Electronic Journal of Biotechnology ISSN: 0717-3458
http://www.ejbiotechnology.info
DOI: 10.2225/vol16-issue2-fulltext-3 *RESEARCH ARTICLE*

Comparison of haploid and doubled haploid sugar beet clones in their ability to micropropagate and regenerate

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Received August 6, 2012 / Accepted February 6, 2013
Published online: March 15, 2013
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Abstract

Background: Haploid plant material is considered as recalcitrant to organogenesis, propagation, and maintenance *in vitro*. However, sugar beet (*Beta vulgaris* L.) breeders utilizing doubled haploid (DH) technology in their breeding programs indicate that sugar beet haploids may be cultured *in vitro* as well as diploids. Thus in this paper the *in vitro* performance of haploid and the doubled haploid sugar beet of various origin was evaluated. The DHs were derived from haploids by diploidization and twelve such haploid and corresponding DH clone pairs were obtained thus the comparison included haploid and DH clones that had identical allelic composition and differed only in their ploidy level.
Results: The genotypes differed in shoot morphology and susceptibility to blackening during culture *in vitro*, but no significant differences were observed between the haploids and DHs. The micropropagation rate was, on average, higher for the haploids than DHs. Viability of the midrib and petiole explants after a 6-week culture was highly genotype dependent, but not affected by explant ploidy level. However, regeneration efficiency depended on both the genotype and ploidy level. The explants of several haploids regenerated more frequently and developed more adventitious shoots than the corresponding DHs thus overall efficiency was higher for haploids.
Conclusions: The results obtained indicate that most of the haploids used in the comparison performed similar to or even better than DHs. This suggests that sugar beet haploid material can be successfully used not only for the production of DHs, but also maintained *in vitro* and utilized in projects requiring haploid tissues as the source material.

Keywords: adventitious buds, axillary shoots, *Beta vulgaris,* organogenesis, ploidy.

INTRODUCTION

Haploid plants may occur spontaneously or develop as the result of apomixis and chromosome elimination after interspecific or intergeneric hybridization, and after induction of gametogenesis in microspore, anther, ovule or ovary culture (Palmer and Keller, 2005; Murovec and Bohanec, 2012). In sugar beet most of these methods fail or are inefficient. Microspore culture led to induction of proembryoid structures or callus. In another culture callus, roots or plants were obtained but the tissue was diploid and their gametophytic origin was not confirmed (Gürel et al. 2008). Only gynogenesis has been reported as the pathway for successful production of sugar beet haploids. Unfertilized ovules are excised from ovaries of male sterile or fertile donor plants and cultured *in vitro.* Gynogenic embryos originating from the egg cell convert into shoots with a haploid chromosome number (Gürel et al. 2000). A single set of chromosomes in haploids is doubled by treating shoot meristems with anti-mitotic agents or by culturing the shoots on media supplemented with such compounds. This results in the

development of doubled haploid (DH) shoots that are completely homozygous (Dhooghe et al. 2011). In conventional breeding, plant homozygosity is obtained after several cycles of inbreeding that is time consuming, particularly in biennial crops and does not ensure complete homozygosity in case of allogamous species like sugar beet. Thus the production of DHs offers a time-saving approach to obtaining pure breeding lines (Dunwell, 2010; Chen et al. 2011). Although gynogenesis is highly laborious and relatively expensive it has been implemented in sugar beet breeding programs for the development of DH lines required for the creation of new hybrid varieties (Biancardi et al. 2010).

Haploid and DH plant material is also invaluable in basic and applied research. The mono-allelic and homozygous states at each locus allow the unmasking of recessive genes, including those conferring undesirable traits. They are therefore convenient for mutant identification and selection. Haploids are also utilized in cytogenetic research, reference genome sequencing, and genetic linkage analysis (Ferrie and Möllers, 2011; Yang et al. 2011). Achievements in plant genetic engineering have recently opened new potential applications for haploids. Genetic modification of haploid tissue and its subsequent diploidization allows the development of transgenics that are homozygous instead of hemizygous at the modified locus (Chauhan and Khurana, 2011).

