Haploid embryogenesis in capsaicinoid forms of soft-flesh *Capsicum* spp.

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Summary

The investigated material involved three pungent soft-flesh forms selected from a *Capsicum frutescens* L. x *C. annuum* L. hybrid. Capsaicinoid contents, determined by the HPLC technique in the fruit pericarp, amounted to 100 $mg \cdot kg^{-1}$. The anthers were cultured *in vitro* employing the method described by Chambonnet (1988) for *C. annuum* L. with modification involving an increase in kinetin content in R_1 medium to 0.2 $mg \cdot dm^{-3}$. The efficacy of embryogenesis ranged between 0.25% and 4.12%, related to the ratio of the number of obtained plants to the number of cultured anthers, depending on genotype and the employed medium. Conversion of the embryos into plants and the general efficacy of androgenesis were highest in one line. In all of them, cytometrically determined DNA content in cells of leaves in the obtained regenerates amounted 1C.

Key words: *DNA content, genotype response, haploid, kinetin*

INTRODUCTION

Interspecific hybrids within the *Capsicum* genus may provide the source of interesting genetic variability. The potential usefulness of *C. frutescens* L. and *C. annuum* L. hybrids reflects specific traits of the two species, i.e. morphological and technological variability in the former and high productivity of the latter. *C. frutescens* L. typically manifests soft-flesh forms of mature fruits and a variable content of capsaicinoids. Results of several studies presented in reviews [1, 2] unequivocally indicate chemopreventive, chemoprotective and anti-carcinogenic effects of the mentioned compounds.

The soft-flesh genotypes resulting from interspecific hybridization and, first of all, the genetically stable lines developed from them, provide a valuable initial material in the creation of innovative culivars. The breeding aims to obtain highly efficient forms useful in pharmaceutical and food industries as a raw material for the production of nutraceutics [3].

Among the techniques assuring rapid genetic stabilization of hybrid material, the induced androgenesis followed by diploidization of haploid plants deserves particular attention. The technique has been successfully employed in several plant species, regarded to be nutraceutical ones [4]. For *C. annuum* L., efficient procedures have been developed to obtain androgenic embryos and plants. However, the infrequent publications related to *C. frutescens* L. and to hybrids between the abovementioned species do not unequivocally precise the conditions for running the cultures or the suitability of individual genotypes [5].

The present study aimed to evaluate the usefulness of three capsaicinoid, soft-flesh lines, selected from a *C. frutescens* L. x *C. annuum* L. hybrid, for obtaining haploids in *in vitro* culture of anthers.

MATERIAL AND METHODS

Three lines selected from a *C. frutescens* L. x *C. annuum* L. hybrid were used as the investigated material. They were characterized by soft flesh of mature fruits and by presence of alleles conditioning synthesis of capsaicinoids. Capsaicinoid content was estimated by the HPLC technique, described by Collins et al. [6], using Perkin Elmer Series 200 chromatograph equipped with detector set by excitation at 280 nm. The concentration of capsaicin and dihydrocapsaicin in the fruit pericarp was very similar in each of the lines and did not exceed the level of 100 $mg \cdot kg^{-1}$. As a rule it was observed twice higher content of capsaicin than of dihydrocapsaicin. The fruit characteristics is shown in table 1.

Table 1.

Characteristics of soft-flesh fruit of *Capsicum* spp. lines

feature	line		
	34/2/24	34/2/33	34/2/33/2
weight [g]	28	17	18
length [mm]	42	35	63
width [mm]	45	35	28
seed weight [g]	2.55	1.84	1.97
shape coefficient	0.93	1.00	2.25
dry matter content [%]	15.0	13.8	14.5
capsaicin content in pericarp [$mg \cdot kg^{-1}$]	67	57	65
dihydrocapsaicin content in pericarp [$mg \cdot kg^{-1}$]	27	38	29

The plants being the source of explants were cultured in a foil tent. Flower buds were collected at the stage when sepals of the calyx showed a length similar to that of crown petals, taking this level of morphological traits as an information on advancement of microspore development. At the time they reached the late uninucleate phase.

