THE EFFECT OF GENOTYPE AND MEDIUM ON PLANT REGENERATION FROM ANDROGENIC EMBRYOS

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A B S T R A C T

The regeneration process of the plants obtained from androgenetic embryos is a very important stage in obtaining homozygotic lines from anther cultures. The efficacy of regeneration depends above all on genotype. Another important factor influencing the regeneration process is the composition of the medium. Different authors recommend various modifications of the media used to regenerate plants from androgenetic embryos. Some researchers obtain whole plants on the regeneration media, while some others carry out this process in two stages: first regenerating shoots, and then stimulating root formation.

In the present study, an effective method of plant regeneration from anther-derived embryos was used to secure sufficient plant material for creating homozygous lines. The experiments were carried out on two species: cabbage and carrots. The aim was to determine the effect of genotype and medium composition on the efficiency of the regeneration process.

The genotype was the factor which had the greatest impact on plant regeneration from androgenic embryos in both cabbage and carrots. In cabbage, the highest number of shoots regenerated from embryos was obtained with 'Kamienna Głowa'. In carrots, the highest number of plants were formed with 'Feria F_1' and 'Narbonne F_1'.

When B5 medium (Gamborg et al., 1968) with 20 mg/l sucrose without hormones and and B5 with 20mg/l sucrose and 20 mg/l kinetine were used, shoot production was more intensive in cabbage. B5 with 20mg/l sucrose and 20 mg/l kinetine was useless for regenerating carrot embryos. In carrots secondary embryogenesis took place during the regeneration of embryos on media without hormones.

Key words: head cabbage, carrot, androgenesis, homozygous lines

INTRODUCTION

The main advantages of F_1 cultivars are higher yield, better quality, and more uniformity. Traditional cultivars are being replaced on the market by new hybrid cultivars. The initial stage in creating hybrid cultivars is developing homozygous lines, which is difficult and very time-consuming with conventional breeding methods. Obtaining homozygous lines from anther culture can shorten the long inbreeding process. The first step in this kind of breeding program is obtaining androgenetic embryos by means of anther culture. The next step is regenerating plants. Difficulties with regenerating plant from androgenetic embryos have been described by several authors (Keller and Armstrong, 1977; Lichter, 1982; Pink et al., 1995; Górecka, 1998).

The aim of this work was to examine the effect of cultivars, and regeneration media on the plant regeneration process from cabbage and carrot embryos obtained in anther culture

MATERIAL AND METHODS

The experiments were carried out on two cultivars of head cabbage: 'Kamienna Głowa' and 'Sława z Enkhuizen' and on three cultivars of carrot Feria F_1', 'Narbonne F_1' and 'Splendid F_1'.

With cabbage, anther cultures after embryo formation were maintained in a growing room at 24°C under continuous light. In two or three days, the green embryos were transferred to four regeneration media. Albinotic embryos were discarded.

Regeneration media were based on either MS or B5 medium (Murashige and Skoog,1962; Gamborg et al., 1968):

MS-1: MS with 10 $g \cdot L^{-1}$ sucrose 10 $g \cdot L^{-1}$ and 0.5 $g \cdot L^{-1}$ activated charcoal;
MS-2: MS with 20 $g \cdot L^{-1}$ sucrose, 1 $mg \cdot L^{-1}$ BA and 0.001 $mg \cdot L^{-1}$ NAA;
B5-1: B5 without amino acids and hormones with 20 $g \cdot L^{-1}$ sucrose;
B5-2: B5 without amino acids with 20 $g \cdot L^{-1}$ sucrose and 20 $mg \cdot L^{-1}$ kinetin.

With carrots, anther cultures after embryo formation were maintained at in a growth chamber at 27°C under continuous light. When the embryos turned green, they were transferred to five regeneration media. The same media were used for regeneration of carrot androgenetic embryos as for cabbage but all media contained 20 $g \cdot L^{-1}$ of sucrose. In addition the medium MS without hormones (MS-3) was used. All the media were a adjusted to pH 5.8.