Several studies have demonstrated that haploid plants are characterized by weaker vigour and slower growth and are smaller than diploid or tetraploid plants (Riddle et al. 2006; Froelicher et al. 2007). Furthermore, the development and growth of haploids *in vitro* encounters considerable problems that make tissue maintenance and regeneration difficult and can even lead to tissue decay (Aleza et al. 2009). The poor performance of haploid tissue may be related to the enhanced expression of recessive, lethal, or sub-lethal genes in comparison with heterozygous material (Germana and Chiancone, 2001). In haploids, the lack of a homologous set of chromosomes also means that the plants are infertile (Ferrie and Caswell, 2011).

The above facts suggest that haploid sugar beet material is recalcitrant to organogenesis, propagation, and maintenance *in vitro*. Sugar beet haploids are only cultured *in vitro* for a very short time and are usually no longer of interest to breeders if their diploidization fails. There are also no available reports comparing haploids and DHs with regard to their ability to micropropagate or regenerate *in vitro*. However, breeders utilizing doubled haploid technology in sugar beet breeding programs communicate that haploids may be cultured *in vitro* as well as diploids. Thus, in this study, in order to verify the effect of ploidy level on haploid performance *in vitro*, haploid and DH clones derived from these haploids were compared.

MATERIALS AND METHODS

Plant material

Haploid and DH sugar beet (*Beta vulgaris* L.) shoots derived from these haploids were developed and provided by the Kutnowska Hodowla Buraka Cukrowego breeding company, Poland. Haploids were produced via gynogenesis *in vitro* from unpollinated ovules of eight heterozygous donor plants selected from populations of different pedigree. The shoots obtained were micropropagated to get clones of haploids. Then the shoots of each clone were separated into two subclones. The first subclone remained untreated and further cultured to keep haploid shoots while the second subclone was treated with colchicine for chromosome doubling. Homozygosity of the produced DH shoots was confirmed by isozyme analysis. In consequence, plant material comprised pair of clones, the haploid together with the DH clone derived from it, which originated from the same ovule and differed in tissue ploidy level only. Twelve such pairs were used in this work; clones marked in the text by the letters A-C came from the same donor plant but from different ovules. Additionally to these 12 pairs, nine haploid clones (Nos. 148-156) derived from donor plants of other populations were used for evaluation of their regeneration ability.

Ploidy level assessment

Tissue ploidy level was assessed by flow cytometry. For this purpose young leaves were chopped by a razor blade in 2 ml of nucleus-isolation buffer (12.1 g l^{-1} TRIS, 0.5 g l^{-1} $MgCl_2 \cdot 6H_2O$, 5.0 g l^{-1} NaCl, 1.0 ml l^{-1} Triton X-100) containing 1 mg 4',6-diamidino-2-phenylindole (DAPI). Samples were passed through a nylon filter and their fluorescence intensity was measured on a Partec PA II flow cytometer

DOI: 10.2225/vol16-issue2-fulltext-3

(Partec GmbH, Germany). Plants with a confirmed haploid (1n = 9) and diploid (2n = 18) chromosome set were used as standards. Ploidy level of the standards was confirmed by chromosome counting. Young leaf meristems were incubated in 2 mM 8-hydroxyquinoline for 5 hrs and fixed in 3:1 methanol:acetic acid solution. Subsequently, leaves were digested in a mixture of cell wall-degrading enzymes consisted of 2% cellulase and 1% pectinase at 37ºC for 40-70 min. Then tissues were put on slides and squashed with forceps. Chromosomes were counted using the Nicon Eclipse e600 light microscope with an x40 objective in phase contrast.

Shoot micropropagation

The haploid and DH shoots were maintained in aseptic *in vitro* culture and their rosettes were divided into separate shoots with intact meristems every 3 weeks. The shoots were cultured on 0.7% agar-solidified Murashige and Skoog (1962) salts and vitamin medium (MS; Duchefa Biochemie) supplemented with 0.3 mg l^{-1} 6-benzylaminopurine (BAP; Sigma), 0.1 mg l^{-1} 1-naphthaleneacetic acid (Sigma), 0.3 mg l^{-1} thiamine (Sigma) and 30 g l^{-1} sucrose; pH 5.8. After autoclaving, 200 mg l^{-1} cefotaxime (Polfa, Tarchomin) was added to prevent bacterial contamination. Micropropagated shoots were cultured in 500 ml containers at 25 ± 1ºC under a 16 hrs photoperiod of 1:1 Daylight (Philips) and Fluora (Osram) fluorescent tubes (55 µmol m^{-2} s^{-1}). Six shoots were enclosed in each container with three replications per clone. The micropropagation rate (the number of shoots developing from the donor shoot) was recorded for 18 individuals in each clone during each shoot transfer to fresh medium. Shoot response to culture conditions was assessed by visual inspection of their appearance in terms of shoot vigour and tissue blackening.

