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Short communication

Treating donor plants with 2,4-dichlorophenoxyacetic acid can increase the effectiveness of induced androgenesis in *Capsicum* spp.

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ABSTRACT

One of the factors conditioning the embryogenic development of microspores in cultures *in vitro* is 2,4-dichlorophenoxyacetic acid (2,4-D), used as component of an induction medium. The aim of the experiment was to evaluate of the effect of 2,4-D applied to donor plants F_1 hybrids of *Capsicum* spp. on embryogenesis through anther culture. The research material included six hot and sweet F_1 *Capsicum* spp. hybrids. Each of the six hybrids plants were subjected to either no 2,4-D application or application of 2,4-D in water solution at rate 1 mg L^{-1}, a day before transferring anthers into culture. The hybrids differed in their response to treatment, in one half of the hybrids 2,4-D application resulted in an increase in the number of embryos and plantlets. The highest number of embryos (127) and plantlets (47) was obtained in culture of anthers from treated plants of (SF4 × 'Luba')F_1 hybrid. The plants produced as a result of the conversion of the embryos differed in their ploidy. On average, two-thirds of them were haploid, irrespective of the experimental variant, with strong differences among the various hybrids. The pre-treatment of donor plants with 2,4-D can be an additional factor enhancing the embryogenic properties of microspores in culture *in vitro*.

1. Introduction

The results of numerous studies into induced androgenesis point to the fact that the plant genotype is the most crucial factor determining the effectiveness of cultures *in vitro* of anthers or microspores (Rodeva et al., 2004; Forster et al., 2007; Dunwell, 2010; Germana, 2011; Irikova et al., 2011; Cheng et al., 2013). The yield quality of the donor plant are not always correlated with the adequate androgenic efficiency. The results reported by Mityko and Fari (1997) demonstrated that a high regeneration potential in sweet-type wax forms of *Capsicum annuum* L. In the same species slightly less effective were the green-blocky cultivars and the lowest androgenic response was noted in hot pepper forms, some of which showed no response at all. Similar observations were made by Koleva-Gudeva et al. (2007), who also evaluated modifications of the culture conditions and the medium composition. The study showed that the cultivars reacted variously. Creating a universal and efficient system for all the genotypes seems very difficult or even impossible. Each of the heterozygotic plants can show a different response (Nowaczyk et al., 2009). An interesting solution for hot forms was presented by Supena et al. (2006). In their research into the improvement of the technology of induced androgenesis, they used a shed microspore culture protocol for haploid production. Indonesian cultivars and breeding lines showed a positive response to the procedure and its authors suggested that it can be a potential tool for the production of double haploids for hot pepper forms. In a later report (Supena and Custers, 2011) they reported that the method was applicable for a limited pool of genotypes.

Among the factors influencing on the process of embryogenesis, a key role is played by growth regulators. As early as in 1955, Haccius described the detailed effect of 2,4-D on the multiplication of the embryos of *Eranthis hiemalis* L. *in vivo*. Treating seeds with 2,4-D soon after seed shedding from the plants resulted in the number of twin embryos increasing. An exogenous application of the growth regulator on *C. annuum* L. flower buds (Nowaczyk and Nowaczyk, 1996) resulted in the increase the number of twin embryos obtained. Parthenogenic embryogenesis was also induced, which resulted in an increase in the share of haploid embryos accompanying zygotic embryos. A similar effect of 2,4-D was noted on other species of *Capsicum* genus (Jędrzejczyk and Nowaczyk, 2009). Dumas de Vaulx et al. (1981) desmonstrated that 10^{-8} g mL^{-1} 2,4-D in combination with kinetin (10^{-8}) in water solution gave superior results than 10^{-7} 2,4-D plus kinetin (2×10^{-6}). The literature describing modifications of the

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Table 1
General characteristics of *Capsicum* spp. parental forms and F_1 donor hybrid plants.

Genotype	Average yield of mature fruits from plant		Fruit characteristics			
	Weight [kg]	Number	Average weight [g]	Taste	Pericarp structure	Wall thickness [mm]
SF4	0.62 ab	64 c	10 a	hot	soft	2.73 a
SF9	0.46 a	51 c	9 a	hot	soft	2.64 a
ATZ	1.05 c	13 a	81 c	sweet	hard	4.01 b
Luba	1.03 c	5.0 a	206 ef	sweet	hard	6.07 c
Mino	1.04 c	5.5 a	189 e	sweet	hard	5.50 c
Sono	1.08 c	9.2 a	118 d	sweet	hard	5.10 c
(SF4 × Luba)F_1	1.10 c	31 b	35 b	hot	soft	3.10 a
(SF4 × Sono)F_1	1.18 c	37 b	32 b	hot	soft	3.00 a
(SF9 × Mino)F_1	0.79 b	25 b	32 b	hot	soft	3.10 a
(SF9 × Sono)F_1	1.08 c	36 b	30 b	hot	soft	2.89 a
(ATZ × Sono)F_1	1.25 c	12 a	104 cd	sweet	hard	4.15 b
King Arthur F_1	1.28 c	5.9 a	217 f	sweet	hard	7.50 d

Table 2
Effect of 2,4-D donor plant treatment on number of embryos and plantlets obtained in anther culture of *Capsicum* spp. F_1 hybrids.

