GENETIC FIDELITY OF *BETA VULGARIS* L. RAMETS DERIVED FROM ADVENTITIOUS BUDS ON SHOOT CULTURE PETIOLES

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ABSTRACT

GENETIC FIDELITY OF *BETA VULGARIS* L. RAMETS DERIVED FROM ADVENTITIOUS BUDS ON SHOOT CULTURE PETIOLES

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Genetic fidelity of adventitious buds rising during *in vitro* shoot culture of *Beta vulgaris* L. was studied, based on the comparison between 417 axillary bud derived ramets and 1108 adventitious bud derived ramets. The characters of pollen shedding, seed set, germness, leaf shape, chromosome number, and guard cell length were compared. The segregation of the characters; hypocotyl color, annualism, and germness was studied in the S_1 progeny of some adventitious bud derived ramets and their heterozygous axillary bud derived counterparts. No evidence for genetic infidelity was seen in most of thousand ramets derived from 141 adventitious buds from 24 original clones, or in S_1 progeny from 50 of those adventitious buds, although two aspects were observed. Tetraploid root tips were growing with diploid ones on the same ramets found to be diploid from guard cell measurements. Different concentrations of 6-benzyladenine on this aspect did not result in any corresponding change of tetraploid frequency. Also, three conspicuously narrow leaf ramets from table beet were observed, but in no case were all ramets from an adventitious bud narrow leafed. This narrow leaf aspect did not persist through the second cycle of shoot culture propagation.

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INTRODUCTION

Sugarbeet (Beta vulgaris L. ssp saccharifera) is an outstanding example of a product of plant breeding. It was developed in Europe less than two hundred years ago from fodder beets by mass selection (Palmer, 1918). Since that time, sugarbeet has been bred to increase sugar percentage (from 7-10 to 13-20%).

As a wind facilitated, cross pollinated, largely self-incompatible plant, beets are highly heterozygous. Both Mendelian and cytoplasmic male sterility are available (Smith, 1980). Mendelian male sterility is commonly used in improving populations of self fertile germplasm, while cytoplasmic male sterility is the method of choice for hybrid development and commercial seed production. Sugarbeet behaves as a biennial crop: the first year in the field it grows vegetatively as a rosette producing a large root with a high percentage of sucrose. If carried over to a second year after undergoing overwintering, it would develop a floral stalk, a process also known as bolting. Cuttings of the floral stalk of individual beets can be used for vegetative propagation for breeding purposes, as can split roots, but the detrimental effect of this on seed quantities produced must be considered. Survival of beet plants in the field following seed bearing is an uncommon occurrence.

Sugarbeet breeding programs primarily utilize mass selection, recurrent selection, and family line breeding(Poehlman, 1979). All

these different systems are geared to producing hybrid cultivars, either diploid or triploid. Mass selection is used primarily to increase disease resistance, root size, root shape, crown height, and, in some programs, sucrose percentage. Recurrent selection is used to concentrate genes for particular quantitative characters, and as it involves progeny performance, favors more readily transmissible traits. Although this system involves some visual selection it differs from mass selection in that the visually selected roots are individually test crossed or intercrossed, then their ramets or selfed seed are used to intercross the best combiners from that test.

In family line breeding, the remnant half sib seed of open pollinated mother beets with superior progeny performance such as resistance to disease and high sugar content, is subjected to further selection for subsequent cycles or is increased for testing as a parental line. Family line breeding, like recurrent selection, has the continual problem of producing enough seed on the mother beet to adequately test. In Europe, triploid or anisoploid sugarbeet cultivars have been successfully used for many years (Hornsey, 1975). When commercial hybrid sugarbeet seed is the goal, either diploid or triploid is the main breeding objective. In these programs either single cross, three way cross, or double cross can be used. The three way cross is mainly used in producing commercial seed in the United States. Male sterility, particularly cytoplasmic male sterility, is utilized in the seed parent since seed production is not necessary for sugar production in the farmers' fields. The male sterile line can be maintained by crossing with its equivalent male fertile line, from which it was derived

by backcrossing.

During a breeding program, it may be quite important to maintain unique individual genotype over years, as in the case of cytoplasmic male sterility maintainers (O-types) and elite general combiners. Because beets rarely survive until even preliminary evaluation of their progeny is complete, and because self sterility precludes maintenence of a heterozygous superior transmitter's gene combination intact through its seeds, there has been a need for an efficient means of vegetative propagation in beets to preserve intact gene combinations until evaluation is complete.

Recently, in vitro propagation of crop plants through tissue culture has become widely used. Shoot culture is successfully used in sugarbeet. A variety of in vitro methods for multiplication has appeared for beets (Hussey and Hepher, 1978; Margara, 1977; Coumans-Gilles et al, 1981; Saunders, 1981; and Harms et al, 1983). Seedlings, axillary buds, and the terminal parts of seed stalks are used to initiate the shoot cultures. Some procedures will be explained in detail in the materials and methods section.

By definition, most shoots arising in shoot culture propagation are derived from axillary buds, but in some species such as Beta vulgaris L., Exbury azaleas (Fordham et al, 1982), and Helianthus annuus (Paterson, 1984) adventitious buds also arise, and can often be indistinguishable from axillary buds. At one time, it was presumed that the ramets of both axillary and adventitious shoot origin were genetically identical to the source genotype. However, reports of genetic variation arising from single cell regenerated plants (Cassells

et al, 1983; Larkin and Scowcroft, 1981), protoplast regenerated plants (Karp et al, 1982), callus and embryo explants (Patel and Berlyn, 1981), and , moreover, adventitious bud derived plants (Evans and Sharp, 1983; Norris et al, 1983; Fordham et al, 1982; Hermsen et al, 1981; Patel and Berlyn, 1981; and Van Harten et al, 1980) suggest that complete genetic fidelity through shoot culture adventitius buds should not be presumed.

The origin of the adventitious buds in beets has been studied by Harms et al (1983), who suggested that in table beet adventitious buds arise from single cells of the upper epidermis of petioles. On the other hand Norris et al (1983) emphasized the multicellular origin of the adventitious buds from their work on plant chimeras in African violet.

The main purpose of this study is to test the genetic fidelity of the ramets of in vitro adventitious shoot origin. It is extremely important to determine the reliability of using shoot culture derived ramets in plant propagation during the breeding program. This is particularly so in the context of the somaclonal variation derived from in vitro culture (Larkin and Scowcroft, 1981).

The following main objectives have been established for this study:

1. To examine the stability of chromosome number by screening for polyploidy or aneuploidy through root tip chromosome counts, guard cell length measurement, plant morphology, pollen production, and seed set.
2. To study the genetic fidelity with regard to the Mendelian

characters hypocotyl color, germness, annualness, and any other traits that might arise in the original clones and/or their S_1 progenies.

3. To study the effect of the 6-benzyladenine in the shoot media on the frequency of any chromosome instability.

MATERIALS AND METHODS

Genetic materials:

Both sugarbeet and table beet clones (Beta vulgaris L.) were used in this study.

- The table beet seeds were from a single cultivar, Early Wonder, which, phenotypically, has heavily pigmented hypocotyl and roots (RR YY), and is biennial (bb) and multigerm (MM). The histological development of prolific adventitious bud formation has been studied in another table beet cultivar (Harms et al, 1983).
- The biennial sugarbeet clones were obtained in the form of shoot culture. They represent both adapted as well as exotic germplasm. The following are the established shoot culture clones which were used.
  . EL 36-18: A randomly sampled individual clone from EL 36, a monogerm (mm), type O (ie, cytoplasmic male sterile maintainer) cultivar bred at East Lansing. This particular clone has been in a continuous shoot culture for five years from seedling origin. The clone has red pigmentation of some plant parts (R-, yy).
  . G335-18E: A randomly sampled monogerm (mm) individual with some red pigmentation (R-, yy) from an East Lansing breeding population. It has also been in continuous shoot culture for five years from embryo origin.
  . 80-66: Elite monogerm (mm) individual from East Lansing

breeding population. Lacks red pigmentation (rr, yy).

. P-13: Multigerm (MM) from Poland seed lot segregating for female sterility. Probably has bolting resistant background. It has also red pigmentation (R-, yy).

. 2-294: Monogerm (mm) from East Lansing breeding population. Lacks red pigmentation (rr).

. 6925-O-3: Monogerm (mm), O-type from parental line SP6926-O. Has red pigmentation (R-).

. J-4: Multigerm (M), unstable stigmoid characteristic from Japan. Has red pigment (RR).

. FC701/5-116: Random multigerm (MM) sample from Rhizoctonia solanum tolerant line FC701/5. Lacks red pigmentation (rr).

. 82 J1-13 and 82 J2-11: Two self-fertile clones from crosses of biennial R- partial stigmoid mutants from Japan with an annual rr genetic stock from East Lansing. Both are Rr Bb.

Some working definitions used in this study:

- Ramet: Each plant derived by asexual propagation from the same original seedling or adventitious bud and propagated by shoot culture; asexual propagule.
- Clone: A collective term for shoots and ramets derived asexually from the same original plant.
- Isolate: A collective term for adventitious bud and all shoots or ramets derived from it by axillary shoot propagation. Each may be identical to the original source genotype.
- Germness: Multigerm or monogerm.

Culture methods:

Murashige-Skoog (1962) basal medium (Table 1) was used with 3% sucrose, 0.9% DIFCO Bacto agar, 0.1 mg/l thiamine.HCl, 0.5 mg/l pyridoxine.HCl, 0.5 mg/l nicotinic acid, and 100 mg/l myo-inositol. This MS + 0.25 6-benzyladenine (BA) was used to multiply the shoot culture and to induce and maintain the adventitious buds. This medium has the lab code M20 and was used in 20 x 100 mm Falcon Optilux disposable Petri dishes sealed with parafin film strips. Plates were kept in continuous low intensity (20 to 50 $Em^{-2}sec^{-1}$) fluorescent light at 24 ± 2°C in growth chambers.