Shoot regeneration

Fully developed and vigorous leaves were excised from 3-week-old shoots for the preparation of explants. For this purpose, the leaf blades were discarded and the midrib and petiole were cut into 1 cm long fragments. Ten explants were placed in 90 mm Petri dishes containing MS medium supplemented with 1 mg l^{-1} BAP and 30 g l^{-1} sucrose, pH 5.8 and cultured in the same conditions as for micropropagation. The explants were transferred to fresh medium after 3 weeks of culture, and after an additional 3 weeks the number of surviving (green) explants, the number of explants with regenerating shoots, and the number of regenerated shoots per explant were recorded. The regeneration rate was calculated as the product of the percentage of regenerating explants and the average number of regenerated shoots per regenerating explant. This rate indicates the hypothetical number of regenerated shoots in 100 explants. Each experiment had five replicates and was repeated three times.

Statistical analysis

Mean values are presented with their standard errors. The effect of genotype was verified using a one-way analysis of variance. The percentage data were subjected to arcsin transformation prior to analysis. The significance of the difference between the ploidy levels was tested after defining the contrasts and using the paired t-test. The analysis was performed using the Statsoft Statistica v. 8.0 package.

RESULTS

Morphology

The haploid shoots and shoots of DHs derived from these haploids were similar in size and leaf morphology (Figure 1). Only three haploid clones (Nos. 1, 5B and 6) were considerably bigger than the corresponding DHs throughout the culture period. The shoot size ranged from 2 to 6 cm. Leaf blades were often very narrow, lanceolate, and petiole-like or irregular and more typical of beets depending on the clone. The leaves were green or yellow-green with red coloration visible on the petioles only or on both the petioles and blades in some clones.

Fig. 1 Leaves, shoot rosettes, and midrib or petiole explants with regenerating shoots or developing callus of three haploid and the corresponding DH sugar beet.

Shoot transfer to a fresh medium was required every three weeks as a longer culture time caused leaf senescence and favoured tissue blackening, which inhibited growth and decreased shoot vigour. We distinguished four classes of shoot blackening during the 3-week culture: I^{st} degree - no symptoms or less than 5% leaf area affected; IInd degree – 5-25%, IIIrd degree – 25-75%, and IVth degree – over 75% leaf area affected (Figure 2). Clones differed in their response to *in vitro* culture and were predominantly classified in the I^{st} and IInd classes (Table 1). Only two haploid clones (Nos. 2B and 5B) were considerably more affected. There was no clear relationship between blackening and ploidy level ($r = 0.23$, $P = 0.279$), but the DH clones were more often classified as exhibiting I^{st} degree blackening (42%) than the haploids (33%), although the significance of the difference was not evident ($P = 0.053$). Tissue blackening was also observed on the excised explants exposed to the culture medium. The process usually initiated from part of the explant or shoot that was in direct contact with the medium.

Micropropagation

In all clones, new shoots developed from axillary meristems at the rosette base allowing their micropropagation. The efficiency of micropropagation was highly genotype-dependent and ranged from 1.3 to 5.3 new shoots developing during the 3-week culture (Table 1). The clones obtained from different ovules of the same donor plant (Nos. 2A and 2B; Nos. 8A and 8B) also differed in their micropropagation ability, but only in haploids. In general the haploids developed more new shoots than DHs, at 3.2 and 2.4, respectively ($P = 0.019$). In fact, five out of twelve haploids showed higher micropropagation efficiency than the corresponding DHs whereas the opposite relation was observed for only one DH genotype (No. 2B-DH).