Hybrids and treatment	Number			
	Anthers on medium		Embryos	Plantlets
	CP	R1		
(SF4 × Luba)F_1	365	250	74 d	26 bc
(SF4 × Luba)F_1 2.4-D	366	254	127 f	47 d
(SF4 × Sono)F_1	366	255	98 e	30 c
(SF4 × Sono)F_1 2.4-D	376	252	90 de	23 bc
(SF9 × Mino) F_1	383	259	21 ab	6 a
(SF9 × Mino) F_1 2.4-D	379	258	40 bc	13 ab
(SF9 × Sono)F_1	369	254	49 c	17 b
(SF9 × Sono)F_1 2.4-D	384	256	49 c	17 b
(ATZ × Sono)F_1	384	252	39 bc	6 a
(ATZ × Sono)F_1 2.4-D	382	249	91 de	22 bc
King Arthur F_1	367	253	4 a	3 a
King Arthur F_1 2.4-D	363	257	11 a	2 a
Mean	374 ± 11.6	253.8 ± 3.06	47.5 ± 34.4	14.7 ± 11.4
Mean 2.4-D	375 ± 8.6	254.6 ± 3.83	68.0 ± 42.2	24.2 ± 18.2

concentration of 2,4-D (Irikova et al., 2011) lacks information on the application of 2,4-D right before flower bud collection. The aim of the present research was to evaluate the effect of the treatment of donor plants with 2,4-D on the effectiveness of induced androgenesis in F_1 hybrids of *Capsicum* spp.

2. Materials and methods

2.1. Origin and characteristics of plant material

Six F_1 *Capsicum* spp. hybrids were used in this study. The mother parent of first four of them were two selected hot, soft-flesh type lines, SF4 and SF9 derived from an interspecific cross between *Capsicum frutescens* L. × *C. annuum* L. The selection within the hybrid populations resulted in a production of homozygotic soft-flesh genotypes, marked SF4 and SF9, demonstrating the adequate level of technological features allowing for their use as a raw material for the production of puree or creating F_1 hybrids for a similar purpose. The paternal parents were one of three sweet cultivars (*C. annuum* L.): 'Luba', 'Mino' and 'Sono'. The fifth hybrid was produced by crossing the sweet breeding line, ATZ, with the 'Sono' cultivar. The final hybrid was the commercial cultivar of annual pepper 'King Arthur F_1' (Seminis Poland) grown as a vegetable. Table 1 presents the general characteristics of hybrids providing the most essential technological features, as well as their capsaicinoids level.

2.2. Culture conditions of donor plants

The donor plants were grown in an unheated foil tunnel under the conditions typical for the production of pepper, at the experimental station the Department of Genetics, Physiology and Plant Biotechnology in Bydgoszcz (53°N, 18°E). Fertilization and irrigation were performed following the agronomic guidelines for annual pepper. The plants were neither pruned or applied with any chemicals and were in good health and vigour during study.

2.3. 2,4-D treatment of donor plants and bud collection

2,4-D treatment and the collection of flower buds following it were performed at the stage of intensive plant flowering and the early fruit setting, at four dates between July 1 and 15. Five groups consisting of 10 plants each of the six hybrids were labelled for the study. The first of the five groups was applied no 2,4-D while the four remaining groups were applied accurately once with a water solution of 2,4-D at mg L^{-1} on the whole plants in the evening. The buds were collected in the morning the following day. The cultures were established four times every other fifth day. In defining the correct bud development stage, the commonly applied criterion of flower bud morphology was assumed, which is the same length of the sepals and calyx petals. For each of the hybrids the degree of advancement of microsporogenesis was confirmed by the microscopic acetocarmine test. Most microspores were at the uninucleate phase with the presence of 3–12% of binucleate cells.

2.4. Anther culture

Initiating and maintaining cultures followed the procedure applied by Dumas de Vaulx et al. (1981) and described in the form of a protocol of media (CP, R1 and V3) composition by Chambonnet (1988). Anthers from two buds were placed in 9-cm Petri dish containing CP induction medium. Having completed an 8-day process of incubation in the dark at 35 °C, cultures were maintained for 4 days in the growth chamber (25 °C, 12-h night 12-h day; 40 μmol m^{-2} s^{-1}). An infection was noted in a number of anthers. Anthers from uninfected dishes were only transferred to the R1 medium. As a result of this decision, each of the hybrids at the successive stage of the experiment was represented by explants on 21 dishes for the control and 21 dishes for anthers from the plants treated with 2,4-D. The number of emerging embryos was noted.

2.5. Acclimatization of the plants

The plantlets, which were the effect of the conversion of normally developed embryos, were transferred onto the V3 medium with no growth regulators. Having reached the height of 5–7 cm,