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Cytogenetical analysis on aneuploids obtained from pollenclones of rice (*Oryza sativa* L.)

Chu Qiren, Zhang Zhenhua and Gao Yuanhua
Shanghai Academy of Agricultural Sciences, Shanghai, China

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Summary. Rice aneuploids were obtained from 1,715 pollenclones with a mean frequency of 10.2% in anther culture (1983 to 1985). Among the aneuploids obtained, the frequency of primary trisomics ranged from 5.4% to 6,7%, tetrasomics from 1.1% to 1.7% monosomics from 0.9% to 1.3%, nullisomics from 0.5% to 1% and double trisomics from 0.5% to 0.7%. The chromosome complements of those aneuploids were identified by pachytene analysis on the absolute length of the extra chromosomes. Pollen clonal aneuploids showed a different range of variation in agronomic characters from dihaploids of the same origin but the phenotypic variations ressembled those found in aneuploids created by conventional breeding methods. The meiotic chromosome behavior of PMC revealed various chromosomal aberrations of aneuploids: loose pairing, trivalents, univalents, straggling chromosomes, bridges and laggards.

Key words: Aneuploids – Pollen clones – Pachytene analysis – Chromosomal aberration

Introduction

Rice aneuploids have been obtained in only frequencies by many various approaches (Chang 1964; Nayar 1973; Khush 1975, 1984). Primary trisomics could be separately identified from the progenies of rice triploids (Ramanujam 1937; Yunoki and Masuyama 1945; Karibasappa 1961; Katayama 1963; Sen 1965; Hu 1968; Iwata et al. 1970; Watanabe and Koga 1975; Sur 1975; Singh and Khush 1979; Khush et al. 1984). Other aneuploids in rice, such as monosomics, nullisomics, and double trisomics, have been found by investigators (Seshu 1963; Khush 1973, 1975, 1984).

With the development of biotechnology in haploid culture and tissue culture of higher plants, various aneuploids have been induced by in vitro methods in many species: *Tabacum, Petunia, Brassica, Dutara* and *Atropa.* Aneuploids have also been regenerated from such important crop species as *Triticum estivum* (Hu et al. 1978, 1980) and *Oryza sativa* (Chu 1983 b; Chu et al. 1984; Chu et al. 1985).

A complete set of primary trisomics of rice could be used to locate specific markers on certain chromosomes, to clarify the independence of the 12 linkage groups suggested by Nagao and Takahashi (1963), to identify each linkage group associated with each chromosome and to evaluate the results from conventional linkage analysis and from the reciprocal translocation linkage studies (Iwata and Omura 1971, 1975, 1978). The other aneuploids involving monosomics and nullisomics could be used to establish addition and substitution lines, by which disease resistant genes and other desirable characters may be introduced from the wild species of rice into rice cultivars.

Plants derived from callus have been called 'calliclones' (Skirvin 1978). It therefore seems appropriate that in vitro regenerated plants derived from anther culture be referred to as 'pollenclones'. Present studies aim to investigate the inducing frequency of various aneuploids of rice pollenclones, to determine the extra chromosome for corresponding aneuploids by pachytene analysis, to compare the morphological differences of pollenclonal aneuploids with those from conventionally created aneuploids, and to study the chromosome behavior of PMC at meiosis of the pollenclonal aneuploids.

Materials and methods

Nineteen parents, including variety 'HuaHanZao' and their 34 F_1 hybrids, were anther cultured in N6 media with a 2 mg/l

2,4-D supplement. The induced calli were transferred into MS media (supplemented by 0.5 mg/l IAA and NAA) and plantlets were regenerated in 1982–1983. A total of 290 pollenclones were obtained. The pollen population inoculated was enlarged to produce 1,213 plants in the same de-differentiation and differentiation media used in 1983–1984. The anthers of variety 'CX112' were inoculated to regenerate 212 pollenclones in 1984–1985. A total of 1,715 plants were derived from the pollenclones. These were morphologically and cytologically identified to determine the frequency of various aneuploids. Based on pachytene analysis of the extra chromosomes of aneuploids, cytological observations on PMC of aneuploids were made. The methods for slide making and staining followed Chu (1982, 1983a, 1984, 1985). Chromosomal behavior of PMC at meiosis for those aneuploids in vitro regenerated was analyzed on the basis of 5 panicles per each pollenclone.

