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Induction of plants from anthers of *Beta vulgaris* cultured *in vitro*

J. H. ROGOZIŃSKA, M. GOŚKA AND A. KUŻDOWICZ

Laboratory of Plant Physiology, Institute of Agriculture, Technical-Agricultural Academy and Laboratory of Plant Cytology and Anatomy; Department of Sugar Beet and Other Root Crops, Institute of Plant Breeding and Acclimatization, Bydgoszcz

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Abstract

The influence of growth substances, saccharose and yeast extract on the differentiation of monogerm sugar beet and polygerm fodder beet anthers is studied. Callus and roots were found to form on the anthers. After subculture, callus derived from a well determined combination of growth substances differentiated into buds, from which plantlets were obtained in unlimited numbers. After rooting, they were transferrred to the soil where they continued to grow. This suggests the possibility of an adaptation of this method in vegetative propagation of beets.

INTRODUCTION

Sugar beet belongs to the type of plants the anthers of which do not differentiate easily (Banba and Tanabe, 1972; Atanassov, 1973; Welander, 1974; Rogozińska and Gośka, 1976). According to the literature, only Banba and Tanabe (1972) succeeded in inducing buds in one anther from which they obtained a single not rooted plantlet.

In our previous paper (Rogozińska and Gośka, 1976), based on ca. 15 000 anthers, only callus and root formation was reported. The continuation of our investigation is aimed at determing the possibilities of obtaining plants from anthers by further modifying the composition of the medium as well as the varieties of the beets.

MATERIAL AND METHODS

Beet anthers were derived from plants grown in the greenhouse and in the field. The anthers were isolated in March and April 1976 (*Beta vulgaris* L. ssp. *vulgaris* convar. *crassa* Alef. provar. *altisima* Döll. 2n, monogerm, sugar beet) and in June and July 1976 (*Beta vulgaris* L. provar. *crassa* Alef. 2n, polygerm, fodder beet). The same method of anthers isolation was used as previously (Rogozińska and

Gośka, 1976) and the anthers were in the stage of tetrads and young microspores. The Linsmaier and Skoog (1965) medium was modified with saccharose, yeast extract and growth substances: benzylaminopurine (BAP), kinetin (K), 6-(3-methyl-2-butenylamino/purine (2iP), zeatin (Z) and naphthalene-2-acetic acid (NAA), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA); greenhouse material — in 10 combinations, and field material — in 22 combinations. The cultures were maintained at a temperature of ca. 25° in a photoperiod of 16 h light (ca. 1500 lux) and 8 h dark.

Stock of shoot clusters derived from anther callus were maintained routinely, employing the best growth substances combination for shoot multiplication. The stock were recultured at 4- to 6-week intervals.

In preparing propagules for their transfer to soil, clusters of shoots were divided into single-shoot units and recultured.

The results presented here are based on 2880 anthers of monogerm sugar beet 960 authers of male-sterile lines derived from plants — grown in the greenhouse and on 10 880 anthers derived from polygerm plants — grown in the field.

The microscopical observations were carried out in orcein stained material using the squash method.

RESULTS AND DISCUSSION

The results obtained from the culture of monogerm sugar beet anthers (derived from grennhouse material) are shown in Table 1. On media containing 20 μM K + 10 μM IAA and on medium containing 5 μM BAP + 25 μM NAA, only callus was formed (0,4%). On medium containing 25 μM BAP + 5 μM NAA the percentage of anthers producing callus was the highest (7,5%) and moreover root formation (0,8%) occurred. This callus, after the first subculture on medium of the same composition, underwent differentiation into buds (Fig. 1*A*).

The results obtained with polygerm fodder beet anthers (derived from plants grown in the field) are presented in Table 2. From the 10 combinations of growth substances and 12 combinations of saccharose, only some anthers exhibited callus formation. The rate of anthers producing callus was in the range of 0,4-4,6%. The highest percentage of anther differentiation was obtained on medium containing 25 μM BAP + 10 μM NAA. In some cases, root formation occurred. The appearance of roots was usually observed after two weeks from inoculation, and the highest percentage of roots (1,2) was obtained on medium containing 25 μM BAP + 15 μM NAA.

