Nucleolus Organising Chromosomes in *Brassica* and Their Bearing on the Phylogeny of the Genus

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Gates (1942) recognised a numerical correspondence in the nucleoli and satellites and their bearing on determining the ploidy of the taxon. Although later workers like De Wet (1953) and Stebbins (1964) did not report such a correspondence in the materials studied by them, in *Brassica* however during the course of the investigation made here on the pachytene chromosomes in the genomes 'a', 'b' and 'c' and their hybrids, a numerical correspondence in the number of nucleolus organisers has been observed in the different species studied here and the details are presented in this paper.

Materials and methods

The following species of *Brassica* and *Raphanus* and their hybrids are included in this investigation.

B. campestris var. *oleifera* (n=10), *B. pekinensis* (n=10), *B. juncea* var. *gracilis* (n=18) *B. napus* var. *oleifera* (n=19), *Raphanus sativus* (n=9) and *R. raphanistrum* (n=9).

B. napus var. *oleifera* (n=19) × *B. campestris* var. *oleifera* (n=10),

H₁, F₁ (2n=29),

B. campestris var. *oleifera* (n=10) × *B. napus* var. *oleifera* (n=19),

H₂, F₁ (2n=29),

B. napus var. *oleifera* (n=19) × *B. chinensis* var. *communis* (n=10),

H₃, F₁ (2n=29),

B. juncea var. *gracilis* (n=18) × *R. raphanistrum* (n=9),

H₄, F₁ (2n=27),

B. napus var. *oleifera* (n=19) × *R. raphanistrum* (n=9),

H₅, F₁ (2n=28).

Flower buds for pachytene chromosome studies were fixed in a fresh mixture of 1:3 acetic acid-absolute alcohol at 10 a.m. Chromosomes for pachytene studies were stained with 1% acetocarmine with ferric chloride as mordant. Camera lucida drawings were made from fresh preparations at bench level using 15x × oel. Photographs were made from fresh preparations. Chromosomes were measured with a graduated twine thread.

Results

In the 4 diploid *Brassica* species studied here the species *B. campestris* var. *oleifera* (n=10) and *B. pekinensis* (n=10) contained each two morphologically

distinct nucleolus organising chromosomes designated as types A_1 and A_2 (Fig. 1 and 2) in the haploid set while *B. juncea* var. *gracilis* ($n=18$) and *B. napus* var. *oleifera* ($n=19$) contained 4 nucleolus organising chromosomes each in their haploid sets. Two of these in the latter two species belonged to the 'a' genome and the other two to the 'b' and 'c' genomes respectively (Figs. 3 and 4). These observations agree well those of Röbbelen (1960). Sikka (1940) observed a numerical correspondence in the number of nucleoli and satellite chromosomes at somatic metaphase in the different *Brassica* species studied by him. However the details observed here differ from the report of Sikka (1940) regarding the number of nucleolus organising chromosomes in the different species and from that of Röbbelen (1960) regarding the morphology of one of the nucleolus organisers and the same are presented here.

1. Sikka (1940) observed two nucleoli and two satellitic chromosomes in each of the three species *B. oleracea* ($n=9$), *B. campestris* ($n=10$) and *B. napus*

Figs. 1-4. Type, genome and species of nucleolus organizing chromosomes.

($n=19$). In the present investigation of *B. campestris* var. *oleifera* ($n=10$) and *B. pekinensis* ($n=10$) both with the 'a' genome two nucleolus organising chromosomes were seen. In *B. napus* var. *oleifera* ($n=19$) 4 nucleolus organisers were seen two of which belong to the two of the 'a' genome, whereas the other two belong to the chromosomes of the 'c' genome.

2. In *B. juncea* Sikka (1940) found three pairs of satellitic chromosomes and 6 nucleoli, while in the present study in *B. juncea* var. *gracilis* ($n=18$) four nucleolus organising chromosomes were seen at pachytene, two of which resemble the two nucleolus organising chromosomes of *B. campestris* var. *oleifera* and the remaining two belong to the 'b' genome of *B. nigra*.

Although these observations largely agree with those of Röbbelen (1960) they differ from his in respect of the nucleolus organising chromosome 10 of *B. napus* var. *oleifera* which corresponds to the second nucleolus organising chromosome of

the 'c' genome. This chromosome possesses a terminal well defined heterochromatic satellite in the complement of *B. napus* var. *oleifera* ($n=19$) (Fig. 4) whereas Röbbelen (1960) observed in *B. oleracea* varieties the distal euchromatic portion of the chromosome serving as a satellite.

Discussion

The two nucleolus organisers in each of these three genomes 'a', 'b' and 'c' did not pair with each other in the three interspecific and two intergeneric hybrids studied here. Therefore, it is suggested that they were derived from two distinct parents.

Fig. 5. Schematic diagram suggesting the probable origin of nucleolus organising chromosome in the 'b' and 'c' genomes from the nucleolus organising chromosome of 'a' genome.

On the assumption that the number of nucleoli or satellites is an index of the ploidy of a taxon (Gates 1942) the three genomes 'a', 'b' and 'c' might be considered as allotetraploids and the species *B. juncea* var. *gracilis* ($n=18$) and *B. napus* var. *oleifera* ($n=19$) as allo-octoploid hybrids having arisen due to natural hybridisation of the genomes 'a', 'b' and 'c'. The number of nucleoli however did not show any correspondence with those of the satellitic chromosomes. Such a lack of correspondence in the number of nucleoli has also been reported in *Danthonia* polyploids (De Wet 1953).