Results

1 Frequency of the various aneuploids derived from pollenclones

A total of 290 pollenclones derived from rice anther culture were morphologically and cytologically observed in 1983 (Table 1): 10.7% were aneuploids. The typical chromosome pattern of dihaploids, haploids and aneuploids derived from pollen plants are shown in Fig. 1. Among the pollen clonal aneuploids counted, primary trisomics (2n+1) account for 6.7%, tetrasomics (2n+2) for 1.7%, monosomics (2n–1) for 1.3%, nullisomics (2n–2) for 1.0% and double trisomics (2n+1+1) for 0.7%. In 1984, 1,213 pollenclones showed a similar distribution frequency for dihaploids, haploids, polyploids and aneuploids. The frequency of aneuploids reached 11.0%. A similar frequency of aneuploids was obtained when 'CX112' was used as the pollen donor in anther culture (Table 1). The mean frequency of aneuploids regenerated in vitro obtained 10.2% in three years of tests. When different parents were anther cultured to produce pollenclonal aneuploids, primary trisomics had the highest frequency (an average of 6.2%). However, the order in inducing frequency of other aneuploids is tetrasomics (1.4%), monosomics (1.0%), double trisomics (1.0%) and nullisomics (0.8%).

2 Primary trisomics regenerated in vitro

Nineteen primary trisomics were obtained from 290 pollenclones derived from 9 pollen donor parents (Table 2). Pachytene analysis on the extra chromosome of triplo plants was made on the basis of the absolute length of chromosome, centromere position, heterochromatin region and arm ratio: 2 plants were triplo 3, 2 plants were triplo 4, 3 were triplo 8, 5 triplo 10 and 3 triplo 12. In 4 other triplo plants the chromosome could not be identified due to the absolute length of the extra chromosome falling in between 2 adjacent chromosomes.

Pachytene analysis on triplo 3 of pollenclones derived from variety 'Fan Xiou 2' is shown in Table 3: chromosome 3 is seen as a trivalent. A typic pachytene chromosome is shown in Fig. 2 where chromosome 10 shows partial association of the trivalent.

Based on the pachytene analysis of the extra chromosome in triplo plants, in 1984 we obtained 80 primary trisomic plants derived from 1,213 pollenclones. Cytological observation on these plants indicated that they belonged to triplo 3, 4, 5, 6, 7, 8, 9, 10 and 12 (Table 4). In 1985, we obtained triplo 2, 6 and 9 when CX112 were used as the pollen donor.

3 Other aneuploids derived from rice pollenclones

Other aneuploids, including tetrasomics, monosomics, nullisomics and double trisomics, were also obtained from rice anther culture. Chromosomal complements of 5 tetrasomics were identified by pachytene analysis, indicating that the extra bivalent involves chromosomes 5, 6, 8 and 12 (Table 5). Morphological markers of the tetrasomics distinguished the former from the dihaploids.

Pachytene analysis was undertaken on the monosomics derived from pollenclones (Table 5). The missing univalents of monoplo plants involved chromosomes 1, 2, 3 and 5. Morphological characters of monosomics are significantly different from the dihaploids originated from same pollen donor.

Table 1. Frequency of dihaploids, haploids, polyploids and aneuploids in rice pollenclones (%)

Material sources	Year	No. of pollen plants	Dihaploids	Haploids	Polyploids	Aneuploids
'HuaHanZao'						
34 F_1	1983	290	34.8	49.7	4.8	10.7
F_1	1984	1,213	36.7	47.2	5.1	11.0
'CX112'	1985	212	35.5	50.4	5.3	8.8
			35.7	48.9	5.1	10.2