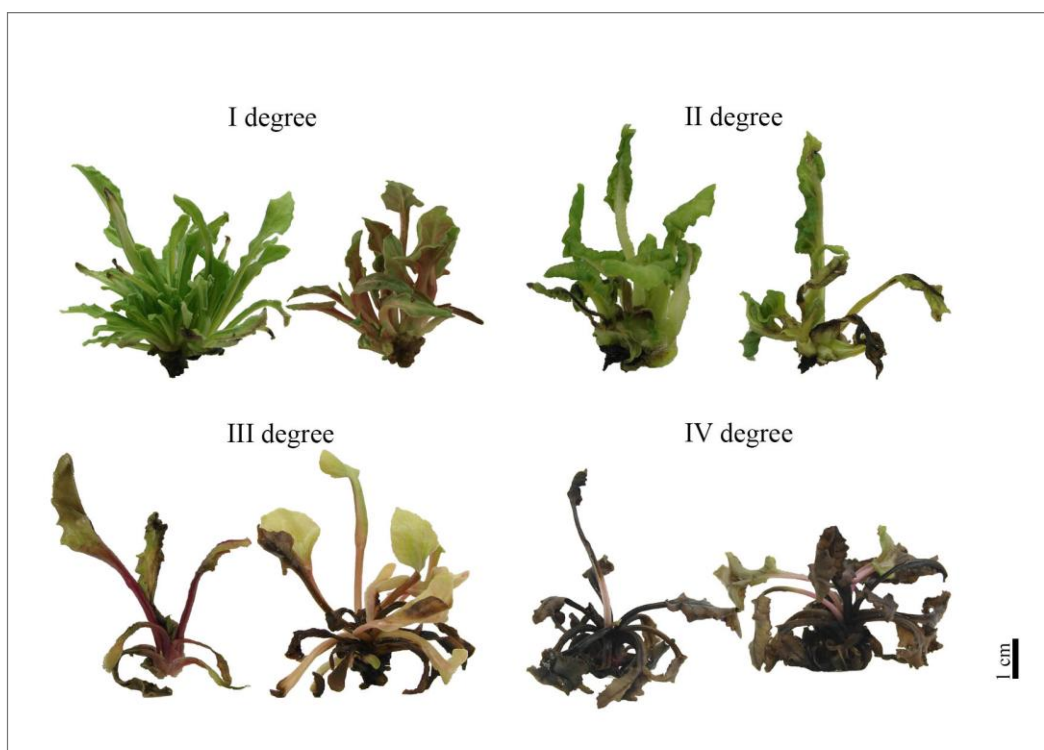

Fig. 2 Classes of shoot response to *in vitro* culture seen as tissue blackening appearing on less than 5% (I[st] degree), 5-25% (IInd degree), 25-75% (IIIrd degree) and over 75% (IVth degree) of the leaf area.

Table 1. Degree of tissue blackening and micropropagation rate (mean ± standard error) of haploid and DH clones.

Clone	Haploid			DH		
	N	Degree of blackening[a]	Micropropagation rate	N	Degree of blackening[a]	Micropropagation rate
1	18	1	1.8 ± 0.3	18	2	1.9 ± 0.3
2A	18	2	2.9 ± 0.3	18	2	2.1 ± 0.2
2B	18	3	1.6 ± 0.3	18	2	2.1 ± 0.2
3	18	2	2.8 ± 0.4	18	1	2.8 ± 0.3
4	18	2	3.7 ± 0.4	18	2	1.3 ± 0.2
5A	18	2	3.4 ± 0.4	18	1	2.4 ± 0.3
5B	18	3	2.8 ± 0.6	18	2	2.8 ± 0.4
5C	18	2	4.8 ± 0.6	18	1	2.8 ± 0.3
6	18	1	5.3 ± 0.6	17	2	4.4 ± 0.6
7	18	1	3.4 ± 0.5	18	1	3.3 ± 0.4
8A	17	1	4.1 ± 0.7	18	2	1.8 ± 0.3
8B	18	2	2.3 ± 0.5	18	1	1.8 ± 0.2
Mean	215		3.2 ± 0.3	215		2.4 ± 0.2

[a]Degree of blackening according to the classes shown in Figure 1.

DOI: 10.2225/vol16-issue2-fulltext-3

Shoot regeneration

Out of all 5110 explants used in the experiments, 68.3% remained green and viable and in 28 out of 33 clones this percentage ranged between 52.0% and 97.3% (Table 2 and Table 3). Lower percent of viable explants (6.3-40.6%) was observed in five out of 21 haploid clones. In two of them (Nos. 148 and 149) over 90% explants showed blackening symptoms and did not survive (Table 3). However, comparison of haploids and DHs derived from these haploids indicated that the percent of viable explants was independent on the ploidy level ($P = 0.264$) (Table 2).