Experiments on the determination of optimal saccharose concs. for anther differentiation were performed on media containing 25 μM BAP + 1 μM IBA. From the investigated saccharose concs. (1-12%), the high-

Table 1

Effect of growth substances and yeast extract on callus and root formation in sugar beet anthers* (*Beta vulgaris* L. provar. *altisima* Döll.)

Treatment						Results	
growth substances μM					yeast extr. %	% of anthers prod. callus	% of anthers prod. roots
BAP	NAA	K	IAA	Z			
0	0	0	0	0	0	0	0
,,	,,	20	10	,,	,,	0.4	,,
,,	,,	0	0	10	0.01	0	,,
,,	,,	10	,,	0	,,	,,	,,
,,	,,	0	25	,,	,,	,,	,,
,,	,,	,,	0	,,	,,	,,	,,
10	,,	,,	,,	,,	,,	,,	,,
5	10	,,	,,	,,	0	,,	,,
,,	25	,,	,,	,,	,,	0.4	,,
25	5	,,	,,	,,	,,	7.5**	0.8

* The number of anthers in each treatment amounted to 240, with the exception of the first, which comprised 720 anthers. The anthers were derived from plants grown in the greenhouse and were isolated on March 28, 29 and April 1, 2, 4, 1976.

** After the first subculture, this callus differentiated into buds.

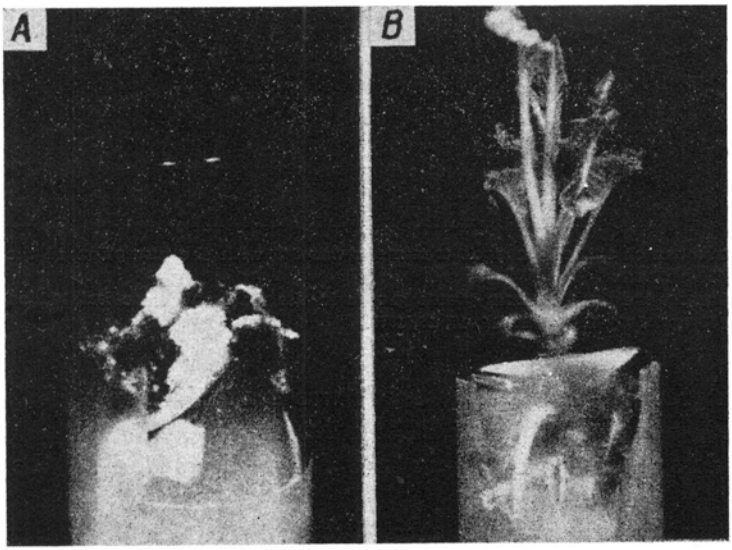


Fig. 1*A*. Differentiation of callus, derived from sugar beet anthers after the 1-st subculture (25 μM BAP + 5 μM NAA). Growth period: July 14 — Sep. 14. 1976. *B* — The rooted plantlet after the 6-th subculture of the differentiated callus (1 μM BAP + 5 μM NAA). Growth period: Dec. 30. 1976 — Feb. 10. 1977.

est percentage of anthers producing callus was obtained on medium containing 5% saccharose.

The same saccharose concs. (1-12%) in combination with 5 μM BAP + 25 μM IBA were tested on anthers derived from flower shoots

Table 2

Effect of growth substances and saccharose on callus and root formation of fodder beet anthers* (*Beta vulgaris* L. provar. *crassa* Alef.)