The discrepancy between the present observations and those of Sikka (1940) and of Röbbelen (1960) may be ascribed to the fact that the terminal heterochromatic satellite in the materials studied by them might have been lost due to mutation in the course of evolution of the species as has been suggested by Gates (1942) in the several materials studied by him. Such a possibility has also been suggested by Sikka (1940) in *Brassica* and De Wet (1953) in *Danthonia* polyploids. Navaschin (1926, 1934) applied the term amphiplasty to such a phenomenon observed by him in *Crepis*. Ramanujam (1937) found in rice varieties the loss of one pair of nucleoli and also the deletion of not only the satellite and thread but also of the nucleolus organizer at the end of the chromosome. Such a loss has also been reported by Jacob (1940) in *Cassia* sps. Gates (1942) suggested a probability that a satellite can be lost also by a gradual diminution until it is below the limits of visibility and that further change in the same direction will lead to its functional and structural diminution.

In polyploids although the terminal nucleolus organiser or the satellite were

lost that would not affect the cytological balance of the plant very severely and the plant would continue to survive. Since the genus *Brassica* is ancient and its exact origin is not yet determined the genus allows for enough time for such a process to take place as has also been interpreted in the case of *Danthonia* (De Wet 1953).

Another possibility is there that the nucleolus organiser chromosome 8 (type A_2) which is having a short arm so short enough at pachytene itself that there is the likelihood of this being not clearly visible in the somatic chromosomes, since the chromosomes of *Brassica* at somatic metaphase are so very small that neither the centromere nor the nucleolus organiser on it could be located with any confidence. It appears from our studies made here that this chromosome 8 is characteristic or is a regular member of the forms with 'a' genome alone and so it is equally likely that in all the forms with 'a' genome with $2n=20$ such as *B. rapa* L., *B. campestris* L., *B. trilocularis* L., and *B. tourneforti* studied by Sikka (1940) the short arm of this may not be easily discernible at somatic metaphase.

Since the number of species investigated here is very small we could not demonstrate the exact origin of any variety or species or the loss or diminution of the size of the satellite. Therefore it is suggested here as a possibility apparent from the observations made here. The two nucleolus organising chromosomes of the 'a' genome are referred here as types A_1 and A_2 and those of the 'b' and 'c' genomes as the types A and C as already stated (Venkateswarlu and Kamala 1971). The chromosomes of the type A_1 of the 'a' genome and the type A of the 'b' and 'c' genomes are found to be morphologically similar except for the number of intercalary chromomeres between the terminal satellite and the proximal heterochromatic segment on the short arm (Figs. 1-4). But the chromosomes of the type A_2 of the 'a' genome and type C of the 'b' and 'c' genomes are quite different in their morphology. The chromosomes of the type C of the 'b' and 'c' genomes seem to be more closely related morphologically with chromosome type C of the 'a' genome. Although the chromosome type A_2 is present in the 'b' and 'c' genomes of *Brassica* such a type of chromosome is also seen in some other genera of Cruciferae like *Diplotaxis muralis*, *Erysimum cheiranthoides*, and *Raphanus* spp. which have been examined here for comparison during this study. It is obvious that this chromosome forms one of the basic types of the haploid chromosomal complement of at least some of the Cruciferae.

It appears from the observations of the pairing of chromosomes in the inter-specific and intergeneric hybrids studied here that the nucleolar chromosome in the 'b' and 'c' genomes might have originated due to a non-homologous translocation between a nucleolar and a non-nuclear chromosome in the original hybrid which gave rise to the 'b' and 'c' genomes. The second nucleolus organising chromosome of the type C in the 'b' and 'c' genomes probably resulted from a non-homologous and unequal translocation involving the terminal portion of the C type of chromosome (i.e. a non-nuclear type) and the short arm of the chromosome type A_2 seen in the 'a' genome (Fig. 5). Such a type of non-homologous translocation has been recorded in *Triticum macha* (Bhaduri and Ghosh 1955). They found an extra pair of nucleolar chromosomes with supernumerary constriction being separated by a small chromosome segment. This has been interpreted by them as derived

through a translocation between two non-homologous chromosomes. Although no translocation configurations could be observed there in *Brassica* species involving the above referred chromosomes, the observation of anaphase bridges in the *Brassica* species by Sikka (1940) supports our above conclusion. Sikka (1940) also suggested that translocations may play a role in the phylogeny of *Brassica*.

The number of nucleoli however does not show any correspondence with those of the satellitic chromosomes. Such a lack of correspondence in the number of nucleoli has also been reported in *Danthonia* polyploids (De Wet 1953).

Although Gates (1942) indicated that the number of nucleoli and the nucleolar regions should proportionally increase in the polyploids he stated that many polyploids are characterised by two pairs of nucleoli. Bergman (1935) showed in *Leontodon incanus* and *L. asperimus* two chromosome pairs bearing satellites and producing two pairs of nucleoli. Stebbins (1964) indicated that many polyploids are known that never produce more than two nucleoli. In the four diploid *Brassica* species studied here only two nucleoli could be seen. Therefore in *Brassica* although the number of nucleolar chromosomes does not seem to deny the theory of Gates (1942) the number of nucleoli could not help in deciding the degree of ploidy of the complement.

Summary

A numerical correspondence is seen in the number of nucleolus organising chromosomes in the 4 diploid *Brassica* species referred in the text. Based on the numerical correspondence the allopolyploid nature of not only of *B. juncea* var. *gracilis* ($n=18$) and *B. napus* var. *oleifera* ($n=19$) but also of the three genomes 'a', 'b' and 'c' has been suggested. The probable origin of the nucleolus organising chromosome of 'b' and 'c' genomes from that of 'a' genome through a reciprocal translocation has also been suggested.

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