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KARYOMORPHOLOGICAL STUDIES ON *PIPER BETLE* L.

JOSEPH JOSE* AND ARUN KUMAR SHARMA

CENTRE FOR ADVANCED STUDY IN CELL AND CHROMOSOME RESEARCH, DEPARTMENT OF BOTANY, UNIVERSITY OF CALCUTTA, CALCUTTA 700019

In the present study thirty three genotypes have been studied, including three wild varieties of *Piper betle* L. The number of chromosome ranges from 2n=42 to 2n=195 and polyploidy is an important factor in evolution. The extensive vegetative propagation of the species has evidently established these polyploid variations in the cultivars. Numerical variations in the same individual are recorded. However, in view of the regularity of chromosome number in majority of the varieties, in spite of certain individual numerical alterations, it appears that the number 2n=78, which is deep seated, has a wide selective value. All the varieties of *Piper betle* L. show a gross uniformity in the karyotype. However, differences in the number of nucleolar chromosomes have been recorded. Notwithstanding the high chromosome number (2n=195) in the wild variety *chava* of *P. betle* L., the number of nucleolar chromosome is not high. It has been suggested that mutations or cryptic alterations of chromosomes are mainly responsible for the evolution and origin of different genotypes of *P. betle* L. However, in the absence of flowering, which is a rare phenomenon in *P. betle* L., vegetative reproduction is of considerable importance.

Piper betle L. (Family Piperaceae) consists of 9 genera and about 1,400 species (Hooker 1885). They are distributed in the tropical and subtropical regions of the world. A large number of distinct varieties are commercially cultivated in India, Sri Lanka and Bangladesh and several wild varieties are also found growing in the forests of the Eastern and Western Ghats. In India alone more than 100 types/varieties are recognised by the cultivators and traders (Khoshoo 1982). The use of betel leaf (*P. betle* L.) is a part of convention, hospitality, a habit and an innocent after meal breath-sweetening practice, involving over one fourth of human race in the world. Despite the very great economic and medicinal importance of this group, it is remarkable that so little attention has so far been paid to the chromosome constitution. Paucity of cytological data on *Piper betle* L. may be largely due to the extremely small size but high number of chromosomes, intraclonal variations and very heavy cytoplasmic contents making critical analysis difficult and time-consuming (Mathew 1958 ; Sharma and Bhattacharyya 1959 ; Dasgupta and Datta 1976 ; Samuel and Bavappa 1981 ; Jose 1982). The present study aims to bridge the gap through a comprehensive investigation on the somatic chromosomes of 33 genotypes, including 3 wild genetic strains. Improved techniques have been adopted (Sharma and Sharma

*Centre for P G study in Botany, Sacred Heart College, Thevara, Cochin 682 013

1980) with a view to understand the number, structure and behaviour of their chromosomes. Such studies may yield valuable data to be utilized for any project involving the improvement of the crop to maximise the yield of aromatic compounds.

MATERIAL AND METHODS

For the study of somatic chromosomes thirty cultivated and three wild varieties of *Piper betle* L. have been selected. These varieties do not belong to the category of any definite botanical taxon since they differ only in certain subtle morphological features of the leaves, texture and taste. In most cases the names of the types indicate the original place of cultivation. For karyotype analysis, temporary aceto-orcein squash preparations was used. Young root tips were collected from cuttings, planted in the experimental botanical garden of the University, were pretreated in different chemical agents for varying periods. Best results were obtained by pretreatment with a mixture of saturated solution of paradichlorobenzene and aesculin (1 ; 1) with a little saponin at 0–5°C for 3–5 minutes and then at 14–15°C for 3½ hours. They were fixed in acetic acid-ethyl alcohol mixture (1 : 2) for 2 hrs, treated in acetic acid (45%) for 10 minutes. The usual schedule for aceto-orcein staining was followed.

OBSERVATIONS

In the present investigation, thirty three taxa have been studied. The normal somatic chromosome number ranges from 2n=42 to 195. However, numerical variations have been recorded in most of the genotypes. The morphology of the chromosome in all the varieties of *Piper betle* L. shows a homogeneity in the extremely short size (2·41 μ to 0·56 μ). The chromosomes decrease in size progressively and bear nearly median or nearly submedian primary constriction. In the karyotypes, ratio of the short arm to the total length of chromosome in percentage or F% was calculated to classify the nature of primary constrictions. On the basis of position of constrictions and lengths, the chromosomes may be classified into the following types.