Table 1.--Murashige-Skoog medium (MS). Inorganic Salts.

Composition	Amount mg/l
KNO_3	1900.000
NH_4NO_3	1650.000
$CaCl_2 \cdot 2H_2O$	440.000
$MgSO_4 \cdot 7H_2O$	370.000
KH_2PO_4	170.000
$Na \cdot EDTA \cdot 2H_2O$	37.300
$FeSO_4 \cdot 7H_2O$	27.800
$MnSO_4 \cdot 4H_2O$	22.300
$ZnSO_4 \cdot 7H_2O$	8.600
H_3BO_3	6.200
KI	0.830
$Na_2MoO_4 \cdot 2H_2O$	0.250
$CoCl_2 \cdot 6H_2O$	0.025
$CuSO_4 \cdot 5H_2O$	0.025

. All salts were reagent grade unless otherwise noted.

In these experiments the medium was adjusted to the pH 5.95

with a few drops of 1N KOH or 1N HCl before adding the agar.

Three to five shoots were placed into each plate containing 35-40 ml of medium.

To induce roots on the shoots, MN-19 medium (MS + 3.0 mg/l naphthaleneacetic acid) was used in 125 ml Erlenmeyer flasks capped by foam stoppers and aluminum foil. Rooting flasks were kept in 80-100 $\mu Em^{-2}s^{-1}$ fluorescent light at 24 ± 2°C in the growth chamber. The same MN-19 medium without the agar was used for culture of isolated roots in flasks shaken mechanically in the dark at room temperature (about 22°C).

Establishment of shoot culture:

Shoot cultures can be established from seedlings, embryos, axillary or adventitious buds, or the terminal parts of floral stalks. Sugarbeet clones were already established mostly from lateral buds of floral stalks. Shoot cultures of table beet were established in the following way:

In August 1982, seeds of Early Wonder were germinated in peat soil in the greenhouse. After ten days most of each cotyledon was trimmed off the seedling and each hypocotyl was cut to give a piece up to two cm long containing the cotyledonary node, small true leaves, and the primary growing point. These pieces were surface sterilized using 15% chlorox and 0.01% sodium laurylsulfate (as a wetting agent). Surface sterilization was made twice each for twenty minutes, then rinsed 5-6 times with sterile distilled water. These shoots were then placed on M20 shoot culture medium. All procedures were performed in a laminar flow hood to give aseptic conditions. The experiments

were begun with 70 different Early Wonder seedlings (seventy individual table beet genotypes) in addition to the previously established sugarbeet shoot cultures. Every individual genotype with its subsequent ramets were designated separately, EW1 Ax, EW2 Ax, ... etc. Only twenty four individual table beet genotypes from the original seventy seedlings were both uncontaminated and growing as in vitro shoots after four weeks. Shoots were then sudivided if axillary buds had grown out and subcultured on M20 for further multiplication with axillary shoots. The adventitious buds that gave rise to the ramets in this study were found on leaf petioles in the shoot cultures (Figure 1). Frequently several adventitious buds were on a single leaf. Adventitious shoots were isolated from the petioles and transferred onto M20 for multiplication by axillary outgrowth. Each adventitious bud and subsequent shoot cultures were given a unique isolate number in addition to its axillary number (for example, EW1 PA1, EW1 PA2; PA= petiole adventitious). In several cases adventitious buds found in shoot cultures derived from previous adventitious buds were isolated to constitute second cycle, and potentially different, genotypes. These second cycle isolates were designated with P_2A. Throughout the study the axillary (Ax) derived ramets were used as control plants.

Both axillary and adventitious shoots that multiplied sufficiently were taken out and separated into smaller shoots of 3-5 leaflets and put onto rooting medium (MN-19). After the development of the roots (3-6 weeks), ramets were transferred to potting mix in Jiffy peat pots and left in the greenhouse for in vivo growth (Figure 2). Some of Early Wonder axillary bud derived ramets were utilized

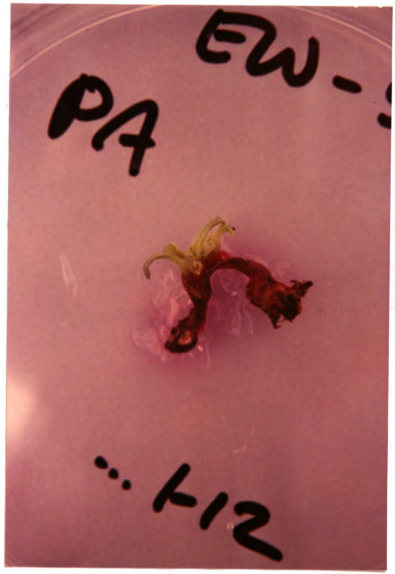


Figure 1.--Adventitious buds grown on a table beet leaf of the genotype EW-53 on the shoot culture medium (M20).

Figure 2.--Potted table beet ramets propagated by shoot culture.

without any comparable adventitious bud derived ramets in order to better sample preexisting variation within this cultivar.

Table 2 shows the number of ramets of different starting clones for both sugarbeet and table beet and their adventitious shoots for experiments 1 and 2. Uneven rooting and plant establishment in pots as well as some contamination problems account for the uneven number of ramets among different isolates.

Experiment 1:

Potted ramets were vernalized in a cold room (4°C) for three months after reaching a height of 10-15 cms. These ramets were placed on a greenhouse room under several incandescent lights for two months to obtain flower stalks (Figure 3). The reproductive characters such as pollen shedding, seed set, and germness were recorded. After this, seed stalks of about 5 ramets of each axillary and adventitius isolate were cut off and reversion to vegetative state was achieved by removal of the incandescent lights during the winter months. Root tip sampling procedures such as described for experiment 3 were then performed.

Experiment 2:

Potted ramets were transplanted in the field for vegetative growth in June 1983 (Table 2). The purpose of this experiment was to study some morphological characters and to observe whether there was any easily detectable mutation or unusual growth. Leaf length and leaf width of three fully expanded leaves for each ramet were measured. Leaf shape, the examined character in this experiment, was determined as the ratio between leaf width to leaf length.

Figure 3.--Seed stalks of some sugarbeet ramets propagated through shoot culture.

Table 2.--The number of ramets obtained from each genotype from both axillary and adventitious bud derived shoot culture.

Starting clone		Number of ramets	Number of ramets
Axillary	Isolate	in experiment 1	in experiment 2
EW-1 Ax		20	8
EW-1	PA 3		1
EW-3 Ax		2	
EW-3	PA 1	1	
EW-9 Ax		6	
EW-9	PA 2		1
EW-9	PA 3	2	
EW-9	PA 5	1	
EW-10 Ax		8	6
EW-10	PA 3	2	2
EW-10	PA 6	2	6
EW-10	PA 7		2
EW-10	PA 8	1	2
EW-10	PA 9	2	3
EW-10	PA 10		2
EW-10	PA 12	3	3
EW-10	PA 13	3	3
EW-10	PA 14		4
EW-10	PA 15		2
EW-10	PA 17		8
EW-11 Ax		14	1
EW-11	PA 2	2	3
EW-11	PA 3		3

Table 2.--Continued.

Starting clone		Number of ramets	Number of ramets
Axillary	Isolate	in experiment 1	in experiment 2
EW-20 Ax		25	7
EW-20	PA 5	4	
EW-20	PA 6	2	
EW-20	PA 7	3	1
EW-20	PA 9		2
EW-20	PA 10	5	
EW-20	PA 11	2	
EW-20	PA 17		3
EW-20	P_2A 2-6		1
EW-20	P_2A 7-1		10
EW-20	P_2A 7-2		3
EW-20	P_2A 7-4		4
EW-21 Ax		5	
EW-25 Ax		4	3
EW-25	PA 1		3
EW-25	PA 4		1
EW-25	PA 12		1
EW-25	PA 13		1
EW-37 Ax		3	
EW-39 Ax		1	2
EW-39	PA 2		1
EW-39	PA 3		1
EW-39	PA 22		4

Table 2.--Conti-ued.

Starting clone		Number of ramets	Number of ramets
Axillary	Isolate	in experiment 1	in experiment 2
EW-40 Ax		2	
EW-40	PA 4		4
EW-40	PA 5		3
EW-40	PA 11		2
EW-40	PA 12		2
EW-44 Ax		15	
EW-44	PA 1	1	
EW-44	PA 2	1	
EW-44	PA 3	1	
EW-44	PA 4	1	
EW-53 Ax		8	
EW-55 Ax		6	1
EW-55	PA 7		2
EW-55	PA 8		6
EW-55	PA 9		1
EW-59 Ax		16	3
EW-68 Ax			2
EW-68	PA 2		1
EW-70 Ax		6	1
EW-70	PA 3		1
EW-70	PA 7		1

Table 2.--Continued.

Starting clone		Number of ramets	Number of ramets
Axillary	Isolate	in experiment 1	in experiment 2
EL 36-18 Ax		13	30
EL 36-18	PA 2	2	6
EL 36-18	PA 3	19	13
EL 36-18	PA 4	1	6
EL 36-18	PA 5	9	15
EL 36-18	PA 6		6
EL 36-18	PA 18	5	16
EL 36-18	PA 23		6
P-13	PA 2	12	11
P-13	PA 3	5	
P-13	PA 4	22	6
P-13	PA 5	25	4
80-66 Ax		4	
80-66 Ax	PA 3	3	3
80-66	PA 4	3	8
80-66	PA 5	2	5
80-66	PA 7		2
80-66	PA 8		3
2-294 Ax		12	9
2-294	PA 1	17	18
FC701/5-116Ax		5	11
FC701/5-116	PA 1	9	6
FC701/5-116	PA 2	3	11
FC701/5-116	P_2A 1-1	5	

Table 2.--Continued.