In the studies, the protocol worked out for *C. annuum* L. by Chambonnet [7] was used. The procedure was supplemented by an additional element in the form of an augmented level of kinetin (KIN) in R_1 medium. The anthers were placed in Petri dishes with Cp medium and were incubated in an air-conditioned chamber at +35°C, in the dark, for 8 days. For subsequent 4 days the culture was conducted in R_1 medium, in variants containing 0.1 or 0.2 $mg \cdot dm^{-3}$ kinetin, at +25°C and 12/12 photoperiod. The experiments were performed in three replications with 10 Petri dishes in each of the genotype and kinetin treatment with number of anthers from two flower buds. The obtained plants were cultivated in V3 medium, with no growth regulators (+25°C, 12/12 h). Ploidy of the obtained plants was established by analysis of DNA content in leaf cell nuclei. For this purpose, a Partec CCC flow cytometer, equipped with Mercury Lamp HBO100, was used. Numbers of cell nuclei which contained 1C and 2C DNA were estimated. Diploid plants of *C. annuum* L. variety were used as a standard. The analysis followed the procedure described by Galbraith [8]. The data of androgenesis effectiveness is presented as the percentage of plantlet in the number of cultured anthers.

RESULTS AND DISCUSSION

The investigated material involved the lines selected from a *C. frutescens* L. x *C. annuum* L. interspecific hybrid. The genotypes differed in weight and size of the fruits. The high weight of the seeds represented an interesting trait: they accounted for 10% of the fruit weight. This information indicated a high fertility of the investigated lines. In the capsaicinoid-less, standard, i.e. hard-flesh cultivars of *C. annuum* L., the weight of seeds did not exceed 3% of the fruit weight [9]. Thus, the evaluated lines could provide initial material for production of F_1 hybrids.

The androgenic response of the studied genotypes proved to be variable (tab. 2). In the standard R_1 medium, i.e. at the kinetin level of 0.1 $mg \cdot dm^{-3}$, seven plants were obtained from 15 embryos of the 34/2/33/2 line. Thus, less than half of the embryos underwent conversion. The effectiveness of androgenesis, calculated as the ratio of developed plants to the number of anthers displayed, was relatively low. Moreover, an noticeable effect of genotype on the efficacy of haploid embryogenesis was noted. The observation corroborates results of the most recent studies on sweet and pungent forms of *C. annuum* L. [10]. The studies were conducted using an inductive medium identical to that used in our experiments and the same conditions of the culture . It seems important to note that in quoted experiments 4 out of 9 varieties of studied genotypes yielded no androgenic response, including

both of the capsaicinoid forms. The authors presented the conclusion that the pungent cultivars of *Capsicum* genus are poor or non-responsive genotypes compared to sweet and bell cultivars. Against such a point of view, the 34/2/33/2 line can be regarded as representing a good source of androgenic haploids.

Table 2.

Androgenic response of *Capsicum* spp. pungent, soft-flesh lines in anthers *in vitro* culture

genotype and kinetin treatment	number			embryogenesis effectiveness (%)
	anther	embryo	plantlet	
R_1 0.1 mg KIN				
34/2/24	340	3	1	0.29
34/2/33	400	1	1	0.25
34/2/33/2	310	15	7	2.25
R_1 0.2 mg KIN				
34/2/33	350	3	2	0.57
34/2/33	330	3	2	0.30
34/2/33/2	340	19	14	4.12

Results of others studies [11-13], both on somatic and genetic embryogenesis, indicate that conversion embryos into plants represents the crucial point in the process. Only some of them develops into fully matured, normal plants. Therefore, from a practical point of view the information on the efficacy in the form of the relationship between the number of developed plants and the number of anthers seem more interesting. This manner of androgenic response presentation has been employed in the present study. In the second part of the study the androgenis response was examined at the altered content of kinetin in R_1 medium. A clearly higher conversion level was noted for the 34/2/33/2 line, resulting in an almost 100% increase in the effectiveness of androgenesis compared to cultures with the lower kinetin level. The suggestion which might be presented on this basis can be formulated as follows: a potential increase in the efficacy of androgenesis due to altered conditions of culture may include genotypes with a natural propensity for such an increase.

