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The effect of silver nitrate on *in vitro* embryogenesis in pepper (*Capsicum annuum* L.) anther culture

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ABSTRACT. Anthers from different Bulgarian pepper lines, cultivars and F1 hybrids were cultivated *in vitro* on C, Cm and MS nutrient media with and without silver nitrate under controlled conditions. Different explants' responses to the investigated media were expressed: callusogenesis, indirect organogenesis and direct embryogenesis with and without regenerants development. High embryo formation on C and Cm media without silver nitrate, and on MS medium supplemented with $AgNO_3$ occurred indicating the greater importance of basal medium than the presence of silver nitrate in the tested concentration.

Key words: anther culture, basal media, callusogenesis, direct embryogenesis, indirect organogenesis, pepper, silver nitrate.

Abbreviations: MS — Murashige and Skoog nutrient medium, $AgNO_3$ — silver nitrate.

Introduction

Pepper breeding by traditional methods is difficult because of cross-fertilization proceeding under certain conditions. To obtain doubled haploid lines of microspore origin through *in vitro* anther culture seems to be very perspective for preserving genetic stability of initial breeding material.

Wang et al. (1973), Kuo et al. (1973) and George and Narayanaswamy (1973) were the first in successful plant regeneration via pepper anther culture.

The effect of different supplements incorporated in the media was investigated: activated charcoal (Vagera, 1984; Vagera and Havranek, 1985; Morrison et al., 1986;

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Pandeva and Zagorska, 1986; Pandeva et al. 1990; Luz et al., 1996, 1997; Boyachi, 2001; Comlekcioglu et al., 2001; Ellialtioglu et al., 2001), carrot extract (Vagera, 1984; Pandeva and Zagorska, 1986; Zagorska and Pandeva, 1986; Pandeva et al. 1990; Ellialtioglu et al., 2001), coco-nut milk (Wang et al., 1981), $AgNO_3$ (Luz et al., 1996, 1997, 2000; Comlekcioglu et al., 2001, Buyukalaca et al., 2004).

The aim of this study was to investigate the effect of added to the nutrient media $AgNO_3$ on the response of cultivated *in vitro* anthers from Bulgarian pepper lines, cultivars and hybrids.

Materials and Methods

The donor pepper plants 4 lines; No No 145, 146, 1312 and 1924; 5 cultivars; Zlaten medal, Hebur, Stryama, Albena and Kourtovska kapija; and 2 F1 hybrids; 1647×1962 and 1647×1969) created in Maritsa Vegetable Crops Research Institute — Plovdiv, were grown in the greenhouse. The anthers were detached from flower buds of equal sepals and petals length, i.e. at cell mononuclear developmental phase (Sibi et al., 1979).

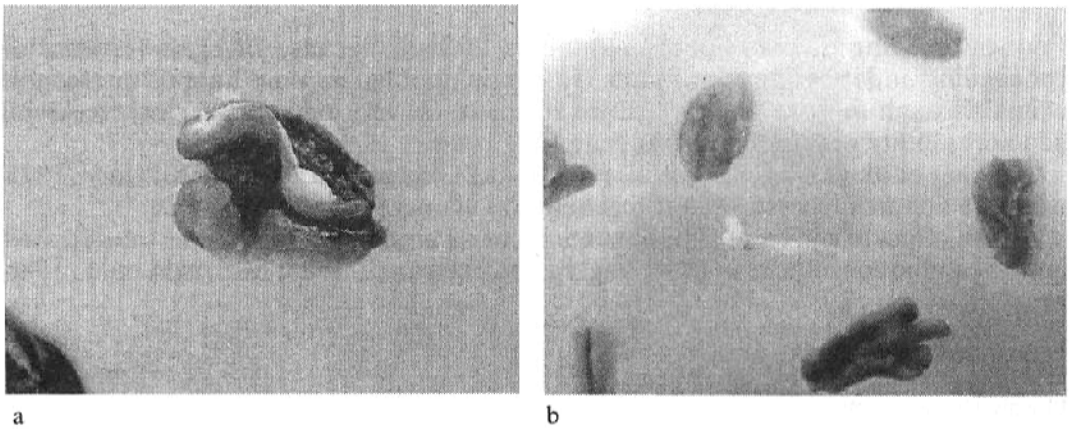
After being sterilized in 5% NaOCl for 10 min and rinsed in sterile distilled water, three times for 15 min the anthers were cultivated on 3 basal media — C (Dumas de Vaulx et al., 1981), Cm (Sibi et al., 1979) and MS (Murashige and Skoog, 1962) with and without $AgNO_3$ — 5 mg/l. In the first 8 days the explants were cultivated in the dark at 36 °C and then transfered to 25 °C and 16:8 h photoperiod (Dumas de Vaulx et al., 1981).