Starting clone		Number of ramets	Number of ramets
Axillary	Isolate	in experiment 1	in experiment 2
6926-0-3 Ax			2
6926-0-3	PA 1		3
6926-0-3	PA 5		2
G335-18E Ax			21
G335-18E	PA 1		2
G335-18E	PA 2		3
G335-18E	PA 3		1
G335-18E	PA 4		3
Total number of ramets		356	370

Three ramets with conspicously narrow leaf shape (Figure 4) were transferred along with their normal counterparts to the greenhouse to be examined further.

Experiment 3:

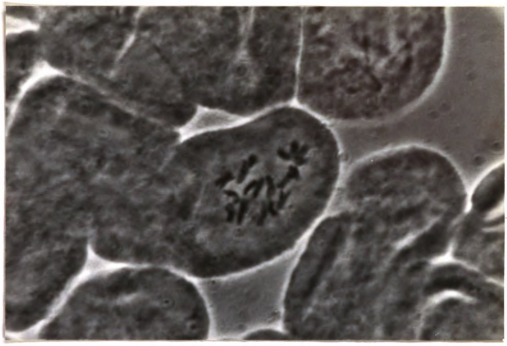
The examination of root tip chromosome number and guard cell length of a sampling of the ramets from both axillary and adventitious shoots was the object of this experiment.

Root tip samples were collected in mid-morning (between 8:00 and 9:00 AM during summer and between 9:00 and 10:00 AM during winter). About 10-15 fresh, robust root tips from each ramet were placed in vials containing 0.01% cycloheximide for 1.5 hours in the refrigerator in order to contract the chromosomes. Following this, the solution in each vial was replaced with 3:1 absolute alcohol to glacial acetic acid and the vials left for 24 hours in the refrigerator to fix the chromosomes in their stages. Both 1N and 3N HCl were used for hydrolysing the roots. The solution was used for 10 minutes at 60° C in a water bath. 3N HCl gave better results. Following hydrolysis, roots were kept in Feulgen stain at least two hours. The phase contrast microscope with oil lens was used for chromosome counting, which was achieved at the pro-metaphase stage (Figure 5 a&b).

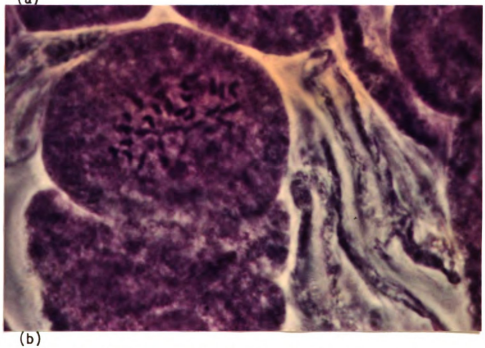
These procedures for chromosome counting were also used for the five ramets of each isolate noted in experiment 1. Data for all chromosome counting were pooled.

Because of the appearence of some tetraploid cells in root tips, microscopic examination of guard cell length was conducted to indicate ploidy of the leaves. A simple light microscope with 40X lens was used

Figure 4.--A narrow leaf ramet of the genotype EW-1 Ax as compared with its neighbor normal leaf of the same genotype.



(a)



(b)

Figure 5.--(a) A normal diploid cell of a sugarbeet genotype (FC701/5-116 Ax) propagated by shoot culture. (b) A tetraploid cell of the same genotype (some chromosomes are not clear because of the focusing.

and its scale units were calibrated. The procedure was executed by peeling the lower epidermis, spreading it on a slide with a drop of water, covering with a glass cover slip, and measuring the longtudinal diameter of the guard cell. The test was always conducted in the afternoon. Thirty readings of five fully expanded leaves for each ramet so tested were taken.. For comparison with guard cell lengths of known diploid, triploid, and tetraploid sugarbeet cultivars, the range and the average of a total 100 guard cells of 10 plants of each ploidy were calculated. It was found that:

for 2n: guard cell length ranged from 7-10 units,

for 3n: " " " " " 10-12 units,

and for 4n: guard cell length ranged from 12-15 units.

The same 2n, 3n, and 4n sugarbeet plants were used also in the cytological studies as mentioned above to determine any mixture of ploidy levels in root tips, as was noticed for certain ramets derived from axillary and adventitious buds.

Experiment 4:

In this experiment, investigation was concentrated on the narrow leaf aspect observed in three ramets mentioned in experiment 2. The three ramets were taken from the field together with their normal counterparts which did not have narrow leaves. Three kinds of analysis were done:

a. Cytological examination of root tips.
b. Flowering and fertility studies.
c. Reestablishing shoot cultures from these ramets to test the persistence of this narrow leaf character after a propagation cycle.

a. The cytological studies:

The three abnormal ramets were from the following clones; EW-1 Ax, EW-10 PA 17, and EW-20 P_2A 7-4. The number 1 was added to the designation of each normal leaf ramet and the number 2 to the designation of each narrow leaf ramet. The six ramets were potted in fresh soil to induce new roots, which were available in ten days. Samples of about 10 root tips were taken and prepared as before.

b. The flowering and fertility studies:

The six ramets were put in a cold room (4°C) for three months for vernalization. The plants were then moved into the greenhouse to complete flower induction. Plants grew and flowered normally. All of them shed polled. Because of the great infection of aphids no seed set could be obtained from the six plants. Lack of seed set even from the three normal leaf plants eliminated the possibility of detecting female infertility.

c. Reestablishing shoot cultures:

The last step which was done to these plants was the test of the narrow leaf character persistence for the same clones throughout the other propagation cycle. This was achieved by reestablishing shoot cultures once again, this time from the terminal parts of flower stalks. These parts were surface sterilized as usual and put first on M20 medium either in petri dishes, or in vials for several days prior to transfer to petri dishes. Because of a contamination problem with these parts, they were subcultured several times following repeated disinfection attempts before being rooted and potted. Ramets which grew well in the M20 were cut and transferred to MN19 for rooting.

After that, rooted plants were transferred to the pots containing fresh soil mix to obtain intact plants. Leaf length and leaf width measurements were recorded.

Experiment 5:

The purpose of this experiment was to compare the segregation behavior of the axillary and adventitious derived ramets in their S_1 generation, and to detect any recessive-like mutations in the S_1 progeny. Two different sugarbeet genotypes that gave considerable numbers of adventitious buds were used; 82 J2-11 and 82 J1-13, initially available as axillary shoot cultures.

82 J2 11 is self-fertile, annual, red hypocotyl and monogerm. It is known to be heterozygous at both B locus (for annualness) and R locus (for red hypocotyl and partially red petioles and stems). 82 J1-13 is self-fertile, annual, red hypocotyl and multigerm. It is also known to be heterozygous in both B and R loci.

From existing shoot cultures, the maximum number of adventitious buds was isolated and transferred to shoot culture medium (M20). A total of 50 adventitious buds was obtained from the two genotypes. After both axillary and adventitious buds had multiplied in shoot culture, at least nine shoots of each adventitious or axillary clone were transferred to MN19 medium for rooting. The plantlets of each clone then were transferred to 3" peat pots after developing roots. Ramets were later transferred to 6" plastic pots and kept in the greenhouse for flowering. Being heterozygous for the dominant annualism allele, these ramets flowered after exposure to only incandescent bulb light. Just prior to first flower opening, bags

were placed over the inflorescences to insure self pollination.

Selfing was achieved by the following steps:

1. Just before the first flower opened the plant was sprayed by strong jets of water to clean the surface from foreign pollen and any aphids.
2. The plants were bagged for 3 weeks. Every bag was shaken every 2 days to distribute the pollen inside.
3. After three weeks, the bag was taken off and the plant was left, after cutting and eliminating the floral stalk tips with unfertilized flowers, to mature the seeds.
4. The seeds were harvested after they ripened (i.e. dried to a brown color) and the seeds of different ramets of the same isolate were bulked together.

Seeds were then sown in a greenhouse room and transplanted into beds. After this, the plants were grown in the summer greenhouse under incandescent lighting. Segregation data were recorded for the following characters :

1. The hypocotyl color (red or green).
2. Annualism or biennialism.
3. Germness (multigerm or monogerm).

This experiment had two different objectives: First, to detect any change in segregation pattern which might have resulted from a mutation-like event at the B, M, or R loci. This would be especially true if the mutation arose as a part of chimera and was not expressed in the ramets of the adventitious clones. Second, to detect by segregation any recessive mutations which would not be expected to be seen in the ramets themselves of the adventitious isolates.

Experiment 6:

When it was found from comparison of guard cell lengths of 2n, 3n, and 4n plants of seedling origin that ramets of axillary and adventitious bud origin did not differ in ploidy level, the question was raised whether there is any effect of the medium components on producing tetraploid root cells. Therefore, an experiment was conducted to test the effect of various concentrations of benzyladenine (BA) in the shoot culture medium on the presence of tetraploid cells in the roots of subsequently rooted and potted diploid plants. Two different genotypes were used:

1. FC701/5-116 which showed some completely tetraploid root tips from both axillary and adventitious ramets.
2. 80-66 which did not show any tetraploid cells.

The axillary shoots of these two genotypes were cultured on the Murashige-Skoog shoot culture medium with different concentrations of benzyladenine as follows:

0.10 mg/l,

0.25 mg/l,

0.50 mg/l,

0.75 mg/l,

and 1.0 mg/l.

After the two genotypes were cultured on the prepared media long enough to have some shoots that can be separated, shoots were transferred to rooting medium (MN19). At the time shoots had roots, they were potted in soil in peat pots. After about three to four weeks, root tip samples were taken from 5 ramets for each BA level

for microscopic examination and previously mentioned fixing and staining procedures were followed. Five plants from each treatment for each genotype were used.

RESULTS

Table beet and sugarbeet materials were treated under the same conditions. It was found in general that the table beet, at least the cultivar Early Wonder, is less adapted for shoot culture than sugarbeet: shoots are slower in growth rate, proportion of survival shoots from all cultured buds or shoots is less in table beet than in sugarbeet, and they seem to be less vigorous. However, in general it produces more adventitious buds which are the main object in this research. Although no data was collected on survival after transplanting, rooted table beet shoots were notably weaker for this.

