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INDUCTION AND MICROPROPAGATION POTENTIAL OF SUGAR BEET HAPLOIDS

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The aim of research was obtaining sugar beet haploids via gynogenesis and their micropropagation. Haploids were obtained by ovule culture from fourteen diploid, monogerm, fertile genotypes. On the tested nutrient media genotypes exhibited different gynogenic potential. Eight haploid plant were chosen for further investigation and after development of first leaves put on micropropagation medium. The presence of cytokinin in medium stimulated development of axillary buds, while in some genotypes adventitious buds developed as well. Multiplication rate was not consistent, although number of developed plants grew after each subcultivation. Differences in plant multiplication started to differ after four subcultures. By testing of differences between correlation coefficients, i.e. multiplication rate during six subcultivations, it was determined that they significantly differ between tested genotypes.

Key words: *Beta vulgaris* L., ovule, haploid, micropropagation

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INTRODUCTION

Inbred lines are most important elements in sugar beet (*Beta vulgaris* L.) breeding, but their production by repeted selfing is time consuming although efficient method. An alternative for creating homozygous plants within short period of time is production of dihaploids. In addition, because of their true homozygosity, dihaploids may allow more accurate evaluation of the genetic value than inbred lines, which can result in increased selection efficiency (RAGOT and STEEN, 1992). Dihaploid production requires two steps: creation of haploid plants and doubling of their chromosome complement. Haploid plants have one half of normal plant chromosome number and for their induction is usually used one of the following techniques: (i) male gametophyte culture, i.e. anther or pollen culture, (ii) *bulbosum* technique of embryo culture and (iii) female gametophyte culture, i.e. ovule or ovary culture. Although is androgenesis (anther or pollen culture) most common way to obtain haploids (SAN and GELEBART, 1986), in some species, like sugar beet, haploids can be only induced via gynogenesis (ovary or ovule culture). This technique is based on the fact that in the specific conditions it is possible to induce gynogenesis, i.e. sporophytic development of one haploid nuclei in embryo sac, which will, in absence of pollination, lead to formation of embryo or embryogenic callus from unpollinated ovule (ŠESEK, 1995).

Spontaneous haploids occur in sugar beet populations at frequency of 0.1% (BOSEMARK, 1971), so in seventies many groups tried to produce haploids via anther culture. This method, unfortunately did not prove to be successful since the only results were calli with different ploidy level (ATANASOV, 1973; WELANDER, 1974). Induction of sugar beet haploids via gynogenesis was reported by HOSEMANS and BOSSOUTROT (1983), and in the later research from unpollinated ovule were also obtained roots and calli with different regeneration abilities (BAROCKA *et al*., 1986; GALATOWITSCH and SMITH, 1990). Haploids can be induced at the frequency up to 35% (VAN GEYT *et al*., 1987) and it strictly depends on correlation between genotype and concentrations of phytohormones in nutrient medium (SPECKMAN, 1986, SEMAN and FARAGO, 1990). Although haploids by themselves have no importance in breeding, their transfer in dihaploids and development of completely homozygous lines can be very significant in shortening and simplifying sugar beet breeding process.

Vegetative propagation of sugar beet *in vivo* is possible, but formation of axillary buds is uneven and low (MIEDEMA *et al*., 1980), so micropropagation *in vitro* is widely used method for fast and efficient multiplication. For stimulation of axillary buds usualy is used citokinin, mostly BA or BAP, in combination with low concentration of giberrelic acid (ATANASOV, 1980; SLAVOVA, 1980). Number of plants obtained this way can go up to 10^6 but in research of MIEDEMA (1982) it depended on genotype, while MEZEI and KOVAČEV (1988) estimated that it did not.

In this paper are presented results of investigation on differences in (i) gynogenic response of fourteen sugar beet genotypes, and (ii) morphogenic potential for micropropagation of induced haploids.

MATERIAL AND METHODS

Fourteen S_2 autofertile diploid lines of sugar beet were used as donors for plant explants. Lines were carrying genes for nuclear male sterility, and served for maintaining of cytoplasmic male sterility. They derived from populations made by successive hybridisation and differed in combining abilities and other phenotypis traits.

Parts of inflorescence with unopened buds were taken and sterilized with 0.1% bleach. They were washed out in sterile distilled water and in sterile conditions ovules were taken out and placed on media for haploid induction, in growth chamber on 24±1°C, with photoperiod 16/8. The basic media were by MURASHIGE and SKOOG (MS) (1962) and DE GREEF and JACOBS (PGo) (1979), with different concentrations of growth stimulators (Table 1).