Treatment						Results	
Growth substances μM					Sacch. %	% of anthers prod. callus	% of anthers prod. roots
BAP	NAA	IBA	K	IAA			
0	0	0	0	0	3	0	0
,,	,,	,,	10	5	,,	,,	,,
,,	,,	,,	20	10	,,	,,	,,
10	25	,,	0	0	,,	1	0.4
15	5	,,	,,	,,	,,	0	0
,,	10	,,	,,	,,	,,	,,	,,
25	,,	,,	,,	,,	,,	4,6	0.8
,,	15	,,	,,	,,	,,	2.1	1.2
15	0	10	,,	,,	,,	4.1	0.5
10	,,	25	,,	,,	,,	0.6	0
25	,,	1	,,	,,	1	0.4	,,
,,	,,	,,	,,	,,	2	0	,,
,,	,,	,,	,,	,,	3	0.8	,,
,,	,,	,,	,,	,,	4	0.4	,,
,,	,,	,,	,,	,,	5	3.7	,,
,,	,,	,,	,,	,,	6	0.4	,,
,,	,,	,,	,,	,,	7	0	,,
,,	,,	,,	,,	,,	8	,,	,,
,,	,,	,,	,,	,,	9	0.4	,,
,,	,,	,,	,,	,,	10	0	,,
,,	,,	,,	,,	,,	11	,,	,,
,,	,,	,,	,,	,,	12	,,	,,

* The number of anthers in each treatment was 240 with the exception of the first — 1200 and 9-th — 720 anthers.

The anthers were derived from plants grown in the field and were isolated on June 15, 18, 22, 23, 24, 28, 29 and July 1, 2, 5, 13, 14, 15, 19, 1976.

which outgrew after the main shoot was removed. Of the 3920 anthers inoculated, none underwent differentiation and, after some time, the anthers dessicated. This failure may have been due to the material used.

Investigations were also carried out on the male-sterile lines of sugar beet. It is known that, in normal conditions, the degeneration of pollen occurs in the stage of tetrads. The aim here was to find out if, after the anthers are isolated and transferred onto medium, these cells will exhibit other developmental proprieties. In experiments carried out on 960 anthers, using 240 in each combination, callus was induced on medium with 10 μM BAP + 5 μM IBA (in 5%). This callus did not show organogenetic differentiation.

The callus from the anthers inoculated on medium with 25 μM BAP + 5 μM NAA (Tab. 1, combination 10) was subcultured after three

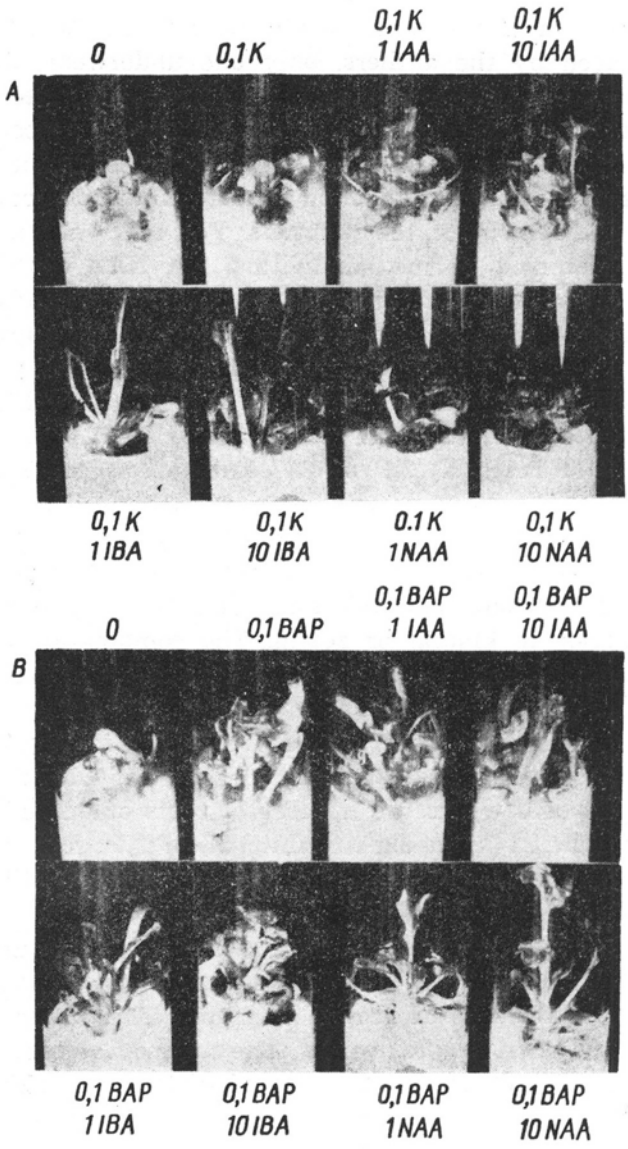
months on medium of the same composition. From the 18 subcultured calluses produced by the anthers, only one underwent differentiation into buds (Fig. 1*A*). This differentiation was observed after two weeks of subculture. This differentiated callus was again subcultured, and multiple buds were formed; however, the growth substance concs. used, which were suitable for the induction of differentiation, proved too high for further development of the plantlets. For that reason, part of the callus was transferred on medium with 5 μM NAA with 0,5, 1 or 5 μM BAP. On these media, further development of the buds into plantlets occurred. The highest conc. of BAP supressed the growth of the plantlets. The plantlets transferred on medium with 5 μM NAA + 1 μM BAP produced roots after 3 weeks of growth (Fig. 1*B*). The plantlets subcultured on various auxin (1 and 10 μM IAA, IBA or NAA) and cytokinin (0,1 μM BAP, K, 2iP or Z) combinations continued to grow and develop showing some growth differences dependent on the combination of the growth substances used (Fig. 2*A*, *B*, *C*, *D*). On control medium without auxin or cytokinin, growth was rather poor. Plantlets grown on cytokinin only, showed somewhat better growth; BAP and 2iP were superior to kinetin or zeatin. The combination of 2iP, Z or BAP with auxin gave similar results in regard to plant growth whereas combinations with K were inferior.