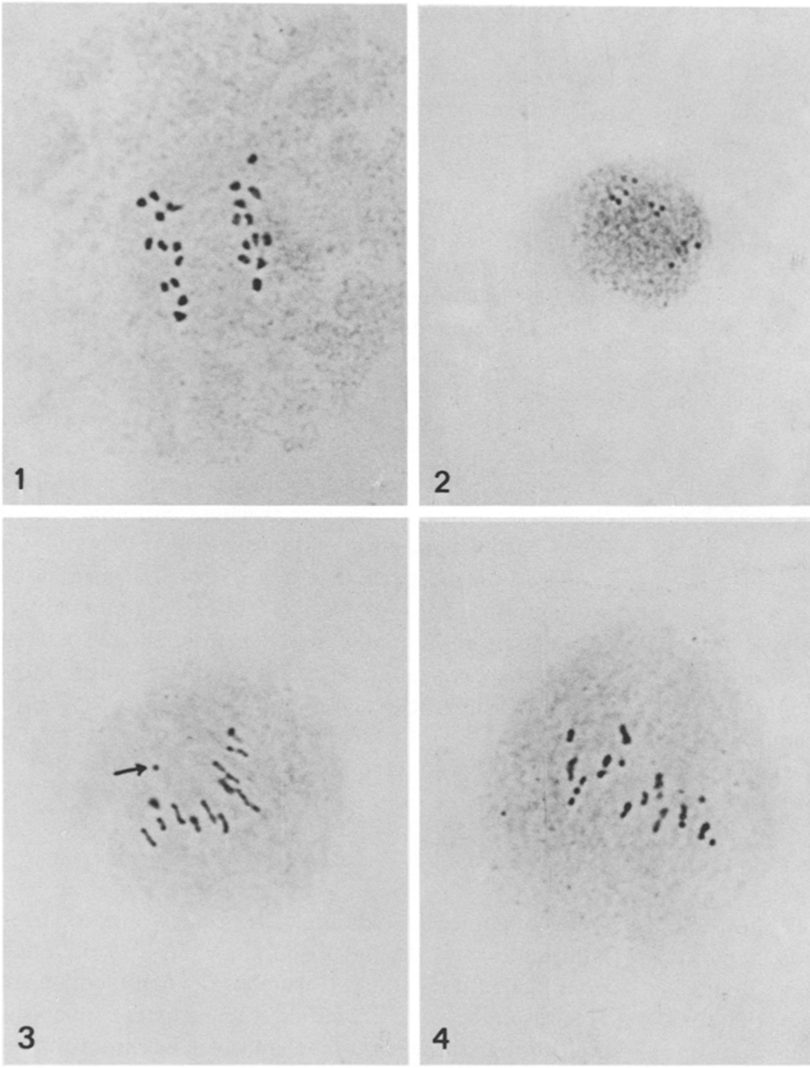

Fig. 1. Chromosome numbers of PMC from pollen plants. *1* Diploid (2n=24); *2* haploid (n=12); *3* primary trisomics (2n+1); *4* double trisomics

A cytological feature of 3 nullisomics is the occurrence of 11 bivalents. Pachytene analysis showed that the missing bivalents are chromosomes 3, 5, and 8 (Table 5). Two double trisomics could be identified as to chromosome complement by pachytene analysis. They have rolled leaves and are highly sterile.

4 Chromosome complement and behavior of PMC in pollenclonal aneuploids

The morphological markers of aneuploids derived from rice pollenclones can be easily distinguished from dihaploids. Chromosomal aberrations can be observed in meiotic cells (Table 6).

The main chromosome aberrations of aneuploids were loose pairing (1.4–19.9%) and univalents (1.4–44.5%) at pachynema; univalents (2.1–48.4%) and ring-of-four (4.7–35.5%) at diakinesis; straggling chromosomes (1.2–54.1%) and tetravalents (0.1–24.5%) at metaphase I; and laggards (2.7–39.1%) and bridges (9.7–11.0%) at anaphase I. Figure 3 shows typical chromosome abnormalities: loose pairing, ring-of-four, straggling chromosomes and bridges.

Discussion

Rice anther culture showed a wide range of variations in chromosome structure and chromosome number. We can obtain such pollen clonal aneuploids as primary trisomics, tetrasomics, monosomics, nullisomics and double trisomics. The induction frequency (10.2%) of aneuploids by this approach is higher than that ob-