Table 1. Nutrient media for haploid induction

Medium	Basic medium	Kin. (mg/l)	BA (mg/l)	NAA (mg/l)	2,4D (mg/l)
K	PGo	0.1	-	-	-
K_1	PGo	0.2	-	-	-
J	PGo	0.3	-	-	-
K_2	PGo	0.4	-	-	-
M	MS	-	0.2	0.6	-
KM	MS	0.1	-	-	-
K_1M	MS	0.2	-	-	-
V	MS	-	0.3	0.1	0.05
Z	MS	-	0.1	0.1	-

Haploid plants were put on micropropagation medium, MS with 0.3mg/l BA and 0.01mg/l GA_3, and were transfered every four weeks. There was six subcultivations and after each the number of plants deriving from axillary shoots was counted. The regression analysis of obtained results was done, and the differences between regression coefficients for each genotype was trested with LSD test.

RESULTS AND DISCUSSION

On media for haploid induction was placed 1176 unpollinated ovules. After one week, they changed color, from perly white to dark brown or black and after 4 to 8 weeks gave rise to haploid plants. Gynogenic potential of tested genotypes significantly differed (Table 2): genotypes 2157, 2185, 2115 and 2197 did not express any potential for gynogenesis, while other ten genotypes gave 18 haploid plants. The highest percent of haploid induction had genotype 2064 (5.21%), and the average induction rate was 2.04%. One ovule in genotype 2046 on media J (0.3 mg/l kin.) developed roots on micropilar end, which never gave rise to plant. Calli developed on five ovules. Those developed from ovules in genotypes 2105 and 2165 did not have regeneration ability, while calli deriving from ovules in

genotypes 2064 and 2086 formed roots that did not develop into the plants. Regeneration ability had only callus from ovule in genotype 2107.

Table 2. Haploid induction in different genotypes

Genotype	Induced			
	Callus	Plant	Root	%
2107	1_Z	1_{KM}	-	3.07
2064	1_K	$2_K+1_{KM}+1_M$	-	5.32
2086	1_K	1_J	-	2.10
2129	-	1_Z	-	0.70
2105	1_J	-	-	1.58
2157	-	-	-	0
2046	-	1_J	1_J	5.00
2165	1_K	1_K+2_J	-	3.11
2079	-	1_K	-	1.56
2185	-	-	-	0
2067	-	2_{K2}	-	1.83
2095	-	$1_{K1}+2_{K2}+1_V$	-	3.64
2115	-	-	-	0
2197	-	-	-	0
Σ	5	18	1	x=2.04

Most haploids were induced on media with cytokinins (K, K_1, K_2, J, KM and K_1M) which indicates that those growth regulators increase embryo yield and therefore induction of haploids as well (LUX *et al*., 1990). The obtained results agree with the fact that roots and calli of different regeneration ability can be induced from unpollinated ovule (POTYONDI and HESZKY, 1992; VAN GEYT *et al*., 1987). Unfortunately, in this research plant regeneration was not achieved although in certain conditions it could be obtained (GALATOWISCH and SMITH, 1990). Although number of obtained haploids agrees with results of other researches it might be increased by using cold pretreatment of unopened flower buds or/and addition of charcoal in the induction medium (GÜREL *et al*., 2000).

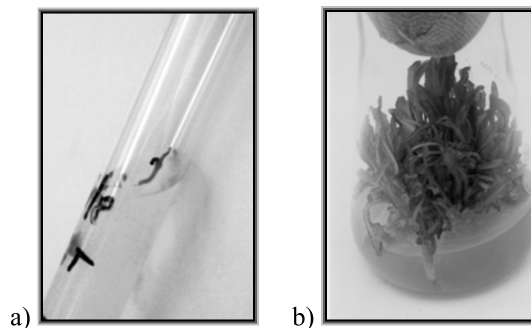

Fig. 1. Haploid plant one week after induction from unpollinated ovule (a) and after micropropagation

For further investigation were selected eight haploids: 1-2064/58, 2-2064/86, 3-2165/31, 4-2067/56, 5-2095/106, 6-2115/47, 7-2115/86 and 8-2115/100. After they were fully formed (Fig. 1a) they stayed one more week on induction medium, and were then transferred on micropropagation medium. After one week on micropropagation medium, leaf stalks started to thicken which indicated begining of formation of axillary buds. In first two subcultivations it took one week for formation of axillary shoots, but in later subcultivations shoots were formed within few days (Fig. 1b). According to differences between regression coefficients, i.e. average growth of number of plants, it can be estimated that genotypes significantly differed in expression of morphogenetic potential for micropropagation (Fig. 2, Table 3).