Type A—Comparatively short chromosome with two constrictions, one median and the other nearly submedian in position (2·41 μ to 1·48 μ).

Type B—Comparatively short chromosome with nearly median to nearly submedian primary constriction and a satellite at the distal end of shorter arm, joined by a SAT thread.

Type C—Short chromosomes (2·04 μ to 0·56 μ) with nearly median to nearly submedian primary constrictions.

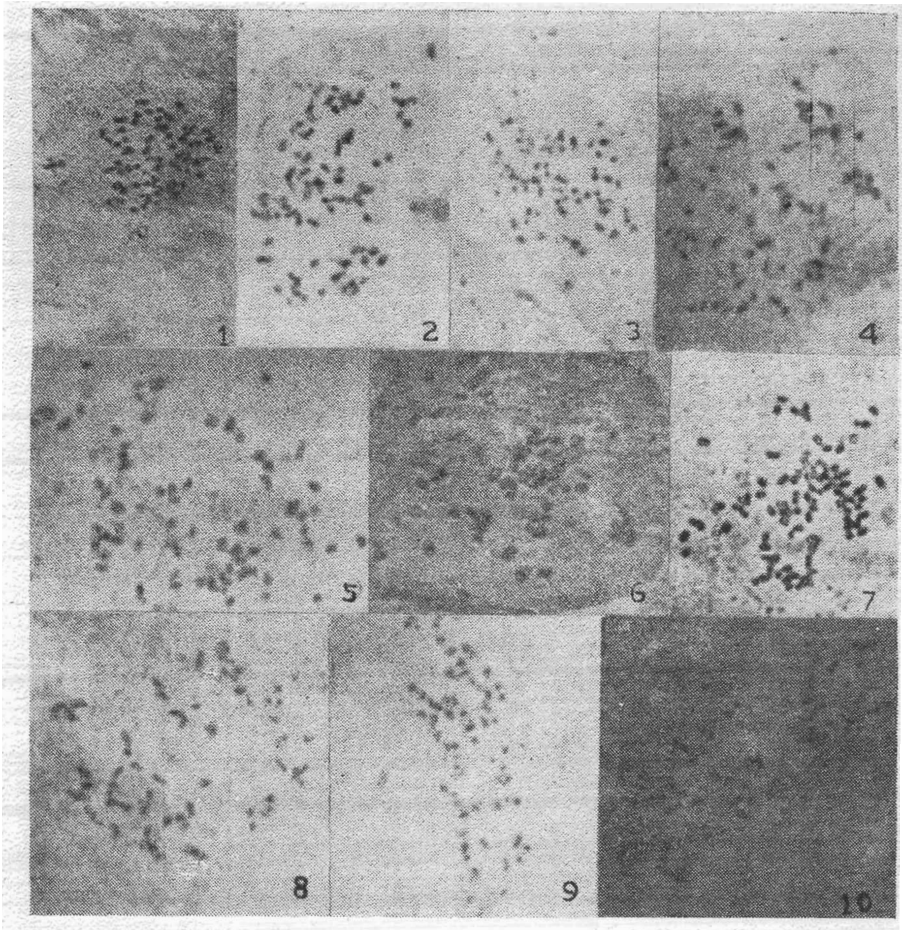
On the basis of the above description of chromosome types, the karyotype of the different genotypes of *Piper betle* L. may be described in the following way (Table 1).

TABLE I. Comparison of the somatic chromosomes of the different genotypes of *Piper betle* L. investigated.