Table 2. Efficiency of shoot regeneration from explants of haploid-DH clone pairs after 6-week culture (mean ± standard error)

Genotype	Number of explants		Percent of viable explants		Percent of regenerating explants		Number of developed shoots		Regeneration rate[a]	
	1n	DH	1n	DH	1n	DH	1n	DH	1n	DH
1	150	150	92.0 ± 4.0	92.7 ± 2.8	0.7 ± 0.7	0.7 ± 0.67	3	3	2.0 ± 2.0	2.0 ± 2.0
2A	150	140	76.7 ± 5.2	55.1 ± 6.3	19.6 ± 3.5	7.9 ± 2.39	136	35	88.9 ± 18.0	25.0 ± 8.2
2B	150	150	40.6 ± 7.4	64.7 ± 9.4	4.7 ± 1.6	13.3 ± 4.33	40	56	26.7 ± 12.2	37.3 ± 13.3
3	150	150	82.7 ± 6.6	78.0 ± 5.7	33.9 ± 5.7	7.3 ± 2.28	302	45	199.5 ± 48.1	30.0 ± 12.4
4	150	150	54.2 ± 5.3	52.0 ± 6.8	1.3 ± 1.3	0.7 ± 0.67	6	5	4.0 ± 4.0	3.3 ± 3.3
5A	150	130	94.0 ± 3.2	84.0 ± 3.1	11.3 ± 4.1	10.5 ± 4.13	134	68	89.3 ± 44.7	47.8 ± 19.6
5B	150	150	76.0 ± 7.0	74.8 ± 3.0	3.3 ± 1.6	1.3 ± 0.91	23	20	15.3 ± 8.3	13.3 ± 9.1
5C	150	150	70.7 ± 10.8	77.8 ± 5.6	3.3 ± 1.6	0	33	0	22.0 ± 11.9	0
6	150	150	75.3 ± 5.8	69.2 ± 4.4	18.0 ± 2.8	9.6 ± 2.48	107	37	71.3 ± 21.4	23.7 ± 7.1
7	150	150	97.3 ± 2.1	95.3 ± 2.4	3.9 ± 1.6	1.3 ± 1.33	24	5	15.4 ± 7.1	3.3 ± 3.3
8A	150	140	26.0 ± 4.8	54.3 ± 8.9	14.0 ± 3.1	4.9 ± 1.96	35	10	23.3 ± 6.0	7.0 ± 3.2
8B	150	130	35.6 ± 8.2	53.6 ± 7.2	2.0 ± 1.1	0	6	0	4.0 ± 2.4	0
Total/Mean	1800	1740	68.4 ± 2.4	70.8 ± 1.9	9.7± 1.1	4.7 ± 0.7	849	284	46.8 ± 7.3	15.9 ± 2.7

[a]Number of developing shoots per 100 explants.

Adventitious buds appeared along the explants on their adaxial side after 2-5 weeks of culture and developed into shoots that could be excised and further cultured. The number of shoots developing on explants depended on the genotype. In the most responsive clone (No. 150) 47.7% of explants regenerated (Table 3) while two DH clones (Nos. 5C-DH and 8B-DH) did not develop any shoots at all (Table 2). Comparison of 12 haploid–DH pairs showed that half of the haploid clones regenerated more frequently than DHs while only one DH clone (No. 2B-DH) had more regenerating explants than the corresponding haploid (2B-1n), 13.3% and 4.7%, respectively. On average, the percent of regenerating explants was two-fold higher for haploids (9.7%) than for DHs (4.7%) ($P < 0.001$).

In total, 1643 new shoots were counted on the explants and, on average, one regenerating explant produced from 1.0 to 10.0 shoots depending on the clone. Comparing haploid-DH clone pairs, 75% of new shoots developed on the haploid explants and the mean number of shoots on the haploid explants (4.7 ± 0.5) tended to be higher than that on the DH explants (3.6 ± 0.4), although the difference was not significant at $P = 0.05$.

For 20 out of 33 clones used in this work, the regeneration efficiency ranged from 10.0 to 89.3 shoots per 100 explants. Ten clones had efficiency below ten shoots and two other clones did not produce any shoots at all and thus their regeneration rates were zero (Table 2 and Table 3). One haploid clone (No. 3-1n) showed two-fold higher regeneration than the second most efficient clone, producing almost 200 shoots per 100 explants. Its regeneration rate was also almost seven times higher than that of the corresponding DH clone (No. 3-DH, 30 shoots per 100 explants). The haploids regenerated with a higher efficiency than DHs ($P < 0.001$), even when the most efficient haploid (No. 3-1n) was excluded

from the comparison ($P < 0.003$). Clones originating from various ovules of the same donor plant had considerably different regeneration efficiencies.