With cabbage, embryos were checked after three weeks for normal development, organogenesis, and callus formation. Then the explants obtained were transferred to fresh medium. Every type of growth was subsequently cultured, including normally developing embryos, shoots, and abnormal growths on the embryos. During the second passage, initials of rosettes, small rosettes and callus were subcultured only on MS-2 and B5-2. Larger rosettes were cultured on rooting medium (B5 + with 30 $g \cdot L^{-1}$ sucrose and 1 $mg \cdot L^{-1}$

IAA. If the shoots had not rooted in three weeks, the old callus and senescent leaves were cut off, and the shoots were again cultured on rooting medium.

With carrots, embryos were checked during the first passage at twelve weeks for callus aggregations, initial rosettes, initial shoots, rosettes, complete plants and secondary embryos.

RESULTS

With cabbage, some embryos grew, forming cotyledons and shoots. Others formed several shoots or callus. However, many died. Out of 194 cabbage embryos cultured on regeneration media, 31 grew and produced shoots, 73 formed callus, and 68 died. In 'Kamienna Głowa', during first passage, 17.1% of the embryos formed shoots, 40.0% formed callus, and 34.7% died. In the moderately late cultivar 'Sława z Enkhuizen', only 8.3% of the embryos developed shoots (Tab. 1).

Table 1. The influence of the cultivar on the regeneration of head cabbage embryos obtained in anther culture on MS-2 and B5-2 medium*

Cultivar	Embryos								
	placed on the regeneration medium (No.)	producing callus		producing shoots		died		non developed	
		No.	%	No.	%	No.	%	No.	%
Sława z Enkhuizen	24	5	20.8	2	8.3	9	37.5	8	33.3
Kamienna Głowa	170	68	40.0	29	17.1	59	34.7	14	8.2

Shoot formation was best on B5-2. The proportion of embryos forming callus was highest on MS-2. The proportion of embryos which formed shoots was the lowest and the proportion of embryos which died was the highest on MS-1 (Tab. 2).

Table 2. Regeneration of head cabbage embryos obtained in anther cultures on different media

Medium*	Embryos								
	placed on the regeneration medium (No.)	Producing callus		producing shoots		died		non developed	
		No.	%	No.	%	No.	%	No.	%
MS-1	36	12	33.3	4	11.1	18	50.2	2	5.5
MS-2	73	35	47.9	11	15.1	16	21.9	11	15.1
B5-1	40	12	30.0	6	15.0	17	42.5	5	12.5
B5-2	122	38	31.1	20	16.4	52	42.6	12	9.8

* MS-1 – MS (Murashige and Skoog 1962) without aminoacids and hormones + sucrose 10 $g \cdot L^{-1}$ + activated charcoal 0.5 $g \cdot L^{-1}$
MS-2 – MS without aminoacids + sucrose 20 $g \cdot L^{-1}$ + BA 1 $mg \cdot L^{-1}$ + NAA 0.001 $mg \cdot L^{-1}$
B5-1 – B5 (Gamborg et al.1968) without aminoacids and hormones + sucrose 20 $g \cdot L^{-1}$
B5-2 – B5 without aminoacids + sucrose 20 $g \cdot L^{-1}$ + kinetin 20 $mg \cdot L^{-1}$

During the second passage, the shoots, callus and fragments of developing embryos produced new shoots intensely only on B5-2 and MS-2. Larger shoots formed usually roots on the rooting medium, although there were differences in rooting between genotypes.