Experiment 1:

Table 3 shows no difference has been detected between axillary derived ramets and their adventitious derived counterparts for pollen shed, seed set, or germness. Some of the sugarbeet ramets of the starting clones; P-13 PA 4, P-13 PA 5, EL 36-18 Ax, EL 36-18 PA 3, and EL 36-18 PA 5 did not flower with the same proportion as the other clones or as table beet clones, although all ramets underwent vernalization at the same time. P-13 is thought to have a bolting resistance selection background from Poland. Pollen shedding and seed setting would eliminate the possibility of male or female sterility. The germness readings indicate that there has been no change between the axillary shoots and their adventitious ones.

Table 3.--Number of ramets for the characters: flowering, pollen shedding, and seed setting and occurrence of mono- or multigerm in axillary and adventitious ramets.

Starting clone		Total number of ramets	Number of ramets			Germness
Axillary	Isolate		Flowering	Pollen shedding	seed set *	
EW-1 Ax		20	15	15	15	multi
EW-3 AX		2	2	2	2	"
EW-3	PA 1	1	1	1	1	"
EW-9 Ax		6	5	5	5	"
EW-9	PA 3	2	2	2	2	"
EW-9	PA 5	1	1	1	1	"
EW-10 Ax		8	8	8	8	"
EW-10	PA 3	2	2	2	2	"
EW-10	PA 6	2	2	2	2	"
EW-10	PA 8	1	1	1	1	"
EW-10	PA 9	2	2	2	2	"
EW-10	PA 12	3	3	3	3	"
EW-10	PA 13	3	3	3	3	"
EW-11 Ax		14	14	14	14	"
EW-11	PA 2	2	2	2	2	"
EW-20 Ax		25	21	21	21	"
EW-20	PA 5	4	4	4	4	"
EW-20	PA 6	2	1	1	1	"
EW-20	PA 7	3	3	3	3	"
EW-20	PA 10	5	5	5	5	"

Table 3.--Continued.

Starting clone		Total number of ramets	Number of ramets			Germness
Axillary	Isolate		Flowering	Pollen shedding	seed set *	
EW-20	PA 11	2	2	2	2	multi
EW-21 Ax		5	5	5	5	"
EW-25 Ax		4	4	4	4	"
EW-37 Ax		3	1	1	1	"
EW-39 Ax		1	1	1	1	"
EW-40 Ax		2	-	-	-	-
EW-44 Ax		15	9	9	9	"
EW-44	PA 1	1	1	1	1	"
EW-44	PA 2	1	1	1	1	"
EW-44	PA 3	1	1	1	1	"
EW-44	PA 4	1	1	1	1	"
EW-53 Ax		8	8	8	8	"
EW-55 Ax		6	5	5	5	"
EW-59 Ax		16	13	13	13	"
EW-70 Ax		6	6	6	6	"
EL 36-18 Ax		13	4	4	4	"

Table 3.--Continued.

Starting clone		Total number of ramets	Number of ramets			Germness
Axillary	Isolate		Flowering	Pollen shedding	Seed set *	
EL 36-18	PA 2	2	1	1	1	mono
EL 36-18	PA 3	19	4	4	4	"
EL 36-18	PA 4	1	-	-	-	-
EL 36-18	PA 5	9	1	1	1	"
EL 36-18	PA 18	5	5	5	5	"
P-13	PA 2	12	11	11	11	multi
P-13	PA 3	5	4	4	4	"
P-13	PA 4	22	13	13	13	"
P-13	PA 5	25	5	5	5	"
80-66 Ax		4	4	4	4	mono
80-66	PA 3	3	3	3	3	"
80-66	PA 4	3	3	3	3	"
80-66	PA 5	2	2	2	2	"
2-294 Ax		12	12	12	12	"
2-294	PA 1	17	17	17	17	"
FC701/5-116Ax		5	5	5	5	multi
FC701/5-116	PA 1	9	9	9	9	"
FC701/5-116	PA 2	3	3	3	3	"
FC701/5-116	P_2A1-1	5	5	5	5	"

* In all cases seed set was more than 50%.

Experiment 2, the field study:

Table 4 summarizes the leaf shape as indicated by the width/length ratio. It appears that leaf shape in table beets is narrower than in sugarbeets. However, within the counterpart axillary and adventitious pairs, there are no significant differences (Table 5). Data of EW-1 Ax, EW-10 PA 17, and EW-20 P_2A 7-4 in table 4 did not include the narrow leaf ramets of 0.225, 0.232, and 0.241 means, respectively. When the mean and the standard deviation of these three starting clones were calculated considering the narrow leaf ramets, the values were; $\bar{x}$ = 0.355 and δ = 0.183, $\bar{x}$ = 0.417 and δ = 0.83, and $\bar{x}$ = 0.533 and δ = 0.195 for these three clones, respectively. Testing the comparison between the adventitious bud derived ramets and their counterparts axillary derived ramets for this character has been demonstrated by "t" test for each isolate. Table 6 shows that there were few differences between the adventitious derived ramets and axillary derived ramets. Only two values which represent less than 0.06 of all values exhibited significance. These two values can be statistically significant but biologically insignificant. In Table 7 the significance of the three narrow leaf ramets is shown as compared with their normal leaf sisters. Significance was demonstrated at 95% confidence. The study of this character completes the greenhouse study and indicates no difference between the axillary derived ramets and their adventitious derived ramets. The three abnormal ramets were investigated further to detect whether they have any genetic difference. They were all diploid and produced normal quantities of pollen. Insect infestation prevented seed set.

Table 4.--Determination of leaf shape through width/length ratio of three leaves per ramet for both axillary and adventitious bud derived ramets in the field. Values are the mean of all ramets of each clone*. NL means narrow leaf ramet.

Starting clone		Total number of ramets	Mean ratio of clone	δ
Axillary	Isolate			
EW-1 Ax		7	0.373	0.056
EW-1 Ax	NL	1	0.225	
EW-1	PA 3	1	0.461	
EW-9	PA 2	1	0.458	
EW-10 Ax		6	0.467	0.065
EW-10	PA 3	2	0.501	0.067
EW-10	PA 6	6	0.409	0.100
EW-10	PA 7	2	0.433	0.049
EW-10	PA 8	2	0.462	0.001
EW-10	PA 9	3	0.398	0.035
EW-10	PA 10	2	0.433	0.019
EW-10	PA 12	3	0.326	0.079
EW-10	PA 13	3	0.484	0.036
EW-10	PA 14	4	0.395	0.093
EW-10	PA 15	2	0.396	0.020
EW-10	PA 17	7	0.442	0.039
EW-10	PA 17NL	1	0.232	
EW-11 Ax		1	0.538	
EW-11	PA 2	3	0.586	0.035
EW-11	PA 3	3	0.569	0.109
EW-20 Ax		7	0.618	0.057

Table 4.--Continued.

Starting clone		Total number of ramets	Mean ratio of clone	δ
Axillary	Isolate			
EW-20	PA 7	1	0.647	
EW-20	PA 9	2	0.629	0.053
EW-20	PA 17	3	0.531	0.051
EW-20	P_2A2-6	1	0.616	
EW-20	P_2A7-1	10	0.533	0.075
EW-20	P_2A7-2	3	0.507	0.061
EW-20	P_2A7-4	3	0.631	0.019
EW-20	P_2A7-4NL	1	0.241	
EW-25 Ax		3	0.590	0.067
EW-25	PA 1	3	0.699	0.121
EW-25	PA 4	1	0.441	
EW-25	PA 12	1	0.717	
EW-25	PA 13	1	0.564	
EW-39 Ax		2	0.704	0.202
EW-39	PA 2	1	0.609	
EW-39	PA 3	1	0.695	
EW-39	PA 22	4	0.639	0.081
EW-40	PA 4	4	0.508	0.121
EW-40	PA 5	3	0.756	0.178
EW-40	PA 11	2	0.826	0.188
EW-40	PA 12	2	0.461	0.055
EW-55 Ax		2	0.458	0.052
EW-55	PA 7	2	0.469	0.033
EW-55	PA 8	6	0.511	0.106

Table 4.--Continued.

Starting clone		Total number of ramets	Mean ratio of clone	δ
Axillary	Isolate			
EW-55	PA 9	1	0.444	
EW-59 Ax		3	0.492	0.033
EW-68 Ax		2	0.656	0.044
EW-68	PA 2	1	0.494	
EW-70 Ax		1	0.574	
EW-70	PA 3	1	0.710	
EW-70	PA 7	1	0.506	
EL 36-18 Ax		30	0.845	0.073
EL 36-18	PA 2	6	0.902	0.034
EL 36-18	PA 3	13	0.904	0.059
EL 36-18	PA 4	6	0.898	0.037
EL 36-18	PA 5	15	0.888	0.053
EL 36-18	PA 6	6	0.805	0.051
EL 36-18	PA 18	16	0.837	0.066
EL 36-18	PA 23	6	0.864	0.029
P-13	PA 2	11	0.640	0.046
P-13	PA 4	6	0.704	0.058
P-13	PA 5	4	0.631	0.091
80-66	PA 3	3	0.678	0.031
80-66	PA 4	8	0.685	0.052
80-66	PA 5	5	0.659	0.043
80-66	PA 7	2	0.693	0.079

Table 4.--Continued.

Starting clone		Total number of ramets	Mean ratio of clone	δ
Axillary	Isolate			
80-66	PA 8	3	0.676	0.044
2-294 Ax		9	0.746	0.185
2-294	PA 1	18	0.852	0.081
FC701/5-116Ax		11	0.672	0.032
FC701/5-116	PA 1	6	0.698	0.040
FC701/5-116	PA 1	11	0.703	0.075
6926-0-3 Ax		2	0.970	0.035
6926-0-3	PA 1	3	0.979	0.019
6926-0-3	PA 5	2	1.061	0.033
G335-18E Ax		21	0.693	0.021
G335-18E	PA 1	2	0.713	0.032
G335-18E	PA 2	3	0.736	0.091
G335-18E	PA 3	1	0.685	
G335-18E	PA 4	3	0.679	0.074

* The mean ratios of the clones marked above do not include the narrow leaf ramets.