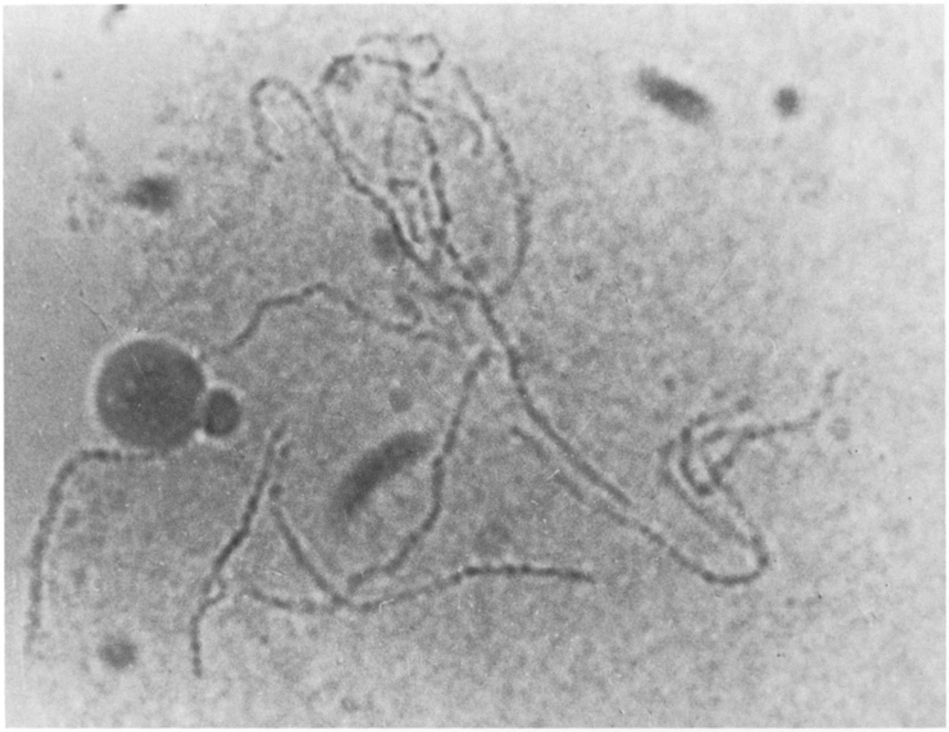

Fig. 2. Pachytene analysis of the extra chromosome from primary trisomics. Note the partial association of the trivalent (chromosome 10) found in triplo 10 derived from 'Su Geng 2'

Table 2. The extra chromosomes of 19 primary trisomics from pollenclones and their morphological markers

Variety as pollen donar	Extra chromosome	Main morphological markers		
		Growth habit	Leaf characters	Other outstanding characters
'Fan Xiou 2'	III	Many tillers	Light green, short flag leaf	Long awned
	IV	Dwarf	Dark green, thick leaf	Late flowering, highly sterile
'Su Geng 2'	?	Dwarf	Semi-rolled leaf	Awned, lax panicle
	VIII	Few tillers	Narrow, semirolled leaf	Short ligule
	X	Weak, slender	–	Small grains, fertile
'M9'	IV	Dwarf, slow growth	Short, thick leaf	Sterile
	?	Bushy with many tillers	Light green leaf	Awned
	XII	Tall status	Long leaf	Lax panicles
'Song Qian'	III	Dwarf, many tillers	Light green leaf	Awned
	X	Dwarf, weak	Semirolled leaf	Small grains
'Dong Nong 333'	X	Dwarf	Rolled leaf	Small grains
'Su Geng 3'	VIII	Fewer tillers	Narrow leaf	Round grains, short panicle
	?	Pseudonormal	Pseudonormal long	Semisterile, awned
	XII	spreading habit	droopy leaves	
'72-597'	VIII	Dwarf, fewer tillers	Thick, dark green leaf	Round grains
	X	Dwarf, small	Normal	Hairy ligule
'2010'	?	Pseudonormal	Normal	Normal
	XII	Tall, many tillers	Droopy leaves	Short awned
Xiong Ji 9	X	Dwarf, weak	Light green leaf	Small grains

Table 3. Pachytene analysis on triplo 3 of pollen plants derived from 'Fan Xiou 2'

Chromosome	Absolute length (μ)	Relative length (%)	Arm ratio	Chromosome type
I	65.1+0.5	13.8	0.79	M[b]
II	60.1+0.4	12.7	0.81	M
III[a]	54.4+0.6	11.5	0.59	SM
IV	49.7+0.7	10.5	0.64	SM
V	45.2+0.4	9.6	0.77	M
VI	40.3+0.2	8.5	0.61	SM
VII	35.4+0.6	7.5	0.47	SM
VIII	31.7+0.6	6.8	0.19	ST
IX	27.3+0.4	5.8	0.39	SM
X	23.1+0.5	4.9	0.42	SM
XI	21.4+0.1	4.6	0.44	SM
XII	18.7+0.6	4.0	0.61	SM