The results were calculated in percentages to total number of inoculated explants as follow: total responding anthers, explants responding through callusogenesis or direct embryogenesis and with indirect organogenesis for each line, cultivar and hybrid.

Results and Discussion

The following types of explants' response in anther culture were established: callusogenesis without regenerative structure formation, indirect organogenesis without development of formed structures and direct embryogenesis with development of some embryos to regenerants (Fig. 1a, b; 2 a, b). Most of the direct embryo-structures survived to heart-shaped phase.

The data presented in Table 1 show better embryogenic response of the anthers cultivated on C medium without $AgNO_3$. On this medium 6 of the studied genotypes



a b

Fig. 1. Direct embryos in anther culture of pepper (*Capsicum annuum* L.):
a — globular phase; b — cotyledone phase

Table 1
Anther response on C medium without and with $AgNO_3$

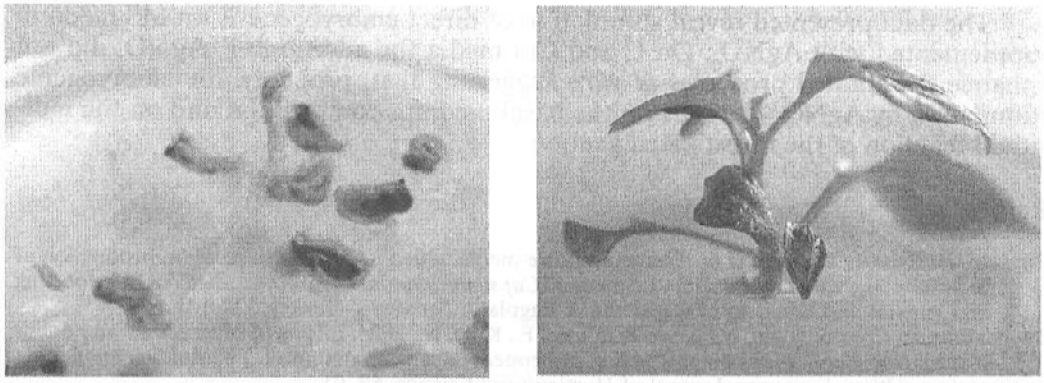
Medium	C							C + $AgNO_3$						
genotype	planted	responding (%)				regenerants (%)		planted	responding (%)				regenerants (%)	
	anthers	total	direct embryos	callus	indirect org.	to anthers	to embryos	anthers	total	direct embryos	callus	indirect org.	to anthers	to embryos
N 145	290	12,07	0	12,07	0	0	0	435	9,2	3,45	5,75	0	0	0
N 146	245	10,2	0	10,2	0	0	0	220	38,64	0	38,64	0	0	0
N 1312	390	1,28	1,28	0	0	0	0	350	5,71	2,86	2,86	0	0	0
N 1924	255	27,45	0	27,45	0	0	0	250	0	0	0	0	0	0
Zl. Medal	405	44,45	4,94	38,27	1,23	0	0	75	0	0	0	0	0	0
Hebur	260	21,15	7,69	13,46	0	0	0	235	21,28	0	21,28	0	0	0
Stryama	230	26,09	10,87	15,22	0	2,17	20	245	0	0	0	0	0	0
Albena	365	36,99	10,96	26,03	0	0	0	-	-	-	-	-	-	-
K. kapiya	205	9,76	0	9,76	0	0	0	215	4,65	0	4,65	0	0	0
1647×1969	740	2,7	0,68	2,03	0	0	0	500	2	0	2	0	0	0
1647×1962	560	15,18	0	15,18	0	0	0	90	0	0	0	0	0	0

Table 2
Anther response on Cm medium without and with $AgNO_3$

Medium	Cm							Cm + $AgNO_3$						
genotype	planted	responding (%)				regenerants (%)		planted	responding (%)				regenerants (%)	
	anthers	total	direct embryos	callus	indirect org.	to anthers	to embryos	anthers	total	direct embryos	callus	indirect org.	to anthers	to embryos
N 145	230	47,83	2,17	45,65	0	0	0	1080	18,06	0,93	16,67	0,46	0	0
N 146	120	20,84	0	20,84	0	0	0	90	44,45	5,56	38,89	0	0	0
N 1312	275	14,55	1,82	12,73	0	0	0	150	6,67	0	6,67	0	0	0
N 1924	290	5,17	0	5,17	0	0	0	-	-	-	-	-	-	-
Zl. Medal	145	3,45	3,45	0	0	0	0	60	8,34	8,34	0	0	0	0
Hebur	290	10,35	3,45	6,9	0	0	0	-	-	-	-	-	-	-
Stryama	90	22,23	5,56	16,67	0	0	0	115	73,91	0	73,91	0	0	0
Albena	280	33,93	7,14	26,79	0	0	0	80	0	0	0	0	0	0
K. kapiya	160	0	0	0	0	0	0	-	-	-	-	-	-	-
1647×1969	565	26,55	0,89	25,66	0	0	0	395	44,3	1,27	43,04	0	0	0
1647×1962	415	19,28	4,82	14,46	0	0	0	195	0	0	0	0	0	0