Table 5.--Test of significance* for the deviation of the mean for the comparison of each axillary and its adventitious derived ramets as compared by their grand means for leaf shape character.

Starting clone		Mean ratio of clone	δ **	Deviation from the mean
Axillary	Isolate			
EW-1 Ax ***		0.355	0.075	0.053
EW-1	PA 3	0.461		0.053
Grand mean		0.408		
EW-10 Ax		0.467	0.048	0.040
EW-10	PA 3	0.501		0.074
EW-10	PA 6	0.409		0.018
EW-10	PA 7	0.433		0.006
EW-10	PA 8	0.462		0.035
EW-10	PA 9	0.398		0.029
EW-10	PA 10	0.433		0.006
EW-10	PA 12	0.326		0.101
EW-10	PA 13	0.484		0.057
EW-10	PA 14	0.395		0.032
EW-10	PA 15	0.396		0.031
EW-10	PA 17***	0.417		0.010
Grand mean		0.427		
EW-11 Ax		0.538	0.024	0.026
EW-11	PA 2	0.586		0.022
EW-11	PA 3	0.569		0.005
Grand mean		0.564		
EW-20 Ax		0.618	0.057	0.041

Table 5.--Continued.

Starting clone		Mean ratio of clone	δ **	Deviation from the mean
Axillary	Isolate			
EW-20	PA 7	0.647		0.070
EW-20	PA 9	0.629		0.052
EW-20	PA 17	0.531		0.046
EW-20	P_2A2-6	0.616		0.039
EW-20	P_2A7-1	0.533		0.044
EW-20	P_2A7-2	0.507		0.070
EW-20	P_2A7-4***	0.533		0.044
Grand mean		0.577		
EW-25 Ax		0.590	0.112	0.012
EW-25	PA 1	0.699		0.097
EW-25	PA 4	0.441		0.161
EW-25	PA 12	0.717		0.115
EW-25	PA 13	0.564		0.038
Grand mean		0.602		
EW-39 Ax		0.704	0.045	0.042
EW-39	PA 2	0.609		0.053
EW-39	PA 3	0.695		0.033
EW-39	PA 22	0.639		0.023
Grand mean		0.662		
EW-40	PA 4	0.508	0.180	0.130
EW-40	PA 5	0.756		0.118
EW-40	PA 11	0.826		0.188
EW-40	PA 12	0.461		0.177
Grand mean		0.638		

Table 5.--Continued.

Starting clone		Mean ratio of clone	δ **	Deviation from the mean
Axillary	Isolate			
EW-55 Ax		0.458	0.029	0.013
EW-55	PA 7	0.469		0.002
EW-55	PA 8	0.511		0.040
EW-55	PA 9	0.444		0.027
Grand mean		0.471		
EW-68 Ax		0.656	0.115	0.081
EW-68	PA 2	0.494		0.081
Grand mean		0.575		
EW-70 Ax		0.845	0.036	0.023
EW-70	PA 3	0.710		0.113
EW-70	PA 7	0.506		0.091
Grand mean		0.597		
EL 36-18 Ax		0.574	0.104	0.023
EL 36-18	PA 2	0.902		0.034
EL 36-18	PA 3	0.904		0.036
EL 36-18	PA 4	0.898		0.030
EL 36-18	PA 5	0.888		0.020
EL 36-18	PA 6	0.805		0.063
EL 36-18	PA 18	0.837		0.031
EL 36-18	PA 23	0.864		0.004
Grand mean		0.868		
P-13	PA 2	0.640	0.040	0.018

Table 5.--Continued.

Starting clone		Mean ratio of clone	δ **	Deviation from the mean
Axillary	Isolate			
P-13	PA 4	0.704		0.046
P-13	PA 5	0.631		0.027
Grand mean		0.658		
80-66	PA 3	0.678	0.013	0.000
80-66	PA 4	o.685		0.007
80-66	PA 5	0.659		0.019
80-66	PA 7	0.693		0.015
80-66	PA 8	0.676		0.002
Grand mean		0.678		
2-294 Ax		0.746	0.075	0.053
2-294	PA 1	0.852		0.053
Grand mean		0.799		
FC701/5-116Ax		0.672	0.017	0.019
FC701/5-116	PA 1	0.698		0.007
FC701/5-116	PA 2	0.703		0.012
Grand mean		0.691		
6926-0-3 Ax		0.970	0.050	0.030
6926-0-3	PA 1	0.979		0.021
6926-0-3	PA 5	1.061		0.061
Grand mean		1.003		
G335-18E Ax		0.693	0.023	0.008

Table 5.--Continued.

Starting clone		Mean ratio of clone	δ **	Deviation from the mean
Axillary	Isolate			
G335-18E	PA 1	0.713		0.012
G335-18E	PA 2	0.736		0.035
G335-18E	PA 3	0.685		0.016
G335-18E	PA 4	0.679		0.022
Grand mean		0.701		

* Significance was demonstrated as 99% confidence, i.e. deviation is compared with 3δ.

** The standard deviation is calculated for each group of axillary and adventitious derived ramets.

***The mean ratios of the clones marked above include the narrow leaf ramets.

Table 6.--"t" test for the comparison between each adventitious isolate with its axillary counterpart for some table beet and sugarbeet starting clones.

Starting clone		Total number of ramets	Mean ratio of clone	"t" test
Axillary	Isolate			
EW-10 Ax		6	0.467	
EW-10	PA 3	2	0.501	0.654
EW-10	PA 6	6	0.409	1.115
EW-10	PA 7	2	0.433	0.654
EW-10	PA 8	2	0.462	0.357
EW-10	PA 9	3	0.398	0.566
EW-10	PA 10	2	0.433	2.429
EW-10	PA 12	3	0.326	2.820
EW-10	PA 13	3	0.484	0.463
EW-10	PA 14	4	0.395	1.330
EW-10	PA 15	2	0.396	5.071**
EW-10	PA 17	7	0.442	0.806
EW-20 Ax		7	0.618	
EW-20	PA 9	2	0.629	0.216
EW-20	PA 17	2	0.531	2.023
EW-20	P_2A7-1	10	0.533	2.429
EW-20	P_2A7-2	3	0.507	2.643
EW-20	P_2A7-4	3	0.631	0.342
EW-25 Ax		3	0.590	
EW-25	PA 1	3	0.699	1.124
EW-39 Ax		2	0.704	
EW-39	PA 22	4	0.639	0.591

Table 6.--Continued.

Starting clone		Total number of ramets	Mean ratio of clone	"t" test
Axillary	Isolate			
EW-55 Ax		2	0.458	
EW-55	PA 7	2	0.469	0.175
EW-55	PA 8	6	0.511	0.570
EL 36-18 Ax		30	0.846	
EL 36-18	PA 2	6	0.902	1.750
EL 36-18	PA 3	13	0.904	2.522**
EL 36-18	PA 4	6	0.898	1.625
EL 36-18	PA 5	15	0.888	1.909
EL 36-18	PA 6	6	0.805	1.281
EL 36-18	PA 18	16	0.837	0.409
EL 36-18	PA 23	6	0.864	0.563
2-294 Ax		9	0.746	
2-294	PA 1	18	0.852	1.927
FC701/5-116Ax		11	0.672	
FC701/5-116	PA 1	6	0.698	1.130
FC701/5-116	PA 2	11	0.703	1.148
6926-0-3 Ax		2	0.970	
6926-0-3	PA 1	3	0.979	0.310
6926-0-3	PA 5	2	1.061	2.022
G335-18E Ax		21	0.693	
G335-18E	PA 1	2	0.713	1.176
G335-18E	PA 2	3	0.736	2.263
G335-18E	PA 4	3	0.679	0.737

** Values are significantly different at 99% confidence.

Table 7.--Determination of the significance of the narrow leaf ramets as compared with their normal leaf ones.

Starting clone		Mean ratio of each ramet	δ	Deviation from the mean
Axillary	Isolate			
EW-1 Ax		0.288	0.056	0.085
		0.356		0.017
		0.318		0.055
		0.225		0.148 *
		0.411		0.038
		0.384		0.011
		0.431		0.048
		0.428		0.055
Grand mean		0.355		
EW-10	PA 17	0.433	0.083	0.017
		0.519		0.103
		0.232 *		0.184 *
		0.437		0.021
		0.430		0.014
		0.457		0.041
		0.427		0.011
		0.395		0.021
Grand mean		0.416		
EW-20	P_2A7-4	0.609	0.092	0.074
		0.639		0.104
		0.248		0.287 *
		0.644		0.109
Grand mean		0.535		

* Values are significantly different from the grand mean at 95% confidence, i.e. deviation is compared with 2δ

Experiment 3:

Results (Table 8) for the microscopic examination have been recorded over the course of a year. They were pooled from three groups of ramets available at different times. All clones were expected to be diploid, based on the background from which they were chosen. Chromosome counting indicated that ramets derived from 24 of 35 clones were indeed diploid. In the eleven other clones, however, some of the root tips were entirely diploid and others were entirely tetraploid although both types of root tips were taken from the same ramet. Thus the same examined root tip showed either diploid or tetraploid cells. On the other hand, no root tip samples of the same ramet gave only tetraploid. That would suggest that the ploidy duplication might occur during the initiation of the secondary roots, possibly during root induction on the shoots on MN-19 medium. The frequency of tetraploid root tips was fairly high (between 12.82 and 42.00%). When a control group of 50 root tips from sugarbeet seed derived plants (5 plants of 2n, 4n, and 3n; parental lines and a hybrid respectively from Nickerson Seeds) were examined microscopically, no tetraploid cells were observed in 2n plants. This result may suggest the effect of the medium on inducing tetraploid cells. This possibility was studied by testing the effect of BA in the shoot culture media on the frequency of tetraploid root tips.

