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BRIEF COMMUNICATION

Production of doubled haploids from androgenic embryoids and plantlets of tobacco

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Abstract

In four experiments, the chromosome doubling effect of acenaphthene vapour was tested on androgenic embryoids and/or plantlets of tobacco. The maximum rate of plants with doubled chromosome numbers (41.3 % diploids plus 2.7 % tetraploids) was recorded when approx. 100 mg acenaphthene in small and sterilized cheese-cloth bags were hanged for 72 h, into the culture jars (6.0 × 11.5 cm), containing 4 - 6 androgenic plantlets newly transferred from culture tubes. Acenaphthene treatments performed at the earlier stages of culture resulted in lower percentages (from 0.0 to 6.3 %).

Additional key words: acenaphthene, *Nicotiana tabacum*.

In tobacco androgenic haploids are produced readily, through anther or isolated microspore cultures. Tobacco (2n=48) is of amphidiploid origin (Goodspeed 1954, Smith 1979) and in its haploids (2n=24) meiotic chromosome behaviours comparable to those of monoploids have been observed (Collins and Sadasivaiah 1972, Emiroğlu 1974). Therefore haploids of tobacco are sterile, and in order to get seed, doubled haploid (DH) forms have to be produced by chromosome doubling.

To produce the DHs of tobacco, 0.1 - 0.4 % aqueous solutions of colchicine can be used to treat anthers before culture (Takashima *et al.* 1995), embryoids during culture (Dal *et al.* 1984) and plantlets after culture (Emiroğlu *et al.* 1987). Diploid tobacco plants have been reconstituted also from the culture of midvein tissues from the aged leaves of haploids (Kasperbauer and Collins 1972, Kasperbauer and Wilson 1979).

Schiltz *at al.* (1975) obtained 34.4 - 64.38 % fertility restitution by applying acenaphthene vapour to the developing buds at the leaf axils of decapitated haploid tobacco plants. But a very low diploidization rate (0.0 - 2.43 %) has been recorded when acenaphthene application was performed on the fourth day of tobacco anther culture (Kefi 1985).

The aim of this study vas to test the chromosome doubling efficiency of acenaphthene at different stages of anther culture and to determine the suitable culture stage and duration for application.

The anthers of *Nicotiana tabacum* L. genotype 59/4 were cultured on Nitsch and Nitsch (1969) medium. Folic acid and biotin were not added and the amount of sucrose was 40 g dm^{-3} (Bürün and Emiroğlu 1985). The pH of the medium was adjusted to 5.5 before autoclaving and the culture tubes (2.5 × 14.5 cm) containing about 25 cm^3 of the medium were sterilized at 121 °C for 20 min.

To induce chromosome doubling, acenaphthene (about 100 mg of acenaphthene crystals in small and sterilized cheese-cloth bags were hanged into the culture tubes and jars for 48 and 72 h) treatments were done at four different stages of culture: when the anthers were placed on the medium, at the ends of the 4^{th} and the 8^{th} weeks of culture and at the end of the 8^{th} week of culture but just after transfer of plantlets from culture tubes into culture jars (6.0 × 11.5 cm).

In further experimental sets acenaphthene treatments were done when embryoids appeared in the dehisced

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anthers. About 25 or 50 mg of acenaphthene was applied for 24, 36, 48 h respectively.

The plantlets which were grown further in culture jars were transferred to pods. Chromosome numbers were determined on young leaves, using Walther's method (Walther 1961). Besides chromosome counting, sizes of stoma guard cells in lower epidermis of leaves and fertility were also considered in distinguishing diploids.

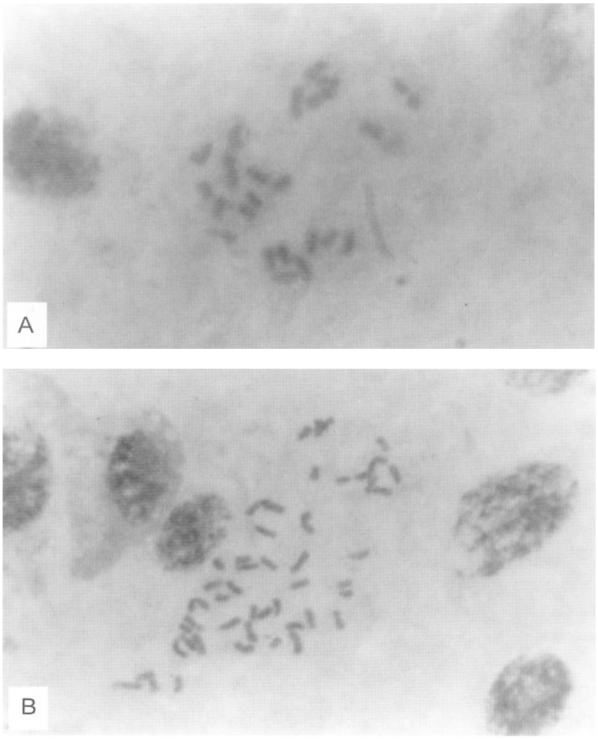

Fig. 1. 2n=24 chromosomes in a leaf cell of a haploid plant (*A*) and 48 chromosomes of a DH plant (*B*) (×600).