Table 3
Anther response on MS medium without and with $AgNO_3$

Medium	MS							MS + $AgNO_3$						
genotype	planted	responding (%)				regenerants (%)		planted	responding (%)				regenerants (%)	
	anthers	total	direct embryos	callus	indirect org.	to anthers	to embryos	anthers	total	direct embryos	callus	indirect org.	to anthers	to embryos
N 145	260	9,62	0	9,62	0	0	0	80	18,75	0	18,75	0	0	0
N 146	105	38,1	0	38,1	0	0	0	115	13,04	8,7	4,35	0	0	0
N 1312	270	5,56	0	5,56	0	0	0	110	9,09	9,09	0	0	0	0
N 1924	135	3,7	0	3,7	0	0	0	-	-	-	-	-	-	-
Zl. Medal	285	12,28	0	12,28	0	0	0	-	-	-	-	-	-	-
Hebur	-	-	-	-	-	-	-	120	8,34	0	8,34	0	0	0
Stryama	90	22,23	0	22,23	0	0	0	-	-	-	-	-	-	-
Albena	250	6,67	0	6,67	0	0	0	125	32	0	32	0	0	0
K. kapiya	85	17,65	0	17,65	0	0	0	-	-	-	-	-	-	-
1647×1969	470	17,02	0	17,02	0	0	0	440	4,55	4,55	0	0	0	0
1647×1962	605	5,79	0,83	4,96	0	0	0	320	3,13	3,13	0	0	0	0



a b

Fig. 2. Regenerants via direct embryos in anther culture of pepper (*Capsicum annuum* L.):
a — plantlet; b — regenerant

responded through direct embryogenesis and most of them (except line 1312) — with callusogenesis.

In the same medium with $AgNO_3$ direct embryogenesis was observed only in lines 145 and 1312.

The regenerative processes were well expressed on C medium without $AgNO_3$ only in Zlaten medal, responding through indirect organogenesis (1,23% of all cultivated explants) and Stryama, responding through development of 20% of direct embryos to plantlets (2,17% of all inoculated explants). In the same medium with $AgNO_3$ the regenerative processes were suppressed.

The data presented in Table 2 show higher number of genotypes (8) responding through direct embryogenesis on Cm medium without $AgNO_3$ compared to the same medium with $AgNO_3$ (4 genotypes). No regenerative processes occurred on Cm media with and without $AgNO_3$.

The data presented in Table 3 show differences compared to these presented in Table 1 and Table 2. The embryogenic answer of the cultured anthers was better expressed on MS basal medium with $AgNO_3$ compared to the medium without $AgNO_3$.

On the MS medium supplemented with $AgNO_3$ the anthers reacted with comparatively higher percentage of direct embryos (from 3,13 to 9,09%) than those grown on the control medium (0,8% only in F1 hybrid 1647×1962).

The answer of anthers from different pepper lines, varieties and hybrids on C- and Cm media with and without $AgNO_3$ was unequal (Tables 1 and 2). Some of the genotypes showed higher embryogenic response on C medium with $AgNO_3$ and lower on Cm medium with $AgNO_3$ (lines 145 and 1312). In other genotypes the direct embryogenesis was better expressed on Cm medium with $AgNO_3$ while on C medium with $AgNO_3$ such response was not observed (line 146, variety Zlaten medal, F1 hybrid 1647×1969).

The results suggest that the basal medium is of great importance for the explants' reaction.

Investigating the effect of $AgNO_3$ in MS, Cm and C media Luz et al. (1996, 1997, 2000) established higher embryogenic induction in pepper anther culture after adding 5 mg/l $AgNO_3$ to the media. Comlekcioglu et al. (2001) studied the effect of silver nitrate on embryo induction in pepper anther culture on MS medium and obtained embryos only in presence of $AgNO_3$ (10 mg/l). Buyukalaca et al. (2004) established highest embryoformation in medium containing 15 mg/l $AgNO_3$.

The data presented reveal stimulation of direct embryogenesis on MS medium supplemented with $AgNO_3$. On C and Cm media the addition of $AgNO_3$ did not enhance the studied processes *in vitro* suggesting that, probably, the embryogenic stimulation by $AgNO_3$ depends on the basal medium composition and on the individual reaction of the investigated genotype.

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