[a] Chromosome 3 is trivalent
[b] M: median chromosome with an arm ratio of 0.67–1.00; SM: submedian chromosome with an arm ratio of 0.34–0.66; ST: subtelocentric chromosome with an arm ratio less than 0.33

tained by conventional selection methods (Iwata et al. 1970). The high frequency of aneuploids in rice anther culture was similar to the findings by Hu (1978, 1980) when wheat anther culture was conducted. The frequency of primary trisomics averaged 6.2%, only triplo 1 and 11 were not found when a total of 1,725 pollenclones were cytologically identified (Table 2). Based on pachytene analysis, we have identified the extra chromosome of triplo 2, 3, 4, 5, 6, 7, 8, 9, 10 and 12. This indicates that if the pollen population inoculated was increased, a complete set of primary trisomics could be established.

Each triplo plants derived from anther culture has its unique morphological markers which can be distinguished from dihaploids and from other triplo plants. Similar phenotypes were found when triplo plants derived from pollenclones were compared to those derived from conventional breeding methods (Iwata et al. 1970; Hu 1968; Sur 1975; Watanabe and Koga 1975; Singh and Khush 1979; Khush et al. 1984).

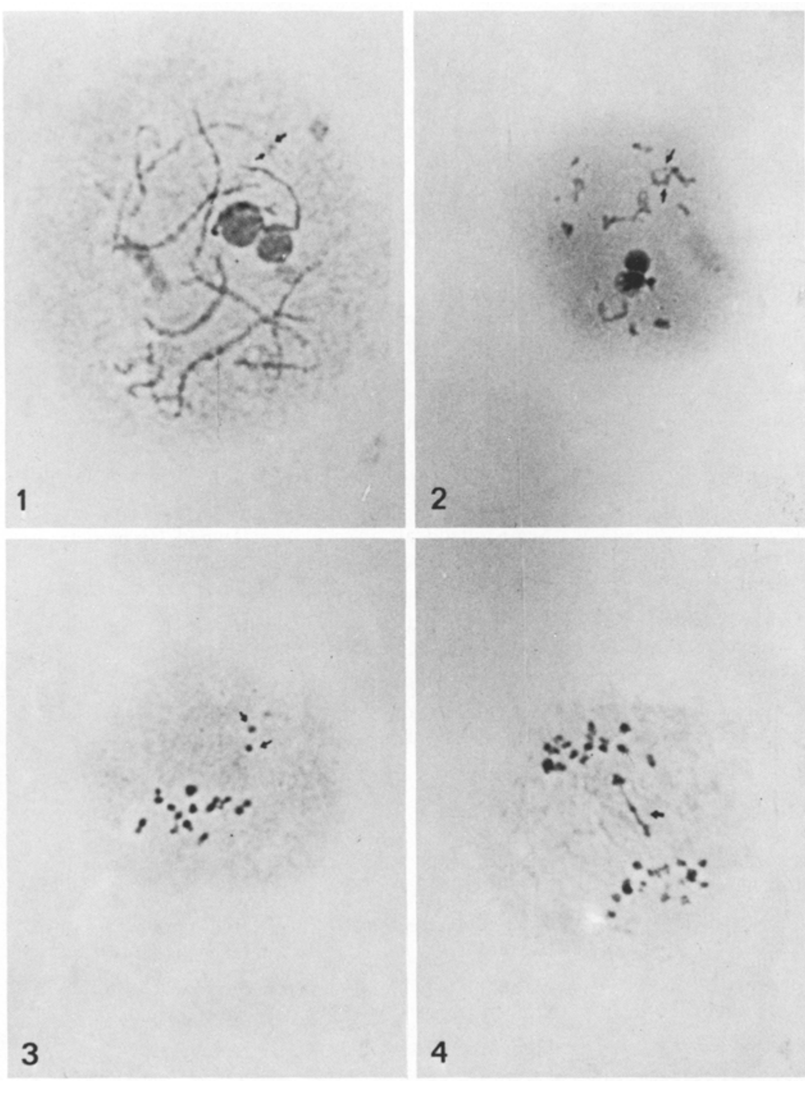

Fig. 3. Chromosomal behaviour at meiosis of the aneuploids derived from pollen plants. *1* Loose pairing at pachynema; *2* ring-of-four at diakinesis; *3* straggling chromosome at metaphase I; *4* bridge at anaphase I