Name of the material	Locality of collection	Alt. in meters	2n	Somatic variation nuclei (2n)	Karyotype formula	Chromosome pairs with sec. constns.	Range of chromosome length (μ)	Total chromosome length (μ)	Total short arm length (μ)	T. F. value (%)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
Piper betle L.										
var. *jhol bangla*	Calcutta	6·0	78	42, 52, 70, 72, 76	$A_6B_2C_{70}$	4	1·85–0·56	78·70	28·15	35·76
var. *pullykody*	Mannackanad	10·0	78	—	A_6C_{72}	3	1·67–0·56	77·96	27·40	35·15
var. *sanchipan*	Mechada	4·5	78	29	A_4C_{74}	2	1·85–0·56	79·62	29·26	36·74
var. *ranipan*	Ramnagar	10·0	58	26, 36, 52	A_4C_{54}	2	2·04–0·56	57·40	20·56	35·81
var. *karpurkanthi*	Mechada	4·5	42	46, 48	A_4C_{38}	2	2·22–0·56	49·62	17·96	36·19
var. *metha-cum-bangla*	,,	4·5	78	—	A_6C_{72}	3	2·22–0·74	85·92	30·93	35·99
var. *metha thackpala*	,,	4·5	78	—	A_6C_{72}	3	2·04–0·56	73·34	27·22	37·12
var. *metha gathpala*	,,	4·5	78	54, 58, 64, 68, 72	A_6C_{72}	3	1·85–0·74	92·96	34·81	37·45
var. *bangla*	,,	4·5	78	26, 52	A_4C_{74}	2	2·41–0·74	86·30	31·30	36·27
var. *gonogathe*	,,	4·5	78	64	A_4C_{74}	2	2·22–0·74	86·30	31·67	36·70
var. *hajurikiamali*	Cuttack	27·0	78	—	A_4C_{74}	2	2·04–0·74	80·74	29·81	36·93
var. *desi-mahoba*	Mahoba	209·0	78	66	A_4C_{74}	2	2·04–0·74	79·62	29·44	36·98
var. *desi-bangla*	Mahoba	209·0	78	—	A_4C_{74}	2	1·67–0·56	68·88	25·93	37·63
var. *kali-bangla*	Bagnan	5·4	78	—	A_8C_{70}	4	1·85–0·74	74·82	27·03	36·14

(*Table I continued*)

Name of the material	Locality of collection	Alt. in meters	2n	Somatic variation nuclei (2n)	Karyotype formula	Chromosome pairs with sec. constns.	Range of chromosome length (μ)	Total chromosome length (μ)	Total short arm length (μ)	T. F. value (%)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
var. *sanchi banaras*	Baraipur	80·0	78	52, 58	A_4C_{74}	2	1·85–0·65	72·40	27·22	37·60
var. *sadadhol*	Mechada	4·5	78	64	A_4C_{74}	2	1·85–0·74	75·92	28·15	37·10
var. *belhari*	Utukur	140·0	78	—	A_4C_{74}	2	1·85–0·74	80·00	30·00	37·50
var. *gachpan*	Utukur	140·0	78	54, 64, 72, 76	A_4C_{74}	2	1·85–0·56	75·92	28·33	37·32
var. *green*	Paramatti-velur	278·0	78	66	A_6C_{72}	3	1·85–0·74	76·66	28·15	36·71
var. *nagoli*	Utukur	140·0	78	—	A_8C_{70}	4	1·85–0·74	80·74	29·26	36·24
var. *kare*	,,	140·0	78	—	A_6C_{72}	3	2·41–0·74	84·82	29·81	35·15
var. *ramtek bangla*	Ramtek	320·0	78	—	A_4C_{74}	2	1·85–0·74	85·56	31·67	37·01
var. *kuljedu*	Utukur	140·0	78	72	A_4C_{74}	2	2·04–0·74	74·82	27·60	36·88
var. *kapoori-pune*	Vadmer Bhariao	586·0	78	—	A_4C_{74}	2	2·04–0·74	77·78	28·89	37·14
var. *bangla banarasi*	Baraipur	80·0	78	—	A_4C_{74}	2	2·04–0·74	75·92	28·33	37·32
var. *bhabna*	Utukur	140·0	78	—	A_4C_{74}	2	2·22–0·74	79·26	29·44	37·15
var. *ramtek kapoori*	Ramtek	320·0	52	58, 72	A_4C_{48}	2	1·85–0·56	48·52	17·78	36·64
var. *malvi*	Utukur	140·0	78	—	A_6C_{72}	3	2·22–0·74	88·14	32·03	36·34
var. *karpuri*	Utukur	140·0	78	—	A_6C_{72}	3	2·04–0·74	84·44	30·37	35·96
var. *deswari*	Mahoba	209·0	78	—	A_6C_{72}	3	2·04–0·74	91·12	32·78	35·98
var. *andaman-wild*	Andaman	79·0	52	—	A_4C_{48}	2	1·85–0·74	53·70	19·44	36·21
var. *gottipan-wild*	Gangtok	1768·0	52	39, 54	A_4C_{48}	2	1·67–0·56	46·30	16·85	36·40
var. *chava-wild*	Rongbull	2127·0	195	52	A_4C_{191}	2	1·48–0·56	173·88	65·02	37·38