The existence of tetraploid root tips raised the question whether the top of the plant was chimeric or mixaploid. This question was approached by measuring the guard cell lengths. Table 9 shows that the average of these lengths ranges from 7.75 to 9.47 units which is

within the diploid controls (from Nickerson Seeds) examined. These results did not indicate any occurrence of tetraploid cells in the shoot system.

Table 8.--Number of diploid and tetraploid cells and the percentage of tetraploid-containing root tips of axillary and adventitious bud derived ramets.

Starting clone		Total number of ramets	Total root tips	Diploids		Tetraploids		% of tetraploid root tips
Axillary	Isolate			Root tips	Cells	Root tips	Cells	
EW-10 Ax		5	35	35	375			
EW-10	PA 17	5	42	42	292			
EW-39	PA 5	4	29	23(4)*	233	6(2)*	139	20.69
EW-44 Ax		5	47	28(5)*	419	19(2)*	233	40.43
EW-44	PA 1	4	32	26(4)*	320	6(2)*	111	18.75
EW-44	PA 2	4	39	39	452			
EW-44	PA 7	4	33	33	413			
EW-44	PA 16	4	37	37	503			
EW-44	PA 17	5	47	38(5)*	425	9(3)*	218	19.15
EW-55 Ax		4	31	31	395			
EW-59 Ax		5	41	27(5)*	276	14(2)*	211	34.15
EW-59	PA 3	4	42	33(4)*	420	9(2)*	187	21.43

Table 8.--Continued.

Starting clone		Total number of ramets	Total root tips	Diploids		Tetraploids		% of tetraploid root tips
Axillary	Isolate			Root tips	Cells	Root tips	Cells	
EW-70 Ax		4	39	34(4)*	429	5(3)*	121	12.82
EW-70	PA 2	4	40	40	532			
EL 36-18	PA 2	5	43	43	542			
EL 36-18	PA 6	5	44	44	487			
EL 36-18	PA 11	5	50	37(5)*	432	13(3)*	223	26.00
EL 36-18	PA 12	5	50	50	635			
EL 36-18	PA 23	5	50	50	701			
EL 36-18	PA 24	5	50	50	825			
EL 36-18	PA 25	5	50	32(5)*	411	18(5)*	343	36.00
P-13	PA 4	5	50	50	735			
P-13	PA 5	5	50	50	862			
FC701/5-116Ax		5	50	29(5)*	359	21(4)*	379	42.00
FC701/5-116	PA 1	5	50	35(5)*	493	15(5)*	255	30.00
FC701/5-116	PA 3	5	50	50	792			

Table 8.--Continued.

Starting clone		Total number of ramets	Total root tips	Diploids		Tetraploids		% of tetraploid root tips
Axillary	Isolate			Root tips	Cells	Root tips	Cells	
80-66	PA 4	5	50	50	837			
80-66	PA 5	5	50	50	903			
80-66	PA 6	5	50	50	962			
80-66	PA 7	5	42	42	635			
80-66	PA 8	5	50	50	823			
J4 Ax		5	50	50	718			
J4	PA 1	4	33	33	479			
G335-18E	PA 1	4	37	37	517			
G335-18E	PA 5	5	42	42	634			
Control seedlings (2n)		5	50	50	640			
" "	(3n)	5	50	50**	721**			
" "	(4n)	5	50			50	496	100.00

* The numbers between parentheses indicate the number of ramets for diploids and tetraploids.
**The numbers of root tips and cells regarding 3n is for triploid not for diploid.

Table 9.--Mean of 30 guard cell lengths measured by calibrating lens in light microscope of five randomly chosen fully expanded leaves of axillary and adventitious bud derived ramets.

Starting clone		Mean of guard cell length	δ
Axillary	Isolate		
EW-1 Ax		7.89	0.532
EW-3 Ax		8.43	0.344
EW-9 Ax		9.01	0.393
EW-9	PA 3	8.49	0.601
EW-10 Ax		7.75	0.742
EW-10	PA 6	7.93	0.635
EW-10	PA 8	7.97	0.573
EW-10	PA 9	8.63	0.379
EW-10	PA 12	7.84	0.805
EW-20 Ax		8.48	0.736
EW-20	PA 5	8.79	0.578
EW-20	PA 6	8.73	0.589
EW-20	PA 7	7.89	0.564
EW-20	PA 11	9.13	0.484
EW-21 Ax		7.93	0.660
EW-25 Ax		7.85	0.771
EW-37 Ax		8.46	0.494

Table 9.--Continued.

Starting clone		Mean of guard cell length	δ
Axillary	Isolate		
EW-44 Ax		8.78	0.491
EW-44	PA 1	7.88	0.385
EW-44	PA 2	8.56	0.706
EW-44	PA 3	8.75	0.671
EW-44	PA 4	7.89	0.832
EW-59 Ax		7.87	0.921
EL 36-18 Ax		9.23	0.494
EL 36-18	PA 2	9.21	0.896
EL 36-18	PA 3	8.83	0.394
EL 36-18	PA 5	8.87	0.911
EL 36-18	PA 18	8.65	0.742
P-13	PA 2	8.75	0.659
P-13	PA 3	8.35	0.588
P-13	PA 4	9.47	0.534
P-13	PA 5	8.95	0.633
80-66 Ax		8.86	0.481
80-66	PA 3	9.39	0.572
80-66	PA 4	8.69	0.740
80-66	PA 5	8.75	0.751
FC701/5-116 Ax		8.73	0.813
FC701/5-116	PA 1	8.49	0.669
FC701/5-116	PA 2	8.84	0.830
FC701/5-116	P_2A1-1	8.65	0.739

Table 9.--Continued.

Starting clone		Mean of guard cell length	δ
Axillary	Isolate		
Control seedlings	(2n) *	8.72	0.875
"	" (3n) *	10.94	0.921
"	" (4n) *	13.37	0.864

* Mean of 100 guard cell lengths.

Experiment 4:

This experiment was carried out to study the narrow leaf ramets. Table 10 shows the measurement of leaf shape as indicated by the width/length ratio. Data are at the ramet level. By demonstrating the standard deviation, no significant differences among the ramets of the same clone have been shown. To show the significance for this character between the same narrow leaf ramets and their normal counterparts, "t" test was achieved as follows:

For EW-1 Ax: $\bar{x}$ of EW-1 Ax 1 is 0.689 and $\bar{x}$ of EW-1 Ax 2 is 0.623

$$\text{"t"} = \frac{0.689 - 0.623}{0.484 - 0.484} = \frac{0.066}{} = 0.136$$

while tabulated "t" at 0.05 (df=11) is 2.201.

So, calculated "t" is not significant for the clone EW-1 Ax.

For EW-10 PA 17: $\bar{x}$ of EW-10 PA 17 1 is 0.610 and $\bar{x}$ of EW-10 PA 17 2 is 0.584

$$\text{"t"} = \frac{0.610 - 0.584}{0.250} = \frac{0.026}{0.250} = 0.104$$

while tabulated "t" at 0.05 (df=23) is 2.069.

So, calculated "t" is insignificant for the clone EW-10 PA 17.

For EW-20 P_2A 7-4: $\bar{x}$ of EW-20 P_2A 7-4 1 is 0.613 and $\bar{x}$ of EW-20 P_2A 7-4 2 is 0.580

$$\text{"t"} = \frac{0.613 - 0.580}{0.433} = \frac{0.033}{0.433} = 0.076$$

while tabulated "t" at 0.05 (df=11) is 2.201.

So, calculated "t" is not sgnificant also for the clone EW-20 P_2A7-4.

Comparing the means of these starting clones with their means in the initial study (Table 5) we find that these means are higher which would suggest that the leaf shape character of table beet is affected by other conditions such as the environmental conditions. We can conclude from this experiment that the narrow leaf aspect is not completely stable if it persists at all.

The possibility of the effect of the environmental conditions on the leaf shape,or, the narrowness, was also studied by transferring some of these ramets to larger pots in order to give the plants better soil and nutrition conditions. However, no visually distinguishable persistence could be observed.

Table 10.--The standard deviation and the deviation from the mean for the leaf shape character of the second cycle ramets and their counterparts normal leaf ramets.

Starting clone		Mean ratio of ramet	δ *	Deviation from the mean
Axillary	Isolate			
EW-1 Ax 1		0.701	0.054	0.012
		0.596		0.093
		0.637		0.053
		0.683		0.006
		0.715		0.026
		0.694		0.005
		0.654		0.035
		0.674		0.015
		0.767		0.078
		0.771		0.082
Grand mean		0.689		
EW-1 Ax 2		0.576	0.041	0.047
		0.648		0.025
		0.645		0.022
Grand mean		0.623		
EW-10	PA 17 1	0.649	0.061	0.039
		0.618		0.008
		0.607		0.003
		0.527		0.017
		0.632		0.022
		0.698		0.088
		0.619		0.009

Table 10.--Continued.

Starting clone		Mean ratio of ramet	δ *	Deviation from the mean
Axillary	Isolate			
EW-10	PA 17 1	0.521		0.089
		0.602		0.008
		0.570		0.060
		0.690		0.080
		0.523		0.087
		0.674		0.064
Grand mean		0.610		
EW-10	PA 17 2	0.621	0.032	0.047
		0.614		0.030
		0.619		0.035
		0.560		0.024
		0.577		0.007
		0.522		0.062
		0.600		0.016
		0.613		0.071
		0.582		0.002
		0.583		0.001
		0.541		0.043
		0.577		0.007
Grand mean		0.584		
EW-20	P_2A 7-4 1	0.687	0.046	0.074
		0.557		0.056
		0.623		0.010
		0.608		0.005

Table 10.--Continued.

