ANTHER CULTURE OF PEPPER (*CAPSICUM ANNUUM* L.) : COMPARATIVE STUDY ON EFFECT OF THE GENOTYPE

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ABSTRACT

There were investigated and compared the in vitro response in anther culture of 16 Bulgarian pepper (Capsicum annuum L.) genotypes: six lines, six varieties and four hybrids developed in Maritsa Vegetable Crops Research Institute, Plovdiv. The anthers reacted with induction of direct embryogenesis or callusogenesis. Only in two of the studied genotypes (line N145 and variety Zlaten medal) the anthers have shown indirect organogenesis. There were established genotypes with good embryogenic ability – varieties Zlaten medal, Albena, Stryama, Hebur, Kourtovska kapiya, F1 hybrids N1647X N668, N1647 X N1962 and lines N1312, N145 and N668. The embryoids obtained from anthers of line N145 and varieties Stryama and Kourtovska kapiya developed to regenerants with formed roots and cotyledons. The results from this study proved that for the first time is achieved a successful induction of embryogenesis in vitro and obtaining plant-regenerants, with microspore origin, of Bulgarian pepper genotypes.

Introduction

The experimental obtaining of haploid plants by androgenetic or gynogenetic development *in vitro* has a great importance for receiving of completely homozygous lines and for shortening the creation process of new varieties in agricultural crops.

The first data for induction of haploidy through pollen embryogenesis in anther culture of pepper *in vitro* were published in 1973 by Wang *et al*. (17) following the research made by George & Narayanaswamy (4) and Kuo *et al*. (6).

The provoking of androgenetic development in cultured anthers depends on a number of factors among which of particular importance is the genotype of the donor plants (3, 8, 10, 11, 12, 14).

Our aim in the present study was to investigate the genotypic differences in reaction of *in vitro* cultured anthers of Bulgarian pepper lines, varieties and hybrids.

Materials and Methods

During the 2001-2003 period donor plants from six lines (No.No 145, 146, 603, 668, 1312 and 1924), six varieties (Zlaten medal, Hebur, Stryama, Albena, Kourtovska kapiya and Maritsa) and four hybrids (F1 No 1647 x No 668, F1 No 1647 x No 1969, F1 No 1647 x No 1962 and F3 No 1647 x No 1962) developed in the Maritsa Vegetable Crops Research Institute, Plovdiv, were grown in greenhouse conditions and used for 30-40 days after formation of the first flower buds (5). Flower buds with pollen in late uninucleate stage by Sibi *et al*. (15), which was identified microscopically, were surface sterilized in 5 % solution of NaOCl, after that washed out threefold in sterile distilled water. The isolated anthers were incubated on agar induction medium as well as in conditions of light and temperature regime according to Dumas de Vaulx *et al*. (2).

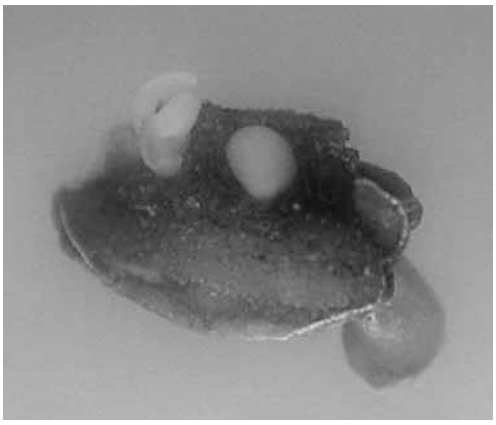

Fig. 1. Direct embryoids in anther culture of pepper (*Capsicum annuum L.*).

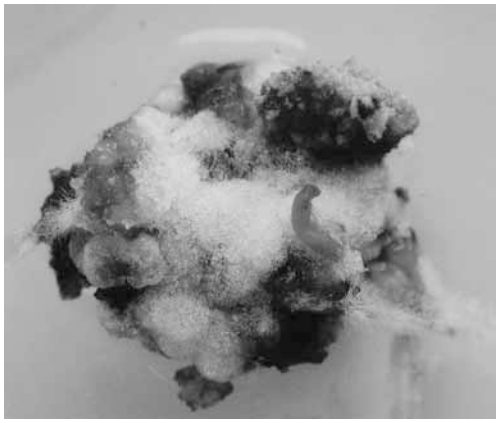

Fig. 2. Indirect organogenesis in anther culture of pepper (*Capsicum annuum L.*).