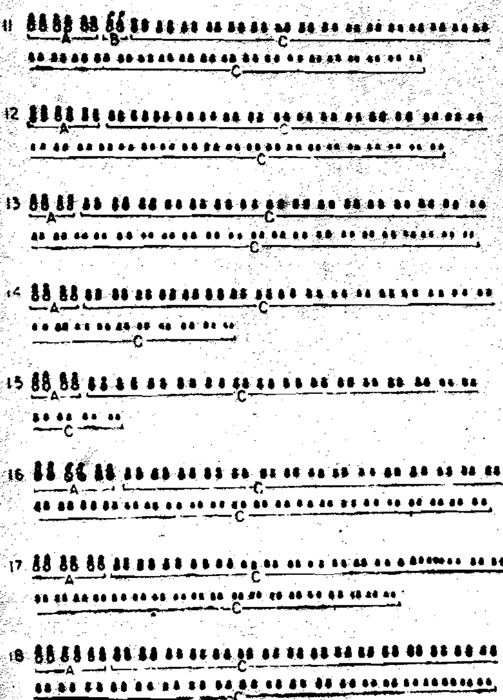


Figs. 1–10. 1–9. Somatic metaphase of *Piper betle* L. varieties *sanchipan*, *metha thackpala*, *metha gathpala*, *bangla*, *sadadohl*, *gachpan*, *karpuri-pune*, *malvi and desawari* respectively. All shows 2n = 78 chromosomes × 1060. 10. Somatic variation nucleus of *P. betle* var. *jhol bangla* 2n = 143 chromosomes (× 905).

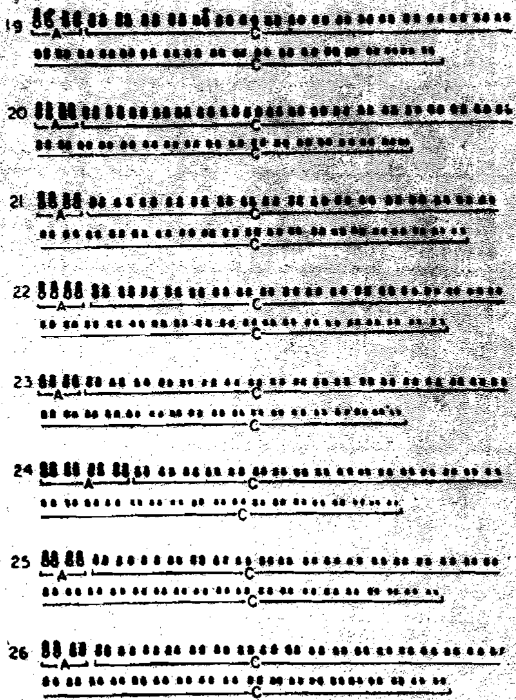
DISCUSSION

(i) Chromosome numbers in the genotypes studied

In the present study thirty three varieties of *Piper betle* have been included within the scope of the present investigation. Majority of the varieties show the chromosome number of 2n=78. In addition, there are 2n=42 chromosomes in the var. *karpurkanthi*, 2n=52 in the varieties *ramtek kapoori*, *andaman-wild*, *gottipan-wild*, 2n=58 in var. *ranipan* and 2n=195 in the wild variety *chava*. *Piper betle* in general, therefore, represents a polyploid species of which certain varieties show higher or lower levels of polyploid series with a basic set of 13 chromosomes. Previous reports have, however, given chromosome numbers as 2n=26 and 52 (Samuel and Bavappa 1981), 2n=32 ; 2n=64 (Sharma and