Table 3. The influence of the cultivar on the regeneration of carrot embryos obtained in anther culture

Cultivar	Embryos										
	placed on the regeneration medium (No.)	producing callus		producing rosettes		producing complete plants		producing secondary embryos		died	
		No.	%	No.	%	No.	%	No.	%	No.	%
Feria F_1	65	4	6.2	15	23.1	28	43.1	2	3.1	16	24.6
Narbonne F_1	29	0	0.0	4	13.8	13	44.8	1	3.4	11	37.9
Splendid F_1	32	4	12.5	0	0.0	6	18.8	1	3.1	21	65.6

With carrots, the efficiency of shoot production on regeneration medium depended on the genotype. In 'Feria F_1' and 'Narbonne F_1', over 40% of the embryos produced complete plants. This was more than twice as many as in 'Splendid F_1'. The proportion of embryos forming rosettes was 23.1% in 'Feria F_1' and 13.8% in 'Narbonne F_1'. In 'Splendid F_1', 12.5% of the embryos formed callus and 65.6% of died (Tab. 3).B5-2 was the worst medium for regenerating carrot embryos; all of the embryos died on B5-2. On MS-2, which contained BA and NAA, carrot embryos regenerated shoots which formed roots on special media containing auxins. Root formation was very slow. The efficiency of shoot production was not high on MS-2, and neither was the efficiency of root production.

Table 4. Regeneration of carrot embryos obtained in anther cultures on different media

Medium*	Embryos										
	placed on the regeneration medium (No.)	producing callus		producing rosettes		producing complete plants		producing secondary embryos		died	
		No.	%	No.	%	No.	%	No.	%	No.	%
MS-1	40	2	5.0	1	2.5	12	30.0	0	0.0	25	62.5
MS-2	20	4	20.0	5	25.0	1	5.0	4	20.0	6	30.0
MS-3	17	0	0.0	1	5.9	7	41.1	2	11.8	7	41.2
B5-1	26	1	3.8	0	0.0	24	92.3	1	3.8	0	0.0
B5-2	28	0	0	0	0.0	0	0	0	0	28	100.0

* MS-1 – MS (Murashige and Skoog,1962) without aminoacids and hormones + sucrose 20 $g \cdot L^{-1}$ + activated charcoal 0.5 $g \cdot L^{-1}$
MS-2 – MS without aminoacids + sucrose 20 $g \cdot L^{-1}$ + BA 1 $mg \cdot L^{-1}$ + NAA 0.001 $mg \cdot L^{-1}$
MS-3 – MS without aminoacids and hormones + sucrose 20 $g \cdot L^{-1}$
B5-1 – B5 (Gamborg et al.1968) without aminoacids and hormones + sucrose 20 $g \cdot L^{-1}$
B5-2 – B5 without aminoacids + sucrose 20 $g \cdot L^{-1}$ + kinetin 20 $mg \cdot L^{-1}$

On media without hormones, secondary embryogenesis took place, followed by conversion to complete plants. The formation of secondary embryos and direct regeneration into plants were very intense, especially on B5-1 (Tab. 4). In 'Feria F_1', an average of 102 plants from one embryo were obtained on B5-1.

DISCUSSION

Different researchers have used different media for regeneration plants from androgenetic embryos. Keller et al. (1975) used B5 and MS media without hormones with 2%sucrose *Brassica campestris* and *Brassica napus.* Takahata and Keller (1991) used B5 medium without hormones for *Brassica oleracea* embryo culture and obtained either direct development of embryos into plants or shoot induction after several passes. Similar results with these media were also obtained by Lelu and Bollon (1990) for *Brassica oleracea* var. *capitata* and *Brassica oleracea* var. *gemmifera*. In our experiments on media without cytokinins, MS-1 and B5-1, we obtained only a few shoots from cabbage embryos.

Nałęczyńska (1991) obtained numerous shoots from androgenetic embryos of *Brassica napus* on B5 medium supplemented with 20 g/l sucrose and 20 mg/l kinetin. In our experiments with head cabbage, the best out of the four media tested proved to be B5-2, which also contained 20 g/l sucrose and 20 mg/l kinetin. With cabbage, the highest number of shoots was obtained on B5-2. However, with carrots, all the embryos died on B5-2.