In the course of the experiments the number of anthers producing callus and embryoids were recorded. The number of embryoids developed to microplants with cotyledons and roots were marked also.

The frequency of both type of reaction and the frequency of reacted anthers were calculated in percentage to the *in vitro* set initial explants. The frequency of the regenerated microplants were presented in percentage both to the total number of anthers and to the number of obtained direct embryoids from the respective genotype.

Results and Discussion

In conditions of the carried experiment the explants from all genotypes reacted with induction of direct embryogenesis (**Fig. 1**) or induction of callusogenesis without development of regeneration structures excluding line No145 and variety Zlaten medal. The regeneration process in these two genotypes probably has a random character since only 0,14 % and 0,27 % respectively of the anthers have shown indirect organogenesis (**Table**, **Fig. 2**). It is very probably in the processes of callusogenesis cells of diploid tissues have been involved. Three of the studied genotypes, one from the group of the lines (No145) and two from the group of the varieties (Stryama and Kourtovska kapiya) respectively, have developed microplants with well formed cotyledons and roots (**Fig. 3**) initiated from direct embryoids.

The data presented by groups of genotypes (Table) show that totally reacted anthers in the lines, varieties and hybrids are with very close values. The highest percentage for this index was recorded in the group of the varieties (12,69 %). In this group is also the highest frequency of direct embryogenesis (2,29 %) compared to genotypes from the other two studied groups. In the lines and hybrids the formed direct embryoids are with very close values (1,38 % and 1,39 %, respectively). There are not observed significant differences in the percentage of anthers with callusogenesis in the three studied groups of genotypes. Indirect organogenesis with equal frequency (0,04 %), was established in the lines and varieties. In these two groups of genotypes the direct embryoids developed to microplants and the process was with higher frequency in the varieties (9,09%).

In comparison of the reaction of anthers from the six studied lines with the highest percentage reacted explants are No.145 (18.16 %) and No.146 (17.23 %). They

TABLE

Frequency of callusogenesis, embryoformation and regeneration in anthers from different pepper genotypes *in vitro*

Genotype	Total number of anthers	Reacted anthers (%)	Reacted with embryogenesis (%)	Reacted with callusogenesis (%)	Reacted with indirect organogenesis (%)	Regenerants to the total number of anthers (%)	Regenerants to the direct embryoids (%)
Lines:							
№ 145	3 580	18.16	1.54	16.48	0.14	0.14	9.10
№ 146	2 235	17.23	0.67	16.55	0.00	0.00	0.00
№ 603	720	2.08	1.39	0.69	0.00	0.00	0.00
№ 668	1 375	8.00	1.45	6.55	0.00	0.00	0.00
№ 1312	2 390	6.49	2.51	3.97	0.00	0.00	0.00
№ 1924	1 335	6.74	0.00	6.74	0.00	0.00	0.00
Total	11 635	12.08	1.38	10.66	0.04	0.04	3.13
Varieties:							
Zl. medal	1 875	20.54	4.00	16.27	0.27	0.00	0.00
Hebur	2 730	8.79	1.47	7.33	0.00	0.00	0.00
Stryama	2 120	13.44	2.36	11.08	0.00	0.47	20.00
Albena	2 825	17.35	2.83	14.51	0.00	0.00	0.00
K.kapiya	1 765	3.97	1.42	2.55	0.00	0.85	60.00
Maritsa	700	7.86	0.71	7.14	0.00	0.00	0.00
Total	12 015	12.69	2.29	10.36	0.04	0.21	9.09
Hybrids:							
F1 1647 x 668	995	11.56	2.51	9.05	0.00	0.00	0.00
F1 1647 x 1969	4 960	13.71	0.91	12.80	0.00	0.00	0.00
F1 1647 x 1962	3 230	12.07	1.86	10.22	0.00	0.00	0.00
F3 1647 x 1962	540	0.93	0.93	0.00	0.00	0.00	0.00
Total	9 725	12.24	1.39	10.85	0.00	0.00	0.00

Fig. 3. Microplant-regenerant via anther culture of pepper (*Capsicum annuum L.*).

