the original material, indicating that the method could be useful in a breeding program.

Additional Key Words: *Beta vulgaris* L., axillary bud, tissue culture, flower, inflorescence

Vegetative propagation of sugarbeet enables the production of clones identical to the parent. Such clones may be used in breeding programs to maintain a genotype constant over different generations, especially useful for combining ability tests (Saunders, 1982; Atanasov, 1980). It has been recognized for some time that regeneration from shoots is regulated by theinfluence of benzyladenine on apical dominance (Saunders and Mahoney, 1982; Coumans-Gilles et al., 1981). This paper reports our work on regeneration from floral structures, and provides a method for successful fixation of heterozygotes.

MATERIALS AND METHODS

Three plant genotypes of differing genetic constitution were studied. Genotype 'AP-59' is self-sterile, with a wide genetic basis. It is both heterogeneous and heterozygous. Genotype '41' is an F1 single cross hybrid with inbred parental lines. Genotype '31' is a self-fertile F1 three-way hybrid that is heterogeneous and heterozygous.

Explant Culture. Explants were obtained in late summer from field-grown plants. Inflorescence segments were placed for 5 min in 70% ethanol, then for 15 min in 0.2% (aq.) HgCl2, then rinsed several times in distilled water. The explants were cultivated on MS medium (Murashige and Skoog, 1962), supplemented with 3% sucrose, 0.7% agar, and 2.2 µM benzyladenine (BA). To avoid vitrification of ramets, after about 14 d, axillary buds were subcultured from floral stalks onto a micropropagation medium that contained MS medium, 3% sucrose, 0.7% agar, and 1.3 µM BA.

After about 30 d of growth, the axillary buds were transferred from the proliferation medium to a root-inducing medium that contained half-strength MS mineral nutrients, 3% sucrose, 0.7% agar, and various concentrations of auxins (NAA or IAA). Four weeks later, after a rapid wash with tap water to eliminate agar, the plants were transferred to soil in pots. This provided a routine procedure for developing rooting plants in the greenhouse.

Chromosome stability was evaluated by the staining method of Bosemark (1971).

Experiments. The initial experiment compared the effect of various stages of floral ontogenesis on the ability to form axillary buds. A factorial design with three genotypes, three ontogenetic phases, and four replications was used. In the early stages of floral ontogeny, all flower tissues develop laterally on the apical meristem, initiated by periclinal division below the surface layer. Phases of ontogenesis were assigned following the system of Kuperman (1963). Unopened flowers from phase

VI, unopened flowers under the protective bracts from phase VII, and fully-opened flowers from phase IX were tested as explants. An abridged description of the floral stages sampled follows:

Stage VI. The flower stalk grows rapidly, but growth of the inflorescence is slow and it does not emerge from the protective leaves. The flowers grow slowly, especially the style and the sepals.

Stage VII. At the time of intensive growth of the sepals and the style, the sepals still cover the stigma and envelop the inner parts of the flower. The inflorescence comes out from the protective leaves; flowers are 1 to 1.5 mm in diameter; the style develops two small stigmata; filaments are still quite short. Pollen tetrads develop, and it is possible to distinguish vegetative and generative cells in the ovary. The petals begin to open.

Stage IX. Full flowering.

The number of axillary buds was determined in the second subculture, on explants treated with benzyladenine for three weeks.

In a second experiment, the morphogenetic regenerative capacity of inflorescence tips, floral axis segments (each including three bracts and three flowers), and individual flowers was tested. The factorial design included three genotypes, four explant types, and four replications (thirty samples per explant type). In this experiment, regardless of the stage of ontogenesis, explants consisted of the inflorescence tip (0.5 or 1 cm long, respectively), floral stalk segments (non-tip), or individual flowers.

RESULTS AND DISCUSSION

The cloning of breeding materials in the second year (the flowering stage) is particularly valuable in sugarbeet breeding. The genotypes tested possessed good phenotypic trains in the seed generation (vigor, seed number, monogerm character, and pollination capacity).