In our research on plant regeneration from androgenetic carrot embryos, we observed intensive formation of secondary embryos on media without hormones, especially in 'Feria F_1'. Induction of secondary embryogenesis and conversion of secondary embryos into plants shortens and simplifies the process of producing plants from androgenetic embryos. Andersen et al. (1990) reported that single plants formed from androgenetic carrot embryos without secondary embryogenesis on B5 without hormones and 20 $g \cdot L^{-1}$ sucrose. Tyukavin et al. (1999) placed carrot embryos obtained from anther culture on MS with 0.1 $mg \cdot L^{-1}$ kinetine and observed secondary embryogenesis. They also reported that secondary embryos appeared even on plants developing from embryos. They also obtained many plants from a single embryoid.

Lelu and Bollon (1990) stated that the predisposition for regeneration in *Brassica oleracea* var. *capitata* and *Brassica oleracea* var. *gemmifera* depended upon genotype. This supports the experiments with head cabbage (Górecka and Krzyżanowska et al., 1997), with Brussels sprouts (Krzyżanowska et al., 1996) and with carrots (Andersen et al., 1990). In the recent experiments, we also observed this tendency.

CONCLUSIONS

- In cabbage and in carrots, the efficiency of plant regeneration from androgenetic embryos depended on the genotype.
- The composition of the medium influenced the regeneration process of androgenetic cabbage and carrot embryos.
- In carrots, secondary embryogenesis took place during the regeneration of androgenetic embryos.

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WPŁYW GENOTYPU I SKŁADU POŻYWKI NA REGENERACJĘ ROŚLIN Z ZARODKÓW ANDROGENETYCZNYCH

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S T R E S Z C Z E N I E

Ważnym etapem technologii uzyskiwania linii homozygotycznych z zastosowaniem kultur pylnikowych jest regeneracja roślin z zarodków androgenetycznych. Sprawia on często duże trudności i wiele zarodków zamiera nie wytwarzając roślin. Efektywność regeneracji zależy między innymi od genotypu. Innym bardzo ważnym czynnikiem wpływającym na proces regeneracji jest skład pożywki. Różni autorzy zalecają rozmaite modyfikacje pożywek do regeneracji zarodków androgenetycznych. Niektórzy badacze otrzymują całe rośliny na pożywkach do regeneracji, inni prowadzą ten proces dwustopniowo regenerując z zarodków pędy, a następnie je ukorzeniają.

Prezentowana praca dotyczy poszukiwania efektywnych metod regeneracji roślin kapusty głowiastej i marchwi z zarodków androgenetycznych, które pozwolą na otrzymanie wystarczającej liczby roślin do wyprowadzenia linii homozygotycznych tych warzyw. Zbadano wpływ genotypu i pożywki na efektywność procesu regeneracji.

Genotyp wpływał w znaczący sposób na regenerację zarodków androgenetycznych zarówno kapusty, jak i marchwi. Z zarodków kapusty odmiany 'Kamienna Głowa' zregenerowano więcej pędów niż z zarodków odmiany 'Sława z Enkhuizen'. Znacznie więcej kompletnych roślin zregenerowano z odmian marchwi 'Feria F_1' i 'Narbonne F_1' niż z zarodków odmiany 'Splendid F_1'.Więcej pędów powstawało, gdy zarodki androgenetyczne kapusty wykładano na pożywkę regeneracyjną B5 (Gamborg i in., 1968) zawierającą 20 g/l sacharozy, bez hormonów oraz B5 zawierającą 20 g/l sacharozy i 20 mg/l kinetyny. Ta ostatnia okazała się zupełnie nieprzydatna do regeneracji roślin z zarodków marchwi. Podczas regeneracji z zarodków androgenetycznych marchwi na pożywkach bez hormonów stwierdzono wtórną embriogenezę.

Słowa kluczowe: kapusta, marchew, androgeneza, linie homozygotyczne