showed also the highest frequency of callusogenesis (16.48 % and 16. 55 %, respectively) not only in the group of lines but also in comparison with all studied genotypes. We observed the lowest percentage *in vitro* reacted anthers in line No.603 (2.8 %) and the lowest frequency of callusogenesis (0.69 %). This is the only line in which was observed direct embryogenesis, stronger expressed in comparison with callus formation. The highest frequency of direct embryogenesis was recorded in line No.1312 (2.51 %) followed by lines No.145 (1.54 %), No.668 (1.45 %) and No.603 (1.39 %). In line No.1924 there was not observed this type of reaction, but only callus formation. Indirect organogenesis showed only line No.145, but the formed structures did not developed to re-

generants. Only in this genotype from the lines the direct embryoids developed to microplants with formed cotyledons and roots. In this line probably exist genetic potential for good regeneration embryogenic ability, which could be effectively used after optimization of anther cultivation conditions.

In comparison of anther reaction from the six studied varieties the highest percentage reacted anthers showed the variety Zlaten medal (20.54 %), followed by the variety Albena (17.35 %). In these two genotypes we observed also the highest frequency of callusogenesis (16.27 % and 14.51 %, respectively). The lowest percentage of reacted anthers (3.97 %) and the lowest frequency of callus formation (2.55 %) in comparison with the other genotypes of this group was registered in the variety Kourtovska kapiya. The direct embryogenesis with the highest frequency was recorded in variety Zlaten medal (4.0 %), followed by the varieties Albena (2.83 %), Stryama (2.36 %), Hebur (1.47 %) and Kourtovska kapiya (1.42 %) and the lowest one in variety Maritsa (0.71 %). A direct organogenesis was watched only in Zlaten medal where the formed structures stopped to develop at different phases. Development of direct embryoids to microplants in Kourtovska kapiya and Stryama was observed with higher frequency in Kourtovska kapiya (Table).

In the hybrids the highest percentage *in vitro* reacted anthers were recorded in F1 No.1647 x No.1969 (13.71 %) and also the highest percentage of anthers reacted with callus formation (12.80 %). In this genotype the direct embryogenesis is lowest expressed (0.91 %). The highest frequency of direct embryogenesis was recorded in hybrid F1 No.1647 x No.668 (2.51 %), followed by hybrid F1 No.1647 x No.1962 (1.86 %), and the lowest one in F1 No 1647 x No.1969 (0.91 %). In hybrid F3 No.1647 x No.1962 we did not observe formation of callus tissue. All *in vitro* reacted anthers from this genotype formed direct embryoids.

In comparison of the reaction of the studied pepper lines, varieties and hybrids the highest frequency of direct embryogenesis showed the variety Zlaten medal (4.0 %), followed by the variety Albena (2.83 %), line No. 1312 (2.51 %) and hybrid F1 No.1647 x No.668 (2.51 %).

Comparatively good embryogenic response was registered in the following studied genotypes: variety Stryama (2.36 %), hybrid F1 No.1647 x No.1962 (1.86 %), line No.145 (1.54 %), variety Hebur (1.47 %), line No.668 (1.45 %) and variety Kourtovska kapiya (1.42 %). From the formed direct embryoids in these genotypes rooted microplants were developed only in varieties Kourtovska kapiya and Stryama and in line No.145.

The results from the present study demonstrate considerable genotypic differences in *in vitro* response of anthers from the studied Bulgarian pepper lines, varieties and hybrids.

Genotypic differences in the answer of *in vitro* cultured anthers of Bulgarian pepper genotypes are reported by Pundeva *et al*. (13), Simeonova *et al*. (16). Differences are established also in study of foreign genotypes – Emerald Giant, Yolo Wonder, Calwonder (12), cv. Feherozon, cv. California Wonder, cv. Greygo, cv. Serrano, LP – 8, LP – 148 (11), Sanliurfa, Kahramanmaras (1). The effect of genotype on *in vitro* response in anther cultures is published also for other crops: barley, wheat (7, 9).

Taking into account the purpose of the *in vitro* cultivation of anthers –developing of haploid regenerants originating from microspores, "preferred" type of reaction is direct embryogenesis because callus could be formed also from somatic tissue. The results received in the present study are very hopeful since in ones of the best Bulgarian pepper varieties – Kourtovska kapiya, Zlaten medal, Stryama and Albena

for the first time is achieved a success in induction of embryogenesis *in vitro* and in some of them are obtained also plant-regenerants. The genotypes which showed such reaction can be included in breeding programs aiming optimization of *in vitro* cultivation conditions for an increase of the percentage of regenerated haploid plants and receiving of pure lines.

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