The rooting ability of these plantlets is shown in Fig. 3. On media containing BAP or K roots were formed after ca. 4 weeks, and after a growth period of 6 weeks the number of roots amounted to 25% for K and 50% for BAP. On media containing Z or 2iP, roots were formed after ca. 3 weeks and the number of roots amounted, after 6 weeks, to 67% for Z and 92% for 2iP.

Some of the rooted plantlets from the 6-th subculture (Fig. 4*A*) were transferred to the soil, where they continued to grow. On Fig. 4*B*, a plant after 4 weeks of growth is shown. The plants grew rather slowly during the first several weeks. The number of passages prior to transfer to the soil can be greatly reduced, though probably not to the same extent as in fodder cabbage anthers, whose plantlets differentiated much faster (Gośka and Rogozińska, 1977).

Cytological observations on root and leaf tips revealed a diploid number of chromosomes in the material tested.

As shown by Margara (1970), the source of the material used is of great importance in inducing buds in sugar beet. Using various parts of the plant, such as flower buds, segments of flower stems, or root fragments, he observed morphogenetical differences. Tissues of flower stems or roots produced callus only. On the contrary, flower buds formed vegetative buds *de novo* (20%) from the callus which developed at the base of the flower stem. Sporadically, also inflorescence stems were produced in the axis of the flower buds.