The application of acenaphthene at the beginning of anther culture lowered the rate of anthers producing plantlets (Table 1). Kefi (1985) also reported similar results. These observations imply the sensitivity of microspores and/or cells to acenaphthene vapour at this stage.

In addition, all of the androgenic plants cytologically examined in the control groups of two experiments (Exp. 1 and Exp. 2) were haploid (Fig. 1). At the end of 8th week, the percentages of plants with diploid chromosome number were low, being 6.3 and 5.9 % for 48 h and 72 h treatments, respectively (Table 1). Percentages of diploids at the 4th week were higher and ranged from 12.3 to 32.3 %. But, the treatment that was done after transplantation of 8-week-old plants into culture jars was the most effective (from 19.6 to 41.3 %). By treating the androgenic tobacco plantlets with acenaphthene before taking them out of the culture jars, Emiroğlu *et al.* (1987) obtained 15.5 % fertility restoration. When the results are compared, the percentage of diploidization is higher in the present investigation, because the plantlets were treated just after transfer into jars where the plantlets began to grow actively.

On the other hand, among treated plants, besides haploids and diploids, haploid-diploid chimeras (3.8 %) and aneuploids (4.8 %) were also found. Tetraploids (2n=96) were observed only in 8 week-old plants group with 72 h treatment.

Treatments of tobacco embryoids in the dehisced anthers with lower amounts of the acenaphthene

Table 1. The effect of acenaphthene (100 mg per culture tube or jar) on chromosome doubling when applied at different stages of anther culture of tobacco for 48 and 72 h (exp. 1 and 2).

	Culture age [week]	Treatment duration [h]	Number of cultured anthers	Anthers producing plantlets [%]	Number of examined plants	Diploid [%]	Tetraploid [%]
Exp. 1		control	90	50.6	27	0.0	0
	0	48	95	12.0	6	0.0	0
	0	72	95	2.9	1	0.0	0
	4	48	99	34.0	45	20.0	0
	4	72	98	43.9	34	32.3	0
	8	48	98	28.6	50	6.0	0
	8	72	100	60.0	9	22.2	0
Exp. 2		control	294	34.8	73	0.0	0
	0	48	294	17.5	16	6.3	0
	0	72	350	27.8	34	5.9	0
	4	48	293	35.7	73	12.3	0
	4	72	297	30.8	47	17.0	0
	8 (tubes)	48	289	37.9	87	9.2	0
	8 (tubes)	72	294	30.2	64	4.7	0
	8 (jars)	48	299	42.1	46	19.6	0
	8 (jars)	72	293	38.5	75	41.3	4

Table 2. The effect of acenaphthene (50 or 25 mg per culture tube) on chromosome doubling when applied to androgenic embryoids for 24, 36 and 48 h (exp. 3 and 4).

	Acenaphthene amount [mg]	Treatment duration [h]	Number of cultured anthers	Anthers producing plantlets [%]	Number of examined plants	Diploid [%]
Exp. 3	control		154	41.0	74	0.0
	50	48	735	35.4	52	10.0
Exp. 4	control		141	44.7	28	3.6
	50	24	89	46.3	19	3.0
	50	36	85	25.4	5	0.0
	50	48	89	42.2	33	5.3
	25	24	92	46.6	12	8.3
	25	36	93	35.2	18	0.0
	25	48	95	46.9	9	0.0

(25 - 50 mg per tube) for shorter time (24, 36 and 48 h) resulted in 0.0 to 10.0 % diploids (Table 2).

In cultivar Karabağlar which is one of the parents of hybrid 59/4, Emiroğlu (1974) reported 2.6 % spontaneous diploid and 5.1 % haploid-diploid chimeras. When we compare our results with the rates of spontaneous diploids (the average of diploids is 3.35 % for 9 different treatments in Table 2 against 7.7 % spontaneous diploids plus chimeras reported), we can state that the amounts and/or the durations of the treatments applied to embryoids were not sufficient to induce chromosome doubling.

From these results, it can be concluded that, acenaphthene treatment at the end of eighth week of culture when the androgenic plantlets are transferred into culture jars is appropriate for chromosome doubling.

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