Table 4. Primary trisomics derived from pollen clones in 1983–1985

Year	Chromosome										
	1	2	3	4	5	6	7	8	9	10	11	12
1983			2	2	?			3		5	?	3
1984			4	12	11	12	5	17	11	5		3
1985		2				2			2			

Table 5. Chromosomal complements and morphological markers of other aneuploids derived from rice pollenclones

Type of aneuploids	Sources of pollenclones	Chromosome complement	No. of plants	Main morphological markers
Tetrasomics	'HuaHanZao'/61	12II + 1II(V)	2	Highly sterile
	'TaiNan 5'/'Shuang' 'Feng 1'	12II + 1II(VIII)	1	Narrow leaves
	'ZongHua 8'	12II + 1II(VI)	1	Lax panicles
	'DongNong 333'	12II + 1II(XII)	1	Awned
Monosomics	'HuaHanZao'/61	11II + 1I(V)	1	Late flowering
	'BaiGanDao'/ 'HuaHanZao'	11II + 1I(III)	1	Dwarf
	'SuGeng 2'	11II + 1I(I)	1	Small panicle
	'SuGeng 3'	11II + 1I(II)	1	Small grains
Nullisomics	'HuaHanZao'/61	12II – 1II(V)	1	Sterile
	'HuaHanZao'/61	12II – 1II(III)	1	Highly sterile
	'TaiNan 5'/ 'ShuangFeng 1'	12II – 1II(VIII)	1	Small grains, sterile
Double trisomics	'ZhongHua 8'	12II + 1I(V) + 1I(VIII)	1	Rolled leaves
	'HuaHanZao'	12II + 1I(VII) + 1I(XII)	1	Highly sterile

Table 6. Meiotical chromosomal behavior of PMC in aneuploids derived from rice pollen plants

	Diploid (2n)	Prim. (2n+1)	Double (2n+1)	Tetra- (2n+2)	Mono- (2n–1)	Nulli- (2n–2)
No. plants observed	17	19	2	5	4	3
No. cells	2,493	2,003	731	1,052	919	518
Pachynema (%)						
Normal	98.3	47.2	67.1	35.5	50.9	31.5
Loose pair	1.4	8.3	18.4	19.9	10.7	1.4
1I	0.3	44.4	14.4	44.5	38.3	1.4
11II	–	–	–	–	–	65.7
Diakinesis (%)						
Normal	99.9	44.7	58.1	40.1	39.2	36.4
1I	0.1	48.4	20.9	24.3	47.1	2.1
Ring-of-four	–	6.8	21.0	35.5	13.7	4.7
11II	–	–	–	–	–	56.8
Metaphase I (%)						
Normal	99.8	57.1	41.5	33.4	45.5	41.4
Straggling	0.1	42.8	54.1	42.1	52.6	1.2
IV	0.1	0.1	4.4	24.5	1.9	1.1
11II	–	–	–	–	–	56.3
Anaphase I (%)						
Normal	99.7	51.8	60.1	55.4	50.2	45.4
Laggard	0.2	37.2	30.1	33.5	39.1	2.7
Bridges	0.1	11.0	9.7	11.0	10.7	–
Others	–	–	–	–	–	51.8

Therefore, the extra chromosome in trisomics may have similar genetic effects on morphological expression.

The present study reveals that the occurrence of tetrasomics, double trisomics, monosomics and nullisomics in aneuploids derived from anther culture. The aneuploids can be tolerant to more than and less than one chromosome. The result supports previous findings in rice by Khush et al. (1984) and in wheat by Hu et al. (1978, 1980).

The high frequency of aneuploids derived from rice anther culture may involve several factors: a growth factor, such as 2,4-D, which in culture media may induce variation in chromosome number and structure of the pollenclones; somatic recombination may take place in the process of plant differentiation. The detailed mechanisms of inducing aneuploids in rice anther culture needs to be further investigated.

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