Table 3. Efficiency of shoot regeneration from explants of haploid clones after 6-week culture (mean ± standard error).

Genotype	Number of explants	Percent of viable explants	Percent of regenerating explants	Number of developed shoots	Regeneration rate[a]
148	80	6.3 ± 3.8	1.3 ± 1.3	1	1.3 ± 1.3
149	80	6.3 ± 3.8	3.8 ± 2.6	3	3.8 ± 2.6
150	260	89.2 ± 3.3	47.7 ± 7.5	177	68.1 ± 11.7
151	180	77.2 ± 5.9	26.7 ± 7.8	52	28.9 ± 8.5
152	220	62.7 ± 7.3	8.6 ± 3.1	19	8.6 ± 3.1
153	180	88.9 ± 4.0	41.1 ± 6.9	103	57.2 ± 10.6
154	250	53.6 ± 7.8	32.4 ± 7.2	113	45.2 ± 10.8
155	260	64.2 ± 5.7	9.6 ± 2.3	26	10 ± 2.4
156	60	78.3 ± 13.3	16.7 ± 8.0	16	26.7 ± 12.6
Total/Mean	1570	65.4 ±2.9	24.5 ± 2.5	510	32.5 ± 3.6

[a]Number of developing shoots per 100 explants.

DISCUSSION

Previous reports on sugar beet performance *in vitro* predominantly concerned diploids (Gürel et al. 2003) and to a lesser extent triploids (Zhang et al. 2001) and tetraploids (Gürel et al. 2001; Yildiz et al. 2007). Such comparisons included materials of various ploidy, but each ploidy level was represented by genotypes of different origin (Detrez et al. 1989; Gürel at al. 2001; Zhang et al. 2001). In contrast, in this work, the pairs of haploids and the DHs derived from these haploids were directly compared; thus these pairs of genotypes had an identical allelic composition and differed only in their ploidy level.

Several studies in other species have reported that diploid and tetraploid genotypes are more vigorous than haploids. For example: apple, citrus, and potato haploids usually had smaller and weaker leaves, slower growth and difficulty in shoot rooting (Riddle et al. 2006; Froelicher et al. 2007; Stupar et al. 2007). Clementine haploids showed weak growth and most shoots died. However, a haploid individual with much more vigorous growth that developed into a normal plant capable of flowering was also identified (Aleza et al. 2009). Also in sugar beet triploid and tetraploid genotypes show more efficient shoot regeneration than diploid ones (Yildiz et al. 2007). However, Gürel et al. (2001) noted that tetraploid explants produced less callus than diploids and that their tissues showed a greater tendency for blackening and the accumulation of phenolic compounds. Haploids have rarely been used and their regeneration efficiency is low, but consistent with the range obtained for other diploid genotypes and similar to that of triploids (Detrez et al. 1989).

The haploid and DH shoots used in this work had a similar morphology although some variation in leaf shape and growth was noted. Leaf abnormalities were observed more frequently in haploids, often manifesting as petiole-like leaves, but the DHs exhibited similar deformations. Morphological leaf deviations were also reported previously regardless of the tissue ploidy level, but further observations of fully developed plants grown in soil indicated that such abnormalities were temporary; this phenomenon was attributed to the effect of cytokinins present in the culture media (Detrez et al. 1989). In contrast to our observations carried out during *in vitro* culture, morphological differences between haploid and DH plants adapted to *ex vitro* conditions or their progeny have been observed in many species. *Ex vitro* grown DH plants were usually 25-30% higher, had longer and wider leaf blades, and were more vigorous than haploids (Riddle et al. 2006; Stupar et al. 2007).