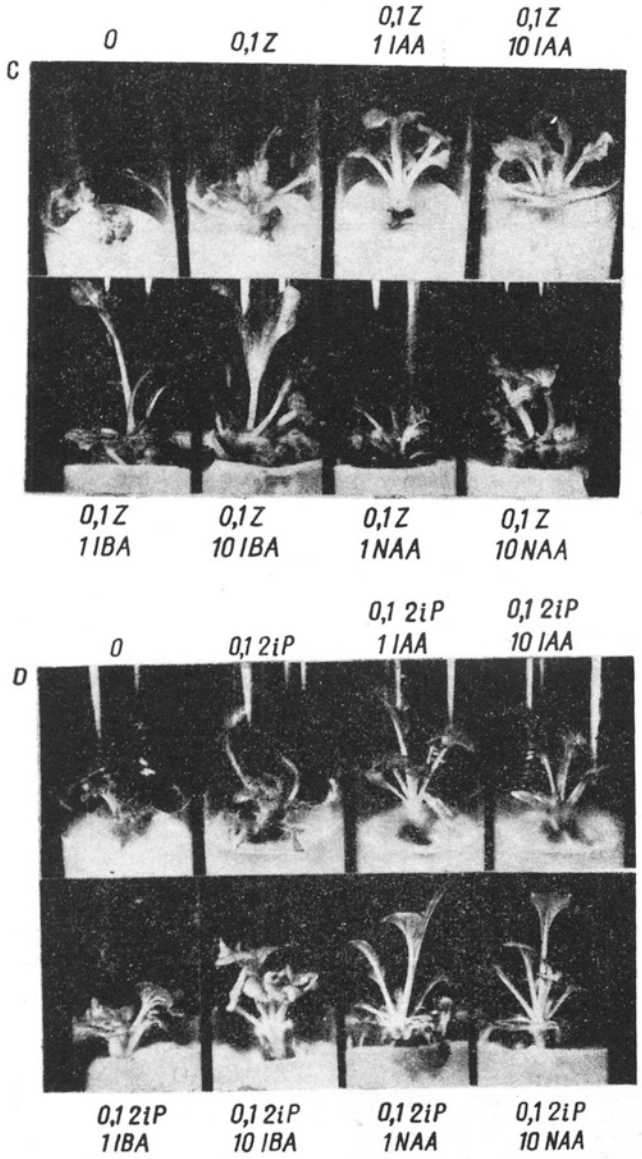


Figs 2 *A, B*. The growth of plantlets on various combinations of growth substances (μM).

A — kinetin + IAA, IBA or NAA; *B* — BAP + IAA, IBA or NAA; 5-th subculture, growth period: Nov. 27. 1976 — Jan. 6. 1977;

In Banba and Tanabe's (1972) studies, mainly callus and root formation was observed and only one anther produced buds. This indicates that it is very difficult to obtain bud differentiation from the anthers of sugar beet.

In our previous study, carried out in 1974 (Rogozińska and Gośka, 1976) on 15 000 anthers and in 1975 (unpublished) also on



Figs 2 *C, D*. The growth of plantlets on various combinations of growth substances (μM)

C — Z + IAA, IBA or NAA; *D* — 2iP + IAA, or NAA; 6-th subculture, growth period: Dec. 30, 1976 — Feb. 10, 1977

15 000 anthers, only callus and roots were produced. The calluses lost their rhizogenetic properties gradually and almost completely, but in their second or third year they are still growing vigorously. Also in a study, carried out last year (1976), on ca. 15 000 anthers, only callus and roots were formed. However, from the callus formed on anthers grown for 3 months on medium with 25 μM BAP + 5 μM NAA, one