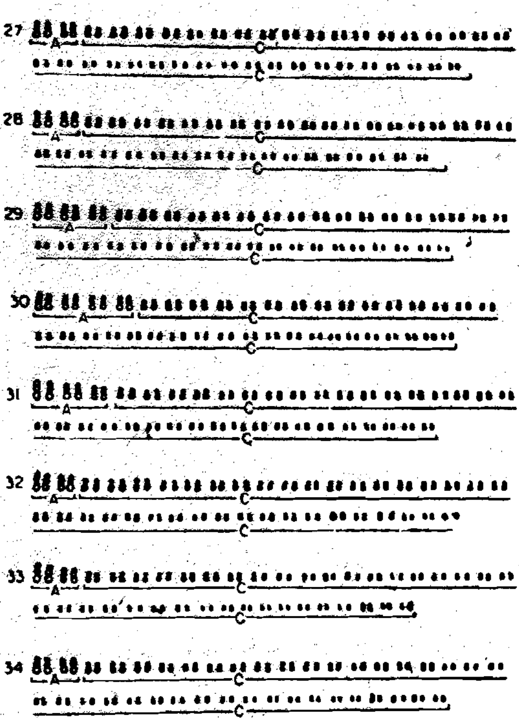


Figs. 11–18. Idiograms showing types of chromosomes present in varieties of *P. betle*, *jhol bangla*, *pullykody*, *sanchipan*, *ranipan*, *karpurkanthi*, *metha-cum-bangla*, *metha thackpala* and *metha gathpala* respectively (× 1575 ca).

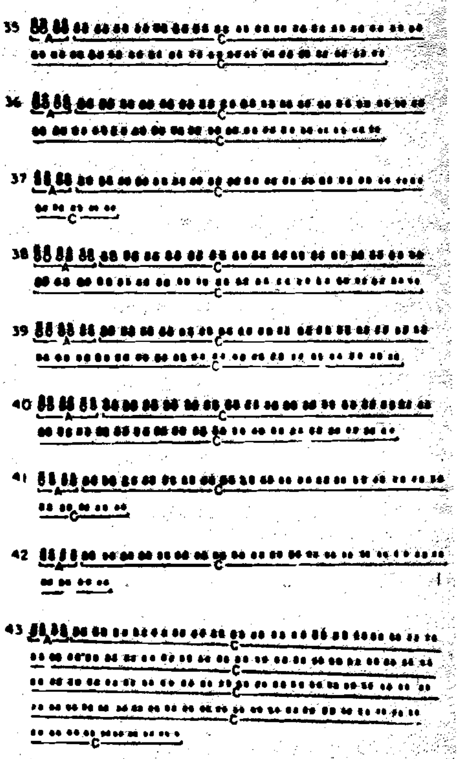


Figs. 19–26. Idiograms of *P. betle* varieties *bangla*, *gonogathe*, *hajurikiamali*, *desi-mahoba*, *desi-bangla*, *kali-bangla*, *sanchi banaras* and *sadadohl* respectively (× 1575 ca).

Bhattacharyya 1959 ; Dasgupta and Datta 1976) and 2n=78 chromosomes (Mathew 1958 ; Jose 1982). However, it is evident that polyploidy had played an important role in the evolution of *P. betle*. The extensive vegetative propagation of this species, which is its only method of reproduction, has evidently established these polyploid variations in cultivars. There are only occasional reports of flower formation after long intervals without, however, any seed formation. It is remarkable that, inspite of vegetative reproduction, there is constancy in the chromosome number in majority of the varieties which may be attributed to judicious selection. Moreover, numerical variations within the same individual have been noted in several varieties namely, *jhol bangla*, *sanchi*, *ranipan*, *karpurkanthi*. *metha gathpala*, *bangla*, *desi-mahoba*, *sanchi-banaras*, *gachpan*, *green*, *kuljedu*, *bhabna*, *ramtek kapoori*, *gottipan-wild* and *chava-wild* (vide Table 1). Such variance has been found principally when the roots were taken from the old stock. Numerical variations have been also reported earlier by Sharma and Bhattacharyya (1959). However, in view of the regularity of chromosome number in majority of the varieties, inspite of certain individual numerical



Figs. 27–34. Idiograms of *P. betle* varieties *belhari, gachpan, green, nagoli, kare, ramtek bangla, kuljedu* and *kapoori-pune* respectively (× 1575 ca).