DOI: 10.2225/vol16-issue2-fulltext-3

Tissue blackening in sugar beet and red beet may begin from the cut edge of the explants or their wounded surface and becomes visible after a few days of culture. Such blackening extends throughout the explant during subsequent weeks (Harms et al. 1983; Yildiz et al. 2007). This phenomenon may result from the accumulation of phenolic compounds (Dan, 2008), and leads to the inhibition of tissue growth and proliferation (Gürel et al. 2001; Yildiz et al. 2007), as in our experiments. Yildiz et al. (2007) reported that the amount of phenolics in sugar beet varied depending on the composition of the culture media, plant organ and genotype. We observed that tissue blackening may also occur at intact sites within the explants as well as on the leaves of micropropagated shoots and thus it is not necessarily related to tissue wounding. The results confirmed the effect of genotype, but the association of blackening with ploidy level was not clear. Although some haploid shoots showed more severe symptoms than the DHs, the differences between the haploid and DH explants were not significant. As tissue blackening is highly adverse for morphogenesis and is genotype-dependent, screening a wide range of genotypes may result in the selection of materials of low susceptibility to blackening. Such selection would increase the percentage of viable explants and thus the overall regeneration efficiency.

Propagation through the induction of adventitious bud development can be achieved using small fragments of leaf or petiole explants. This method also allows genetic engineering of explant cells and the direct production of transgenic shoots. The regeneration efficiency may be high, but much variation is observed depending on several factors like culture conditions, explant source and genotype. Moreover, inter-plant and inter-leaf variation has been reported (Gürel et al. 2000). Grieve et al. (1997) showed that 10-53% of petiole explants developed adventitious buds with, on average, 1.0-2.4 buds per explant, enabling the production of 13-97 shoots per 100 explants depending on the genotype and benzylamine concentration used. Our results were similar although some genotypes responded with a lower efficiency or produced no shoots at all. We also identified a superior haploid genotype with a regeneration efficiency of 200 shoots per 100 explants. Sugar beet is an allogamous species that exhibits inbreeding depression and thus even plants from advanced breeding populations are partially heterozygous (Pedersen and Keimer, 1996). As a consequence, individual haploids possess different allele combinations due to meiotic segregation, even those originating from different ovules of the same plant, and thus the observed variation in regeneration ability may be strongly affected by genetic factors.

The results presented in this work show that haploid material is capable of axillary bud development as well as that of adventitious shoots on the midrib and petiole explants. More importantly, the efficiency of both processes was usually similar or even higher for haploids than for the corresponding DHs derived from these haploids. Therefore, we observed no adverse effects that could be related to haploidy. The results provide no evidence to support the opinion that haploids have lower viability and vigour. The poor performance of haploids and also DHs is usually explained by the expression of recessive deleterious alleles, but may also be due to differences in gene expression, as observed between 1x and 2x potato tissues (Stupar et al. 2007). The superior performance of some haploids over the corresponding DHs may thus reflect the effect of allele dose, as haploids possess only half the number of deleterious alleles, and/or may be the result of differential gene expression. The relatively good performance of the haploids in this work may also be the consequence of unintended preselection at the early stages of their development. The material was obtained after a process involving successful gynogenesis, the clonal propagation of haploids, and their subsequent diploidization, followed by a second round of propagation. Thus the haploid and DH shoots were obtained after several cycles of maintenance *in vitro*. During those stages several shoots were weak, did not respond to propagation or died and were not suitable for producing the haploid-DH pairs required for this study; thus only material of high vigour was used. Nevertheless, our observations indicate that sugar beet haploids can be effectively propagated *in vitro* and utilized as the starting material in, for example, genomic research or genetic transformation, potentially leading to the development of homozygous transgenics.

CONCLUDING REMARKS

In this work we compared for the first time the *in vitro* performance of sugar beet haploid and DH shoots derived from these haploids. The results indicate that haploids may exhibit similar or even superior ability for micropropagation and shoot regeneration compared to DHs. This suggests that sugar beet haploid material can be successfully used not only for the production of DHs, but also maintained *in vitro* and utilized in projects requiring haploid tissues as the source material.

DOI: 10.2225/vol16-issue2-fulltext-3

ACKNOWLEDGMENTS

The plant material used in this study was developed and kindly provided by the Kutnowska Hodowla Buraka Cukrowego (KHBC) breeding company in Straszków, Poland.

Financial support: This work was supported by the Polish Ministry of Agriculture and Rural Development (Grant no. 74/HOR hn-801-22/11).

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How to reference this article:

KLIMEK-CHODACKA, M. and BARANSKI, R. (2013). Comparison of haploid and doubled haploid sugar beet clones in their ability to micropropagate and regenerate. *Electronic Journal of Biotechnology*, vol. 16, no. 2. http://dx.doi.org/10.2225/vol16-issue2-fulltext-3

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