Starting clone		Mean ratio	δ *	Deviation from
Axillary	Isolate	of ramet		the mean
EW-20	P_2A 7-4 1	0.644		0.031
		0.560		0.053
		0.569		0.044
		0.579		0.034
		0.657		0.056
		0.649		0.064
Grand mean		0.613		
EW-20	P_2A 7-4 2	0.588	0.019	0.008
		0.594		0.014
		0.559		0.021
Grand mean		0.580		

Experiment 5, the genetic study of S_1 progeny:

To determine the genetic fiedelity of adventitious buds, there is a need to test their progenies for the presence of any new recessive traits segregating out, or sorting out of chimeric traits from the adventitious bud ramets. Three simply inherited characters were examined in the S_1 progeny of two self-fertile axillary clones and their adventitious derived ramets. Both original clones were heterozygous for hypocotyl color and annualism, and homozygous for either dominant multigermness or recessive monogermness. Tables 11 and 12 show the segregation of the three characters. Applying χ^2 test is to show the significance of the observed ratios from the expected ones. No segregation for any new character was observed. It appears from these tables that:

1. Both characters segregating for their dominant and recessive alleles.
2. Although the grand ratios of the two genotypes for hypocotyl color were not significantly different from the expected ratio, several individual lines showed significant variation in annualism.

This latter case may be due to that the character is more affected by environmental conditions since recording data for annualism did not continue until all possible annuals would flower, that was because of the shortness of time. Germness confirms the similarity of both axillary derived ramets and their adventitious derived ramets. In no case did germness segregate in the S_1 family of any adventitious clone. This indicates that there was no major change at the M locus.

Table 11.--The deviation of segregation ratios of hypocotyl color as tested by χ^2 for the S_1 progeny for 50 S_1 seeds of two sugarbeet genotypes and their adventitious bud derived ramets.

Starting clone		Number of ramets				χ^2
Axillary	Isolate	Observed		Expected		
		Red	Green	Red	Green	
82 J2-11 Ax		27	12	29.25	9.75	0.692
82 J2-11	PA 1	28	13	30.75	10.25	0.982
82 J2-11	PA 2	28	16	33	11	3.029
82 J2-11	PA 3	29	15	33	11	1.938
82 J2-11	PA 4	29	11	30	10	0.133
82 J2-11	PA 5	36	8	33	11	1.090
82 J2-11	PA 6	29	8	27.75	9. 25	0.224
82 J2-11	PA 7	34	12	34.5	11.5	0.089
82 J2-11	PA 8	30	12	31.5	10.5	0.280
82 J2-11	PA 9	30	14	33	11	1.090
82 J2-11	PA 10	33	12	33.75	11.25	0.066
82 J2-11	PA 11	33	14	35.25	11.75	0.573
82 J2-11	PA 12	34	9	32.25	10.75	0.378
82 J2-11	PA 13	34	11	33.75	11.25	0.006
82 J2-11	PA 14	30	12	31.5	10.5	0.285
82 J2-11	PA 15	27	11	28.5	9.5	0.333
Total of ramets for genotype I		491	190	510.75	170.25	3.054
82 J1-13 Ax		35	14	36.75	12.25	0.164
82 J1-13	PA 1	33	15	36	12	1.000
82 J1-13	PA 3	37	13	37.5	12.5	0.027
82 J1-13	PA 4	37	10	35.25	11.75	0.348
82 J1-13	PA 5	31	19	37.5	12.5	4.507 *

Table 11.--Continued.

Starting clone		Number of ramets				x^2
Axillary	Isolate	Observed		Expected		
		Red	Green	Red	Green	
82 J1-13	PA 6	40	9	36.75	12.25	1.149
82 J1-13	PA 8	31	18	36.75	12.25	3.598
82 J1-13	PA 9	36	12	36	12	0.000
82 J1-13	PA 10	37	13	37.5	12.5	0.027
82 J1-13	PA 11	38	11	36.75	12.25	0.171
82 J1-13	PA 12	38	10	36	12	0.444
82 J1-13	PA 13	34	16	37.5	12.5	1.307
82 J1-13	PA 14	37	12	36.75	12.25	0.007
82 J1-13	PA 15	32	15	35.25	11.75	1.198
82 J1-13	PA 16	35	10	33.75	11.25	0.185
82 J1-13	PA 17	39	9	36	12	1.000
82 J1-13	PA 18	34	10	33	11	0.121
82 J1-13	PA 19	31	15	34.5	11.5	0.420
82 J1-13	PA 20	37	10	35.25	11.75	0.348
82 J1-13	PA 21	38	9	35.25	11.75	0.869
82 J1-13	PA 22	37	13	37.5	12.5	0.027
82 J1-13	PA 23	38	16	40.5	13.5	0.617
82 J1-13	PA 24	46	7	39.75	13.25	3.931 *
82 J1-13	PA 25	35	16	38.25	12.75	1.104
82 J1-13	PA 26	37	15	39	13	0.411
82 J1-13	PA 27	40	12	39	13	0.103
82 J1-13	PA 28	38	12	37.5	12.5	0.027
82 J1-13	PA 29	34	10	33	11	1.063
82 J1-13	PA 30	36	14	37.5	12.5	0.240
82 J1-13	PA 31	37	14	38.25	12.75	0.164
82 J1-13	PA 32	37	13	37.5	12.5	0.027
82 J1-13	PA 33	40	9	36.75	12.25	1.149

Table 11.--Continued.

Starting clone		Number of ramets				χ^2
Axillary	Isolate	Observed		Expected		
		Red	Green	Red	Green	
82 J1-13	PA 34	37	12	36.75	12.25	0.007
82 J1-13	PA 35	43	11	40.5	13.5	0.617
82 J1-13	PA 36	42	13	41.25	13.75	0.055
82 J1-13	PA 37	41	12	39.75	13.25	0.157
Total of ramets of genotype II		1337	449	1339.5	446.5	0.019
Total number of ramets		1828	639	1850.25	616.75	1.071

* Ratio is significantly different at P= 0.05, where χ^2 at df = 1 and P = 0.05 is 3.84.

Table 12.--The segregation of germness and the deviation of segregation ratios of annualness as tested by χ^2 for the S_1 progeny of two sugarbeet genotypes and their adventitious bud derived ramets.

Starting clone		Number of ramets				χ^2	Germness
Axillary	Isolate	Observed		Expected			
		Annual	Biennial	Annual	Biennial		
82 J2-11 Ax		26	13	29.25	9.75	1.444	mono
82 J2-11	PA 1	26	25	30.75	10.25	2.934	"
82 J2-11	PA 2	35	9	33	11	0.484	"
82 J2-11	PA 3	28	16	33	11	3.029	"
82 J2-11	PA 4	24	15	29.25	9.75	3.768	"
82 J2-11	PA 5	32	12	33	11	0.120	"
82 J2-11	PA 6	25	12	27.75	9.25	1.089	"
82 J2-11	PA 7	31	15	34.5	11.5	1.420	"
82 J2-11	PA 8	34	8	31.5	10.5	0.793	"
82 J2-11	PA 9	31	13	33	11	0.484	"
82 J2-11	PA 10	22	20	31.5	10.5	11.460 *	"
82 J2-11	PA 11	34	13	35.25	11.75	0.176	"
82 J2-11	PA 12	25	18	32.25	10.75	6.518 *	"
82 J2-11	PA 13	27	18	33.75	11.25	5.400 *	"

Table 12.--Continued.

Starting clone		Number of ramets				χ^2	Germness
Axillary	Isolate	Observed		Expected			
		Annual	Biennial	Annual	Biennial		
82 J2-11	PA 14	25	17	31.5	10.5	5.364 *	"
82 J2-11	PA 15	24	13	27.75	9.25	2.026	"
Total of ramets for genotype I		449	227	507	169	26.530 *	
82 J1-13	Ax	29	19	36	12	5.444 *	multi
82 J1-13	PA 1	28	20	36	12	7.110 *	"
82 J1-13	PA 3	31	17	36	12	2.777	"
82 J1-13	PA 4	31	15	34.5	11.5	1.120	"
82 J1-13	PA 5	27	23	37.5	12.5	11.760**	"
82 J1-13	PA 6	28	21	36.75	12.25	8.333 *	"
82 J1-13	PA 8	35	10	33.75	11.25	0.185	"
82 J1-13	PA 9	23	24	35.25	11.75	17.028 *	"
82 J1-13	PA 10	26	22	36	12	11.111 *	"
82 J1-13	PA 11	26	20	34.5	11.5	8.377 *	"
82 J1-13	PA 12	27	21	36	12	9.000 *	"

Table 12.--Continued.

Starting clone		Number of ramets				χ^2	Germness
Axillary	Isolate	Observed		Expected			
		Annual	Biennial	Annual	Biennial		
82 J1-13	PA 13	30	19	36.75	12.25	4.959 *	multi
82 J1-13	PA 14	27	20	35.25	11.75	7.724 *	"
82 J1-13	PA 15	29	18	35.25	11.75	4.433	"
82 J1-13	PA 16	28	15	32.25	10.75	2.240	"
82 J1-13	PA 17	28	19	35.25	11.75	5.964 *	"
82 J1-13	PA 18	25	18	32.25	10.75	6.518 *	"
82 J1-13	PA 19	26	17	32.25	10.75	4.845 *	"
82 J1-13	PA 20	30	15	33.75	11.25	1.667	"
82 J1-13	PA 21	33	13	34.5	11.5	0.261	"
82 J1-13	PA 22	34	15	36.75	12.25	0.823	"
82 J1-13	PA 23	36	16	39	13	0.923	"
82 J1-13	PA 24	34	19	39.75	13.25	3.327	"
82 J1-13	PA 25	35	15	37.5	12.5	0.667	"
82 J1-13	PA 26	34	17	38.25	12.75	1.889	"
82 J1-13	PA 27	35	17	39	13	1.641	"
82 J1-13	PA 28	33	15	36	12	1.000	"

Table 12.--Continued.