Figs. 35–43. Idiograms of *P. betle* varieties *bangla banarasi, bhabna, ramtek kapoori, malvi, karpuri, desawari, andaman-wild, gottipan-wild* and *chava-wild* respectively. (× 1575 ca).

alterations it appears that the number 2n=78 has a wide selective value possibly due to certain adaptive advantage. In any case, the occurrence of certain varieties with the number other than 78 may be attributed to such somatic variants playing an effective role in the origin of new genotypes through their participation of the formation of daughter shoots. In majority of the cases, however in *P. betle*, such variant nuclei are possibly eliminated during selection.

(ii) Structural patterns of chromosomes in *Piper betle*

As far as the chromosome structure is concerned all varieties of *Piper betle* show a gross uniformity in the karyotype. The chromosomes, in general, are medium sized to small in a graded karyotype. The number of chromosomes with secondary constriction varies from 2 to 4 pairs. Certain varieties, however, differ from each other in the number of chromosomes with secondary constriction, even though the chromosome number remains constant. For example, the varieties such as *sanchipan, bangla, gonogathe, hajurikiamali, desi-mahoba, desi-bangla, sanchi banaras, sadadhol, belhari, gachpan, ramtek bangla, kuljedu kapoori-pune, bangla-banarasi* and *bhabna* have two pairs with secondary constrictions, whereas in *pullykody, metha-cum-bangla, metha thackpala, metha gothpala, green, kare,* 44

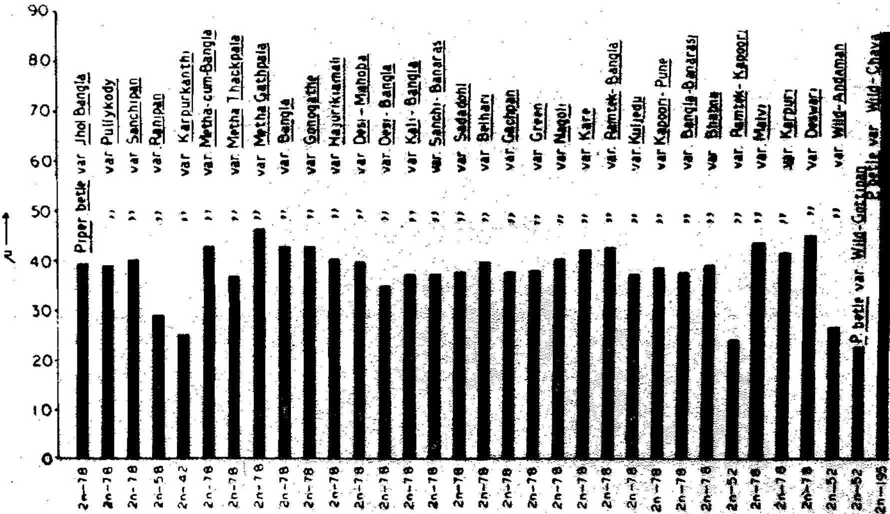


Fig. 44. Histogram showing the total length of chromatin matter in the haploid complement of different varieties of *Piper betle.*

malvi, *karpuri* and *deswari*, three pairs are present. Similarly *johl bangla*, *kali-bangla* and *nagoli* have four pairs of chromosomes with secondary constriction. All these varieties have 2n=78 as the normal somatic chromosome number. Except such variations, uniformity is observed in the karyotype.

The uniformity in the koryotype, with certain exceptions, taken in conjunction with the phenotypic differences, suggests that gene mutations or imperceptible chromosome changes have been principally responsible for the evolution and the origin of different genotypes of *P. betle*. The occasional strains with different numbers of chromosomes with secondary constriction no doubt indicate the importance of minute structural alterations in the origin of some of the strains. The presence of two pairs of chromosomes with secondary constrictions even in a highly polyploid strain like var. *chava-wild* (2n=195) is rather remarkable. It appears that along with polyploidy there must have been structural changes leading to loss of secondary constrictions in some of the chromosomes during the evolution. Amphiplasty, often noted following hybridity, may however, be ruled out in view of the absence of any flower production or sexual reproduction in the cultivated varieties. The cytological data obtained during the present investigation, where the chromosomes have been worked out with the aid of improved techniques, to be utilized for any project involving the improvement of the crop to maximise the yield of aromatic compounds.

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