Editor: Katarzyna Niemirowicz-Szczytt

Title: Progress in Research on Capsicum & Eggplant

Editorial and technical work: Grzegorz Marszałkowski

English language version: verified by Ewa Jarzębska

ISBN 978-83-7244-862-0

Editor-in-Chief: Jan Kiryjow

Address: Nowoursynowska 166, 02-787 Warszawa, e-mail: wydawnictwo@sggw.pl

First edition. Printed 200 copies

Submitted for printing and printed in August 2007.

Printed by: Agencja Reklamowo-Wydawnicza A. Grzegorczyk

Recommended citation style:

M-Ch Daunay, E Jullian, A Whipkey, J Janick 2007. Eggplant and capsicum peppers: historical texts and images. In: Niemirowicz-Szczytt K. (Ed.), Progress in Research on Capsicum & Eggplant. Warsaw University of Life Sciences Press, Warsaw, Poland, pp. 000-000.

Effectiveness of androgenesis induced in anther culture of pepper (*Capsicum annuum* L.)

Koleva-Gudeva Liljana[a], *Trajkova Fidanka* [a] *and Spasenoski Mirko*[b]

[a] Institute of Southern Crops, Goce Delcev, b.b., 2 400 Strumica, R. Macedonia
[b] University Ss. Cyril and Methodious, Faculty of Natural Science and Mathematics, Institute of Biology, Arhimedova 5, P.O. 162, 1 000 Skopje, R. Macedonia
e-mail: liljanak@isc.ukim.edu.mk

Key words: haploid production, *in vitro*, incubation treatment, pepper (*Capsicum annuum* L.)

Abstract

The frequency of obtained androgenic plants depends highly on the genotype; therefore the low rate of haploid recovery limits the utility of anther culture in pepper breeding.

In the present study the effectiveness of induced androgenesis in *in vivo* anther culture of 19 pepper genotypes was investigated and established. The aim of this study was establishment of effective *in vitro* technology for study of haploid and diploid plant regenerants; induction of embryogenesis in pepper anther culture; development of the embryos into regenerants as well as successful adaptation and acclimatization of regenerants from sterile to greenhouse conditions.

The anthers were cultured according to the method developed by Dumas de Valux *et al.* (1981), heat pre-treatment was applied at 35^{o}C for 8 days in dark. The experiment showed that the effectiveness of androgenesis process depends on pepper genotype and the conditions for anther culture maintenance. The direct embryogenesis resulted in embryo formation that developed into plantlets.

After successful acclimatization of the regenerants firstly in climate conditions and after in greenhouse conditions seed material from four pepper genotypes was collected: Piran, Kurtovska kapija SR, Zlaten medal SR and Féherözön.

The collected seed material is an excellent possibility for further breeding processes, cytogenetic and other molecular level research.

Abbreviations: KIN - kinetin; 2,4-D - 2,4-dichlorophenoxyacetic acid

Introduction

Creation of haploids and spontaneous dihaploids in anther culture is a well developed and used method in plant genetics and breeding. Wang *et al.* (1973) obtained the first haploid pepper in anther culture. Haploid morphogenesis of *Capsicum* species was studied by George and Narayanaswamy (1973) and Kuo *et al.* (1973) although the production of haploid plants was very low.

The first successful reproductive method for production of pepper haploids was developed by Dumas de Valux *et al.* (1981). The research on androgenesis was intensive during the last years of the twentieth century, but the regenerants were a mixture of haploid and diploid plants (Kaparakis 1999). In order to increase the effectiveness of somatic embryogenesis and haploid production different stress treatment were used (Mityko 1993, Mityko *et al.* 1995, Mityko and Fari 1997).

If the induction of somatic embryogenesis in culture of anthers when microspores are in the stadium of first pollen division (n=x) is successful, haploid and diploid regenerants will be obtained (Koleva-Gudeva 2003).

Nowadays, androgenesis in *in vitro* conditions is an effective method for induction of haploids (Koleva-Gudeva *et al.* 2007).

Materials and methods

Nineteen pepper genotypes were used as anther-donor plants (Table 1). Anther-donor plants were grown under greenhouse conditions at the Institute of Southern Crops, Republic of Macedonia from 26th May when 4-weeks old seedlings were transplanted into polyethylene pots filled with mixture of soil, sand and perlite. The temperature in the greenhouse was regulated through lateral and roof windows. Also the greenhouse was externally sprayed with a calcium carbonate/calcium oxide (lime) suspension for heat reduction (shading). The greenhouse temperature was maintained at 20 ˚C minimum and 28 ˚C maximum. During growing season the plants were free of pests and fungal diseases. Mother plants were used during the four weeks after the first flower buds appeared. The flower buds were harvested when the corolla was of the same length as the calyx or slightly longer.