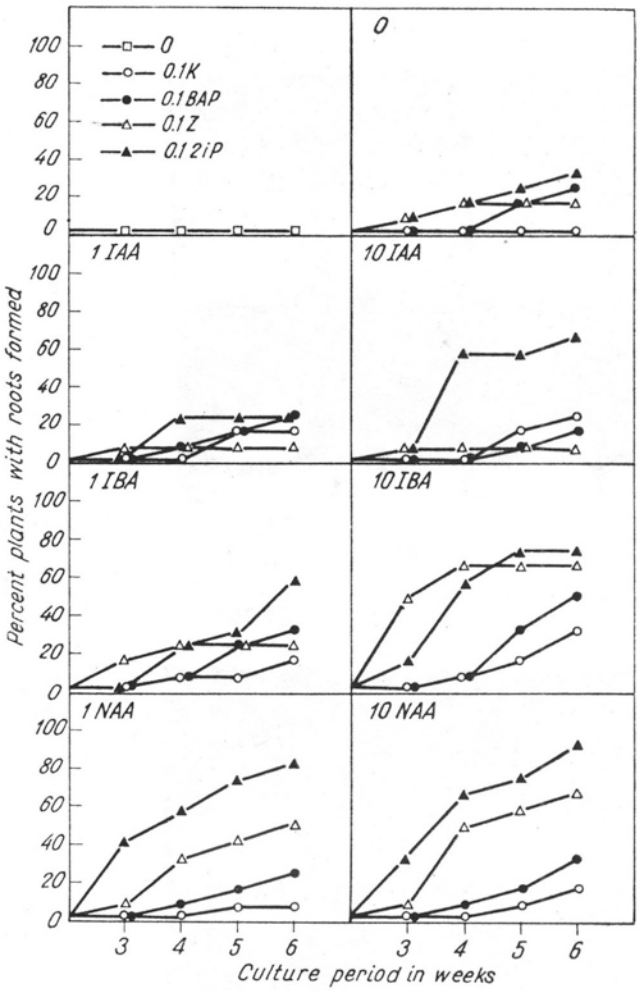


Fig. 3. Effect of growth substances (in μM) on rhizogenesis of sugar beet plantlets.

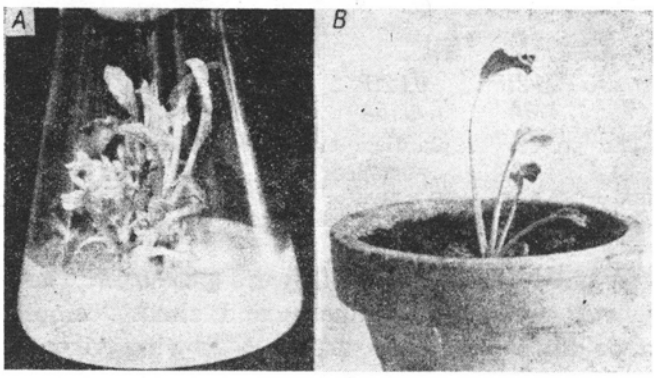


Fig. 4*A*. The plant, before being transferred to the soil (1 μM BAP + 5 μM NAA). 6-th subculture, growth period: Dec. 1, 1976 — Jan. 10. 1977. *B* — The plant after 4 weeks of growth in the soil. Growth period: Jan. 10. — Feb. 11. 1977.

piece (from the 18 calluses) differentiated into buds after the first subculture. The origin of this bud-producing callus was not analyzed anatomically. It probably derived from some anther cells and not pollen grains, since the plantlets were diploids.

From that bud-inducing callus hundreds of differentiated plants are at present being cultured and as soon as roots are produced, are transferred successfully to the soil. Multiple bud induction in sugar beet callus derived from anthers can find application in vegetative propagation of this economically important plant (Rogozińska and Gośka, in press). In this way, as shown in the present investigation, it is possible to obtain unlimited numbers of plants.

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Author's address:
Doc. dr hab. Janina Rogozińska
Institute of Agriculture
Technical-Agricultural Academy;
Bernardyńska Str. 6; 85-029 Bydgoszcz, Poland
Prof. Alfons Kużdowicz and Inż. Maria Gośka
Department of Sugar Beet and Other Root Crops
Institute of Plant Breeding and Acclimatization,
Weyssenhoffa 11, 85-950 Bydgoszcz, Poland

Uzyskiwanie roślin z pylników buraka w kulturze in vitro

Streszczenie

Przeprowadzono badania nad wpływem substancji wzrostowych, sacharozy i ekstraktu drożdżowego na różnicowanie pylników jednonasiennego buraka cukrowego i wielonasiennego buraka pastewnego. Na pylnikach tworzył się kalus i korzenie. Po przeszczepieniu kalusa pochodzącego z określonej kombinacji substancji wzrostowych, uległ on wyróżnicowaniu w pączki, z których uzyskano liczne roślinki. Po ukorzenieniu przesadzono je do gleby gdzie kontynuowały swój wzrost. Sugeruje to na możliwości adaptacji tej metody w wegetatywnym mnożeniu buraka i uzyskiwaniu niezliczonej liczby roślin genotypowo jednorodnych.