Evaluation of the regenerants ploidy was based on analysis of DNA content in leaf cell nuclei. All of obtained plantlets contained 1C DNA in G_1 stage. The share of the nuclei after DNA replication was relatively low (fig. 1). This unequivocally confirms androgenic origin of regenerated plants.

The results we have obtained were similar to the data of other studies [12, 14, 15] documenting a very significant effect of genotype on the efficacy of the induced androgenesis. The particularly favourable reaction in one of our line demonstrates the purposefulness of using androgenic potency evaluation as an additional criterion in selection among the recombinants within the popolations of interspecific, pungent hybrids. The investigation on individual response of the single plants in F_2 progeny of interlines and interspecific hybrids within *Capsicum* genus [16] showed the possibilities of obtaining the genotype with satisfactory androgenic potency.

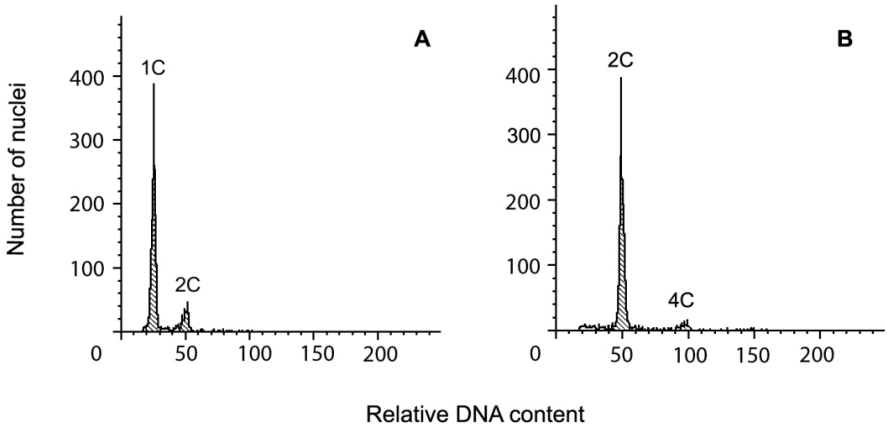

Figure 1. Histograms of relative DNA content; A – haploid plantlet, B – diploid, standard plant

CONCLUSION

The result of the study showed the particular effect of genotype character on androgenetic response of capsaicinoid lines being the results of selection within *Capsicum frutescens* L. and *C. annuum* L. interspecific hybrids and suggests the necessity of the testing the new breeding material from the point of view of androgenic potency.

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HAPLOIDALNA EMBRIOGENEZA U KAPSAICYNOIDOWYCH FORM SOFT-FLESH *CAPSICUM* SPP.

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S t r e s z c z e n i e

Materiałem badawczym były trzy ostre linie soft-flesh wyselekcjonowane z mieszańca *Capsicum frutescens* L. x *C. annuum* L. Zawartość kapsaicynoidów, oznaczona metodą HPLC, w perykarpie owoców sięgała poziomu 100 $mg \cdot kg^{-1}$. Pylniki były prowadzone w kulturach *in vitro* według metody opisanej przez Chamboneta (1988) dla *C. annuum* L. z modyfikacją w zakresie zwiększenia udziału kinetyny w pożywce R_1 do 0,2 $mg \cdot dm^{-3}$. Efektywność embriogenezy wahała się od 0,25% do 4,12%, w relacji liczba wyłożonych pylników – liczba uzyskanych roślin, zależnie od genotypu i pożywki. Konwersja zarodków w rośliny i ogólna efektywność androgenezy była najwyższa u jednej linii. Zawartość DNA określona cytometrycznie w komórkach liści uzyskanych regeneratów wynosiła u wszystkich z nich 1C. Rośliny były zatem efektem haploidalnej androgenezy.

Słowa kluczowe: *efektywność embriogenezy, odpowiedź genotypowa, kinetyna, zawartość DNA*