The developmental stage of the microspores was determined in microscopic slides of acetocarmine squashes. Flower buds were surface sterilized in 70 % ethanol for sev-

Table 1. List of accession code and origin of pepper genotypes used in the experiment

Nr.	Accession	Pepper genotype	Origin of production
1	MK1	Piran	Macedonia
2	MK2	Kurtovska kapija BG	Bulgaria
3	MK3	Kurtovska kapija TR	Turkey
4	MK4	Zlaten medal ŠT	Macedonia - Štip
5	MK5	Kurtovska kapija MK	Macedonia - Strumica 2002
6	MK6	Bonbona	Macedonia - Strumica
7	1	Slatko luta	Macedonia
8	3	Vezana luta	Macedonia
9	4	Sivrija	Macedonia
10	5	Feferona	Macedonia
11	7	Zlaten medal SR	Macedonia - Strumica
12	8	Kurtovska kapija SR	Macedonia - Strumica 2000
13	9	California wonder	Serbia
14	15	Fehérözön	Hungary
15	16	Rotund	Macedonia
16	1H	Pritavit F1	Hungary
17	2H	Tomato shaped sweet	Hungary
18	3H	Tura	Hungary
19	4H	Majori	Hungary

eral seconds, then in 5 % Ca (ClO) 2 + 2-3 drops Tween 20 for 10 minutes, and rinsed three times in sterile distilled water. After the removal of the filaments, anthers from three flower buds were placed in Petri dish (6 cm diameter) with the concave face down touching the culture medium.

The method of Dumas de Valux *et al.* (1981) was used for induction of androgenesis. According to the method, the anthers were cultivated on CP medium + 0,01 mg/l KIN + 0,01 mg/l 2,4-D with incubation of 8 days in darkness at 35±2 ˚C, the following 4 days the anthers were transferred to clime chamber at 25±2 ˚C with photoperiod of 12 h light/12 h dark. Afterwards, the anthers were subcultured on R_1 medium + 0,01 mg/l

KIN and placed in clime chamber at 25±2 °C with photoperiodism 12 h light/12 h dark. Young shoots emerging from the anthers were transferred onto hormone free V_3 media in order roots to be formed.

The plantlets were planted on sterile mixture of perlite : peat : sand (1:1:1) and acclimatized in clime chamber and later placed in greenhouse under cover in order cross-pollination to be barred.

Data analysis

All data on percentage of embryogenic anthers and number of embryos per 100 anthers were subject to analysis of variance (ANOVA), and the mean values were evaluated at the $p < 0.05$ level of significance using Duncan's Multiple Range Test.

Results and Discussion

Pepper is recalcitrant in cultures *in vivo* and the results in cell and tissues cultures are moderate. Anther culture is the only exception from this rule (Mityko and Fari 1997).

Not all genotypes under investigation were able to produce haploid embryos. After the induction period on CP medium for 12 days the anthers were subcultured on R_1 medium where, since the beginning the embryos showed totipotency, progression in development, growth and shoot formation.

The shoots continued the development on V_3 medium, where in absence of phytohormones young plants were formed (Figure 1a). The rooting was also on V_3 medium and well rooted shoots were transferred on sterile mixture of sand : perlite : peat in ratio 1:1:1. In this stage the plants were ready for adaptation and acclimatization in greenhouse conditions.

From 19 pepper genotypes under investigation, 12 possessed potential for formation of direct somatic embryos. The hot genotypes Feferona, Vezena luta, Sivrija and Bonbona and the sweet genotypes Rotund, Kurtovska kapija TU and Kurtovska kapija MK did not show androgenic potential, *i.e.* in anther culture they did not form haploid shoots. There are several factors affecting androgenesis in many species, such as genotypes (Mitykó *et al.* 1995, Rodeva *et al.* 2004), growth of donor plants, pretreatments of anthers (Koleva – Gudeva 2003) and composition of medium (Koleva – Gudeva and Spasenoski 2001).

Figure 1.

a. Development of the embryos into regenerants on V_3 medium.

b. Acclimatization of the regenerants in clime chamber under controlled conditions.

c. - f. Fully developed plants of different pepper genotypes created *via* androgenesis under greenhouse conditions.

According to the classification of Mityko and Fari (1997) for identification of androgenic potential according to the percentage of anthers that give embryos, pepper types are classified into:

- poor androgenic potential - less than 5 % embryogenic anthers
- fair androgenic potential - 5.1 – 15 % embryogenic anthers
- good androgenic potential - 15.1 – 30 % embryogenic anthers
- excellent androgenic potential - over than 30 % embryogenic anthers

The results of our research showed that somatic embryos are formed on CP medium with heat temperature stress (+35 ˚C) which is in concord with the findings of Dumas de Valux *et al.* (1981).

