

METHODOLOGY:

# A technique to obtain metaphasic chromosomes in asparagus (*Asparagus officinalis* L.) spear cells

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## ABSTRACT

Asparagus is a dioic perennial species, which makes it difficult to use conventional methods for chromosome analysis with root tips or pollen mother cells. A technique using metaphasic chromosomes from spear tips was developed. Metaphases were arrested with distilled water at 0°C for 15 to 17 h and the material was then subjected to fixation with ethanol:chloroform:acetic acid 6:3:1; hydrolysis with 5 N HCl for 20 min at room temperature; staining with 3% Giemsa for permanent mounts, or the method of Feulgen and squashing with 1% acetic carmine for temporary mounts.

## INTRODUCTION

Asparagus is a diploid species ( $2n = 20$ ). Breeding of this species involves the use of tissue culture, especially for meristem culture, and the development of new cultivars through anther culture (Doré, 1974). These techniques, however, are often associated with ploidy variation in the regenerated plants (Mercy and Zapata, 1986). Polyploids, aneuploids and chromosome aberrations have been observed in plant or animal cells cultured *in vitro* (Sacristán, 1971). Because of these variations, the newly bred plants must be analyzed and have their karyotype compared to that of normal plants (Thévenin, 1969; Loptein, 1979).

Chromosome analysis of field plants by conventional methods such as mitotic chromosome of

the root tips or meiotic chromosomes in the mother cell of the pollen grain is difficult, because asparagus is a perennial and dioecious species. These difficulties led to the search for an alternative material for ploidy assessment. The fast growing asparagus spear was chosen for its high frequency of mitotic division and high potential for obtaining countable and morphologically analyzable metaphasic chromosomes.

## MATERIAL AND METHODS

Asparagus spears which had grown one to two centimeters above the soil were used. The material was washed in distilled water and then pre-treated. Treatments were made with the spindle inhibitor 8 HQ (8-hydroxyquinoline) at different concentrations, exposure times and temperatures and with distilled water (Table I). The material was collected every hour for analysis. Three different fixing mixtures were tested: (3:1) ethyl alcohol:acetic acid, (6:3:1) ethyl alcohol:chloroform:acetic acid, and (2:1) ethyl alcohol:propionic acid. Hydrolysis in 5 N HCl, at room temperature, was assessed at 20, 25 and 30 min.

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**Table I** - Pre-treatments used to inhibit mitotic division in asparagus spear cells.

Mitosis inhibiting agent	Temperature (°C)	Exposure time (min)
8 HQ 0.002 M or 0.006 M	25 or 4	30
		60
		90
		120
		150
		180
		210
240		
8 HQ 0.002 M or 0.006 M	25 + 4	15 + 15
		30 + 30
		30 + 60
		30 + 90
		30 + 120
		30 + 150
		30 + 180
30 + 210		
Distilled water	0	Hourly exposures from one to 19 h

8 HQ = 8-Hydroxyquinoline.

Giemsa solution at 3% and the Feulgen method were used to stain the chromosomes for permanent and temporary slides, respectively. The material stained with Giemsa was squashed in acetic acid at 45%. The material stained by the Feulgen technique was observed by smear and squash by placing the tissue on a drop of 1% acetic carmine, 2% acetic orcein and 1% lactopropionic orcein.

The results of each treatment were obtained from analysis of thirty cells.

## RESULTS AND DISCUSSION

The spears showed a large number of cells in various phases of cellular division. The best pre-treatments as for condensation and spread were 8 HQ 0.002 M at 4°C for 90 min; 8 HQ 0.002 M at 25°C for 150 min; 8 HQ at 0.006 M at 25°C for 150 min and 8 HQ 0.006 M for 60 min + 90 min at 4°C (Table II). When distilled water at 0°C was used as a spindle inhibitor,

**Table II** - Effects of different pre-treatments and exposure times on mitotic cells of asparagus spears.

Antimitosis agent	Treatment	Interphase	Intact metaphases*	Good metaphases**	Contracted chromosomes***	
8 HQ	0.002 M 25°C	180	30	150	-	
		210	60			
		240	90			
			120			
	0.002 M 4°C	-		30	90	120
				60		150
						180
						210
						240
	0.002 M 25 + 4°C		30 + 150	15 + 15	-	30 + 90
			30 + 180	30 + 30		30 + 120
			30 + 210	30 + 60		
	0.006 M 25°C	-		30	150	180
				60		210
				90		240
				120		
	0.006 M 4°C	-		30	-	150
				60		180
			90	210		
			120	240		
0.006 M 25 + 4°C		15 + 15		30 + 150	-	
		30 + 30	-			
		30 + 60				
		30 + 120				
		30 + 180				
Distilled water	0°C	17, 18 and 19 h	1 to 10 h	14, 15 and 16 h	-	

\*No spindle rupture; \*\*spindle rupture and easy chromosome counting; \*\*\*the chromosomes were too contracted.

the best exposure period to obtain compact chromosomes was 15 to 17 h (Figure 1). This type of pre-treatment was chosen because of low cost, lack of toxicity and excellent results.

Among the different fixing mixtures used, the most efficient was 6:3:1 ethyl alcohol:chloroform:acetic acid. All the others caused refringence. The hydrolysis time for best result was 25 min.

Giemsa solution at 3% for permanent slides and Feulgen staining with squashing in 1% acetic carmine for temporary slides allowed good visualization of the chromosomes, due to superior contrast between chromosomes and cytoplasm. The use of 8 HQ as an inhibiting agent of mitosis in root tips of asparagus is quoted by various authors, though concentrations and exposure times vary (Thévenin, 1969; Marks, 1972; Lopstein, 1979). Distilled water at 0°C has been used on wheat root tips (Melnyk and Unrau, 1961).

## RESUMO

O asparago é uma planta dióica perene, o que torna difícil o uso de métodos convencionais para análise cromossômica com pontas de raiz, ou células-mãe de grãos de pólen. Uma técnica usando cromossomos metafásicos a partir de pontas de turião foi desenvolvida. As metafases foram paralisadas com água destilada a 0°C por 15 a 17 h e o material foi então submetido a fixação com etanol:clorofórmio:ácido acético (6:3:1); hidrólise com HCl 5 N por 20 min à temperatura ambiente; coloração com Giemsa 3% para montagens permanentes, ou pelo método de Feulgen e esmagamento com carmim acético a 1% para montagens temporárias.

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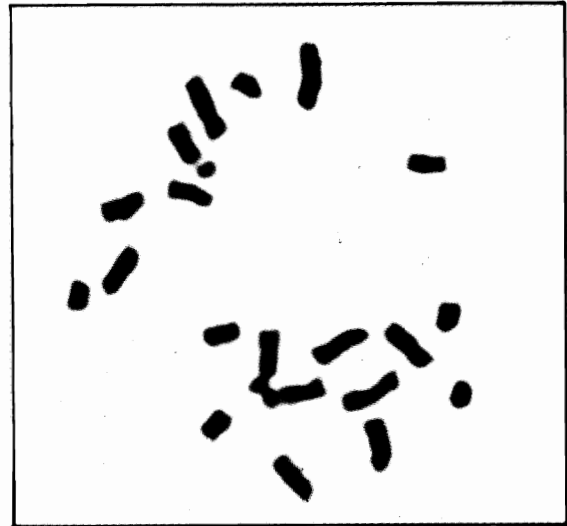


Figure 1 - Normal diploid cell ( $2n = 20$ ) of an asparagus spear pre-treated with distilled water at 0°C for 16 h.

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(Received April 28, 1995)