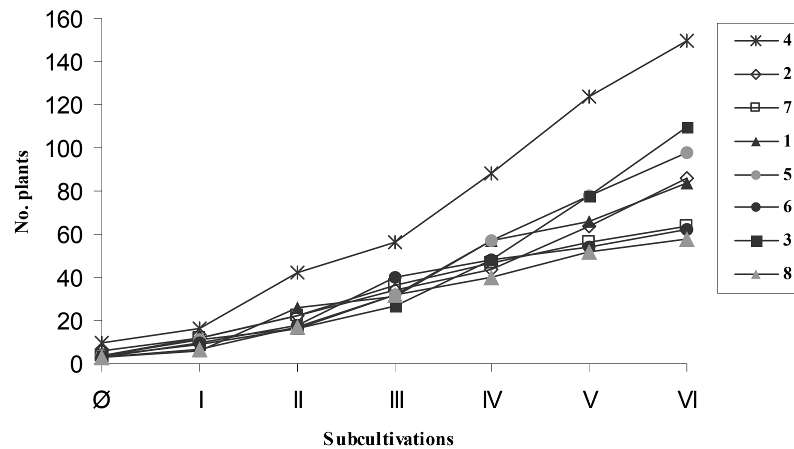

Fig. 2. Increase of haploid plants number during micropropagation

Table 3. Differences in micropropagation rate

Genotype	Regresssion coef.	0.05	0.01
4	23.70	a	a
3	17.05	b	b
5	16.38	bc	bc
1	13.28	cd	bcd
2	12.71	de	cd
6	10.61	de	d
7	10.61	de	d
8	9.61	e	d

At the begining of multiplication genotype 4 (2067/56) had highest micropropagation rate, and maintained it during all time of investigation. Other genotypes could be divided in approximately two groups (i) with high micropropagation potential (numbers 3, 5 and 1) and (ii) with low micropropagation potential (2, 6, 7 and 8). Dinamics of multiplication rates also varied during subcultivations - for example, micropropagation of haploids 1 and 6, was very intensive in first three

subcultivations but it became much lower in last three. Haploids 3 and 5 expressed the oposite behaviour, since their micropropagation rate increased in last subcultivations. Very often happens that micropropagation medium stimulates, beside axillary, adventitious buds as well (HUSSEY and HEPHER, 1977; SAUNDERS, 1982). In this investigation in all genotypes there was more or less intesive formation of adventitious buds on leaf stalks, but it did not have any effect on micropropagation rate.

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INDUKCIJA I POTENCIJAL ZA MIKROPROPAGACIJU HAPLOIDA ŠEĆERNE REPE

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I z v o d

Cilj istraživanja je bio dobijanje haploida šećerne repe putem ginogeneze i njihova mikropropagacije. Haploidi su dobijeni kulturom semenog zametka iz četrnaest diploidnih, monogermnih i muški fertilnih genotipova šećerne repe. Ispitivani genotipovi su na podlogama sa različitom koncentracijom stimulatora rasta ispoljili različit potencijal za ginogenezu. Za dalja ispitivanja je odabrano osam haploida koji su nakon razvića prvog para listova postavljeni na podlogu za mikropropagaciju. Prisustvo citokinina u hranljivoj podlozi je stimulisalo pojavu i razviće bočnih pupoljaka u pazuhu listova, dok je kod nekih genotipova takođe došlo do pojave adventivnih pupoljaka. Porast broja biljaka tokom mikropropagacije nije bio kontinuiran, iako je broj novostvorenih biljaka rastao posle svake subkultivacije. Razlike u intenzitetu multiplikacije biljaka počele su da se značajno ispoljavaju nakon četvrte subkultivacije. Testiranjem značajnosti razlika korelacionih koeficijenata, odnosno intenziteta mikropropagacije ispitivanih genotipova tokom šest subkultivacija, utvrđeno je da se one međusobno statistički značajno razlikuju.

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