Starting clone		Number of ramets				χ^2	Germness
Axillary	Isolate	Observed		Expected			
		Annual	Biennial	Annual	Biennial		
82 J1-13	PA 29	32	20	39	13	5.025 *	multi
82 J1-13	PA 30	37	11	36	12	0.111	"
82 J1-13	PA 31	35	15	37.5	12.5	0.666	"
82 J1-13	PA 32	35	14	36.75	12.25	0.333	"
82 J1-13	PA 33	38	11	36.75	12.25	0.169	"
82 J1-13	PA 34	36	13	36.75	12.25	0.060	"
82 J1-13	PA 35	38	15	39.75	13.25	0.308	"
82 J1-13	PA 36	37	16	39.75	13.25	0.467	"
82 J1-13	PA 37	37	14	38.25	12.75	0.162	"
Total of ramets for genotype II		1133	609	1306.25	435.5	92.161 *	
Total number of ramets		1582	836	1813.5	604.5	118.207 *	

* Ratio is significantly different at P = 0.05, where χ^2 at df = 1 and P = 0.05 is 3.84

Experiment 6, the effect of benzyladenine on root tip chromosome number:

The frequency of tetraploid root tips and the absence of tetraploid cells in root tips from the sample of seed derived plants, prompted an experiment to test the effect of different levels of BA in the shoot culture medium. Two clones were used: 80-66, which had not shown any tetraploid roots before, and FC701/5-116, which had produced some tetraploid roots in both axillary and adventitious clones(Table 8). Table 13 gives the results of this study. No tetraploid root tips were seen in clone 80-66, whereas FC701/5-116 ramets from three of the five treatments (0.25 mg/l, 0.75 mg/l, and 1.0 mg/l) had some tetraploid root tips. To study the effect of BA on tetraploid frequency, regression analysis was achieved. From the analysis of variance of regression (Table 14), we can run the F test for significance as follows:

Calculated $F = \frac{0.023}{0.005} = 4.6$,

while the tabulated $F_{(0.05)\ (1,4)} = 7.71$.

Therefore, the calculated F is insignificant and we can conclude that data from this experiment suggests that there is no major effect of BA concentrations in the shoot culture medium on ploidy level of subsequent roots.

It is worthwhile to mention that during this investigation an attempt was made to culture roots in vitro in the liquid form of the root induction medium (MS plus 3.0 mg/l NAA). The purpose was to see if growth would be strong enough to provide material for chromosome counts. There was a 15-20 times increase in volume of roots, but vigorous potted plants were still thought to be better root tip sources.

Table 13.--Proportion of tetraploid root tips of axillary derived ramets produced from media containing different benzyladenine concentrations.

Benzyladenine levels	80-66				FC701/5-116				Frequency of tetraploid cells
	Diploid		Tetraploid		Diploid		Tetraploid		
	Roots	Cells	Roots	Cells	Roots	Cells	Roots	Cells	
0.10 mg/l	30	213	-	-	30	470	-	-	0.000
0.25 mg/l	30	329	-	-	27	393	3	47	0.107
0.50 mg/l	30	418	-	-	30	356	-	-	0.000
0.75 mg/l	30	376	-	-	25	349	5	72	0.171
1.00 mg/l	30	354	-	-	26	279	4	75	0.212

These cultured roots also were used in the microscopic studies and gave cells completely similar to their original ramets.

Table 14.--The analysis of variance of regression between benzyladenine concentrations and frewuency of tetraploid cells.

Source of variation	df	Sum of squares	Mean of squares	F
Total	4	0.038		
Regression	1	0.023	0.023	4.6
Error	3	0.015	0.005	

DISCUSSION

In vitro vegetative propagation through tissue culture has been increasingly used during the past ten years. One of the methods listed under tissue culture techniques is shoot culture, which involves artificial branching by axillary buds stimulated by cytokinin in the medium. There are references on shoot culture for various species of economic importance (Smith and Murashige, 1970; Murashige, 1974; Kartha, 1975; Pierik, 1975; Pieper and Zimmer, 1976; Sagawa, 1976; Arnold von and Eriksson, 1979; Jones et al, 1979; Skirvin and Chu, 1979; Konar and Singh, 1980; and Paterson, 1984).

Various methods for cloning beets by shoot culture have been reported (Margara,1977; Hussey and Hepher, 1978; Coumans et al, 1981; Saunders, 1981; and Harms et al, 1983). The adventitious buds that grow on the petioles of shoots during *in vitro* propagation would be a supplemental source for multiplication if they produce true copies of the original clone. Very few reports of clearly adventitious buds in shoot cultures exist (Fordham et al, 1982 in blue berry; Harms et al, 1983 in table beets; and Paterson; 1984 in sunflower). Adventitious buds were investigated in this research to determine if they present any danger of genetic change if they are used to increase the number of *in vitro* propagated shoots. In general, adventitious buds can arise in five situations: on leaf parts isolated from intact plants, on leaf parts isolated from shoot culture, on intact plants, on callus, or on intact shoot cultures, which was the case of the material studied here.

Larkin and Scowcroft (1981) have proposed the term "Somaclonal Variation" to describe the genetic infidelity observed among plants

regenerated from adventitious shoots or somatic embryos. They reviewed the species which have displayed this phenomenon: sugar cane, potato, tobacco, rice, oats, maize, barely, Brassica sp., pelargonium, carrots, chrysanthemum, carnation, red clover, sorghum, pineapple, garlic, and lettuce. Genetic infidelity of the adventitious buds regenerated on isolated leaves has been reported. Hermsen et al (1981) found 84.7% of 425 plants of adventitious shoots grown on in vitro cultivated rachis and petiole explants of the F_1 hybrids of Solanum etubersum X S. pinnatisectum were scored as doubled or quadrupled chromosome number. Evans and Sharp (1983) regenerated plants from leaf explants of Lycopersicon esculentum. They obtained autotetraploid plants frequently. Moreover, they reported that there were several monogenic mutations segregating in the progeny of the regenerated plants. There have been no studies of the genetic fidelity of adventitious buds from shoot cultures in beets or any other species. This kind of study was undertaken in the investigation here. Reviewing the reports of somaclonal variation, three levels of sophistication can be noticed. The first level described types of morphological variation in the plants regenerated from tissue culture. The second level involved genetically transmissible variation and detection of recessive variation in self progeny (McCoy and Phillips, 1982). The third level has involved detection of linkage change in regenerated plants.

The research described here on beets was done at the first and second levels. No persistent variation was found in plants derived from 141 individual adventitious buds from 24 original clones of

sources. However, this study is not meant to be a comperhensive answer to the question of somaclonal variation from adventitious buds in beet shoot cultures. Firstly, sample size of individual adventitious buds and wider germplasm sample should be larger, it was difficult to get almost equal or near numbers from different adventitious shoots at the same time through tissue culture techiques because of the vast variation in growth on the media. Secondly, there could be further investigations to detect any possible change in the combining ability among the adventitious ramets or in the isozyme patterns which are useful as molecular markers.

This study was executed by comparing the adventitious bud derived ramets with their counterpart ramets from the axillary buds through several approaches. When morphological and fertility characters were monitored, no aneuploidy was detected, and only three variants in leaf morphology were found. These narrow leaf types did not maintain that abnormal appearance through a subsequent cycle of shoot culture multiplication. In the field these narrow leaf individuals were noteworthy. One narrow leaf plant appeared unexpectedly among ramets of axillary bud origin. Additionally, other ramets derived from the same adventitious or axillary bud were normal in appearance. The narrow leaf character that does not persist might be explained as a carryover from some effect of the first shoot culture or as an epigenetic aspect.

When root tip chromosome number and guard cell length were examined, neither gave any polyploid or aneuploid plants. The fully tetraploid root tips which were found were accompanied by fully

diploid root tips from the same ramet. This indicates spontaneous duplication in somatic cells (polysomaty), probably during the shoot culturing or the initiation of the secondary roots. Some important points must be mentioned. In spite of the occurrence of some tetraploid root tips in some otherwise diploid ramets, there was no indication of tetraploid guard cells in the shoot system. Both axillary and adventitious derived ramets behaved similarly, i.e. the tetraploid root tips were found in some adventitious derived ramets as well as in some axillary derived ramets. One axillary clone displayed a mixture of diploid and tetraploid root tips in two different batches of axillary bud derived ramets.

When S_1 progeny of adventitious bud derived ramets were screened, no offtypes were found as segregates, as would occur if recessive mutants had arisen with the adventitious bud. Furthermore, no major change in segregation pattern of originally heterozygous characters was noticed. If this had happened, it would have indicated a mutation-like change at the heterozygous loci (B, b) and (R, r). No unusual segregation was noted in the families expected to breed true for either monogermness (mm) or multigermness (MM). Segregation for annualness which would be expected to be 3:1 annnual to biennial, did not give this ratio in most cases, with significant deviation (Table 12), even in the control as well as total ratio. The most likely explanation of this deviation for annualness is because of the variability in the microenvironment as well as genetic background among the annual plants. They did not grow at the same rate and therefore, they had not started to initiate the flowering stalks at the same time.

Since the time of the experiment was limited, it is very likely some of the annual plants were classified as biennial ones. The hypocotyl color was closer to the expected ratio, however, some ratios deviated significantly.

There was no promotive effect of higher benzyladenine concentrations used for shoot multiplication when frequency of tetraploid root tips was measured. The origin or maintaining of tetraploid cells in the rooting shoot is still unknown. However, Nitsch et al (1969) reported certain cytokinins such as the substituted phenylureas are effective in producing diploid shoots from haploid Nicotiana.

In concolusion, there was no indication of any genetic infidelity in ramets or progenies derived from adventitious buds in most cases of characters studied in this research. Moreover, the study suggests that the adventitious derived ramets are probably identical to their counterpart axillary derived ramets and there is no reason to exclude these buds during the in vitro propagation of shoot culture.

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