Table 2. Haploid embryo induction from anthers of different pepper genotypes

Pepper genotype	Total number of anthers	Embryogenic anthers (%)	Number of embryos per 100 anthers	Embryogenic response
Féherözön	1502	17.39 a	32.60 bc	Good
Tura	300	17.05 a	17.05 ab	Good
Pritavit F1	330	9.23 abc	9.39 abc	Fair
California wonder	151	6.67 abc	5.67 c	Fair
Zlaten medal SR	1031	6.12 abc	8.97 bc	Fair
Majori	330	5.83 abc	6.73 c	Fair
Piran	823	5.03 abc	34.05 ab	Poor
Zlaten medal ŠT	723	4.29 bc	18.57 bc	Poor
Tomato shaped sweet	360	4.17 bc	4.54 c	Poor
Kurtovska kapija BG	620	2.90 bc	50.55 a	Poor
Kurtovska kapija SR	875	2.73 bc	10.20 bc	Poor
Slatko luta	140	2.43 bc	3.33 c	Poor
Feferona	79	0.00 c	0.00 c	No
Vezena luta	83	0.00 c	0.00 c	No
Sivrija	104	0.00 c	0.00 c	No
Rotund	109	0.00 c	0.00 c	No
Kurtovska kapija TU	236	0.00 c	0.00 c	No
Kurtovska kapija MK	122	0.00 c	0.00 c	No
Bonbona	270	0.00 c	0.00 c	No

Mean within a column followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's multiple range test.

From all 19 genotypes, 12 showed ability for embryo formation (Table 2):

- 2 genotypes with good androgenic potential: Tura and Féherözön;

- 4 gentypes with fair androgenic potential: Pritavit F1, Californian wonder, Zlaten medal SR and Majori;

Table 3. Seed material collected from four pepper genotypes obtained in *in vitro* anther culture

Pepper genotype	Number of plants	Number of seeds per fruit	Total number of seeds
Kurtovska kapija SR	9	31.33	282
Zlaten medal SR	4	72.50	290
Piran	8	26.87	215
Féherözön	11	38.54	424

- 6 genotypes with poor androgenic potential: Piran, Zlaten medal ŠT, Tomato shaped sweet, Kurtovska kapija BG, Kurtovska kapija SR and Slatko luta;

- 7 genotypes do not possess androgenic potential: Feferona, Vezena Luta, Sivrija, Rotund, Kurtovska kapija TU, Kurtovska kapija MK and Bonbona.

Seed material was collected from four genotypes: Kurtovska kapija SR, Zlaten medal SR, Piran and Féherözön (Table 3, Figure 1 c-f). The collected seed material is a good base for further cytogenetic and molecular research and involvement in the process of pepper breeding in order to create better varieties.

Acknowledgment

The authors are indebted to Dr. Lajos Tompos from Produkt, Kutato, Fejleszto, Termelo Kft., Hungary for providing seeds of varieties Pritavit F1, Tura and Majori and genotype Tomato shaped sweet and to Dr. Judit Mitykó from Agricultural Biotechnology Centre, Gödölő Hungary, for providing seeds of Hungarian pepper cultivar Féherözön.

References

Dumas de Valux R., Chambonnet D., Pochard E. 1981. *In vitro* culture of pepper (*Capsicum annuum* L.) Anthers: high rate plant production from different genotypes by + 35 °C treatments. Agronomie 1(10): 859-864.

George L., Narayanaswamy S. 1973. Haploid capsicum through experimental androgenesis. Protoplasma 78: 467–470.

Kaparakis G. 1999. *In vitro* culture of pepper (*Capsicum annuum* L.). PhD theses - Kaparakis Georgis, Aristotle Univ. Hellas, submitted Univ. Nottingham, UK.

Koleva-Gudeva L., Spasenoski M., 2001. The effect of some citokinines on pepper organogenesis (*Capsicum annuum* L. cv. Kurtovska kapija and Zlaten medal) cultured *in vitro*. Yearbook of the Institute of Southern Crops – Strumica, 1, 31-35.

Koleva-Gudeva L. 2003. Anther culture in pepper (*Capsicum annuum* L.), Yearbook of Institute of Southern Crops, Year 3: 95-102.

Koleva-Gudeva L., Spasenoski M., Trajkova F. 2007. Somatic embryogenesis in pepper anther culture: The effect of incubation treatments and different media. Scientia Horticulturae 111:114-119.

Kuo J. S., Wang Z. Z., Chien N. F., Ku S. J., Kung M. L., Hsu H. C. 1973. Investigation of the anther culture in vitro of *Nicotiana* and *Capsicum annuum* L. Acta Bot. Sin. 15 (1), 43–47.

Mityko J. 1993. Obtention of Anther derived homozygous plants in pepper (*Capsicum annuum* L.). OMFB, Hungary No. 93-97-44-0468.

Mityko J., Andrasfalvy G., Csillery G., Fary M. 1995. Anther culture response in different genotypes and F_1 hybrids of pepper (*Capsicum annuum* L.). Plant Breeding 114, 78-80.

Mityko J., Fari M. 1997. Problems and results of doubled haploid plant production in pepper (*Capsicum annuum* L.*)* via anther and microspore culture. Hort. Biotech and breeding, ISHS 1997, Acta Hort. 447: 281-287.

Rodeva V.N., Irikova T.P., Todorova V.J. 2004. Anther culture of pepper (*Capsicum annuum* L.): Comparative study of effects of the genotype. Biotechnol. & Bitechnol. Eq. 18/3, 34-38.

Wang J. J., Sun C. S., Wang C. C., Chein N. F. 1973. The induction of pollen plantlets of *Triticale* and *Capsicum annuum* from anther culture. Sci. Sinica XVI (1), 147–151.