Axillary bud induction from floral explants

The incidence of axillary buds was determined for floral explants taken from three genotypes, at three ontogenetic phases. Genotype, ontogenetic phase, and the interaction (genotype X phase) all were significant in an analysis of variance of the data. Of the three genotypes, '31' produced the greatest number of axillary buds in each ontogenetic phase (Table 1). The three ontogenetic phases also differed in potential for bud induction. More buds were produced in explants from the early phases (VI and VII), during which floral development was not complete, than in phase IX, when the inflorescence was fully open (Table 1). These results

Table 1. Mean numbers of axillary buds induced in cultures of inflorescence explants obtained from three sugarbeet genotypes, at three ontogenetic phases. (Means are averages of four replications.)

Ontogenetic phase	Number of buds produced			
	Genotype			
	AP-59	41	31	Mean
VI	14.0	13.2	18.0	15.1
VII	13.2	13.5	17.5	14.7
IX	6.2	2.8	3.2	4.1
Mean	11.2	9.8	12.9	11.3

Table 2. Mean numbers of axillary buds induced in cultures of various inflorescence explant types obtained from three sugarbeet genotypes. (Means are averages of four replications of 30 samples each.)

Explant source	Number of buds produced			
	Genotype			
	AP-59	41	31	Mean
Inflor. tip, 0.5 cm	31.0	31.2	29.8	30.7
Inflor. tip, 1.0 cm	20.5	19.0	17.5	19.0
Inflor. segment	12.2	10.5	7.2	10.0
Flower	1.2	1.2	1.2	1.2
Mean	16.2	15.5	13.9	15.2

agree with those of Miedema (1982). The significant interaction of genotype X phase arose from the reversal of genotypes 'AP-59' and '31' in number of buds produced in phase IX, in contrast to their relative production of buds in explants from phases VI and VII (Table 1).

Axillary bud induction from inflorescence explants

Our initial experiment showed that floral ontogenetic phase significantly affected number of axillary buds induced in explant culture. With the same group of three genotypes, we

also tested the effect of inflorescence explant location and size ("explant source") on ability of explants to form buds *in vitro* (Table 2). The analysis of variance for these data showed significance for genotype, explant source, and interaction (genotype X explant source). The significant interaction must arise from the magnitudes of data for genotype X source combinations, as data trends were consistent for both genotype and explant source.

As reported previously by Miedema (1982) and Coumans-Gilles et al. (1989), we also found that explants from inflorescence tips produced significantly more buds than those from other parts of the inflorescence (Table 2). Only a few buds were induced from individual flowers (Table 2), and all attempts to stimulate bud growth in the cultures of individual flowers were unsuccessful, regardless of ontogenetic phase. In contrast, axillary buds were induced easily on inflorescence segments between flowers. Our data imply that the morphogenetic capacity of individual flowers is very low, in agreement with the findings of Margara (1970, 1977).

Size (length) of the initial explant from the inflorescence tip influenced the induction of axillary buds in each genotype. Tips 0.5 cm long produced significantly more buds than those 1.0 cm long, but either length of tip explant yielded more buds than did segments of the flower stalk (Table 2).

Root regeneration in micropropagation is achieved relatively easily by use of suitable media. Similar numbers of rooted plantlets were obtained on media with various auxin concentrations, as well as on the medium without an auxin (Table 3). Therefore, the auxin-free medium was selected for use in further research.

Table 3. Effect of auxins on the induction of root primordia (% rooted) in three sugarbeet genotypes. The test medium consisted of half-strength MS elements + 3% sucrose + 0.7% agar.

	PERCENT OF BUDS FORMING ROOT PRIMORDIA Auxin (µM)				
	NAA		IAA		
Genotype	0	2.7	0.05	5.7	Mean
AP-59	88	84	83	68	80.7
31	87	77	77	63	76.0
41	78	67	63	54	65.5
Mean	84.3	76.0	74.3	61.7	74.1

Given the same concentration of auxin, the inflorescence explants differed in bud growth, suggesting that this trait is genetically controlled. After acclimatization, the plants from all explants were phenotypically homogeneous, and cytological analysis showed that they were genetically stable in chromosome number. These results support and extend those of other studies (Mezei and Kovacev, 1988; Novak and Kubalkova, 1982; Slavova et al., 1982).

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