Studies on Nucleolus and Nucleolar Chromosomes in Angiosperms

VI. Nucleolar organization in genus *Tulipa* L. (Liliaceae)

A. K. Koul, Anima Langer and B. A. Wafai

Department of Biosciences, University of Jammu, Jammu-18001, India

Accepted December 17, 1987

Nucleolar organization is ascribed to certain specific chromosomal sites which are visible under the light microscope as secondary constrictions. Literature is, however, replete with instances where such constrictions have not been seen, yet the nucleolar organization is normal. Among angiosperms, *Tulipa* a liliaceous genus, is one such example and even today continues to be controversial in so far as its nucleolar chromosomes are concerned. Varying claims have been advanced from time to time about the number and structure of these chromosomes in different species. The reports vary from those suggesting total lack of visible NORs (Wafai and Koul 1981) to those indicating multiplicity of such regions (Woods 1937). In order to settle the controversy, it has become necessary to re-investigate the karyology and undertake studies on nucleologenesis of the genus. Two varieties of *T. clusiana* DC., namely var. *chrysantha* Sealy and var. *stellata* Regel have been chosen for the purpose; the former is a diploid ($2n=24$) and the latter a tetraploid ($2n=48$).

Materials and methods

Bulbs of *T. clusiana* var. *chrysantha* were collected from natural populations growing at hill slopes of Duksum sanctuary (Dist. Anantnag, Kashmir) and those of var. *stellata* from Pampore plateau and along the bank of Manasbal lake (Kashmir).

Bulbs were nurtured in moist sand at room temperature during November. For karyology, healthy root tips were pretreated either with a saturated solution of para-dichlorobenzene or 0.002 M 8-hydroxyquinoline for 3–4 hrs at 10°C. The root tips were thoroughly washed in running water to remove last traces of the pretreating agent, fixed in acetic-alcohol (1:3) for 24 hrs and stained in acetic-orcein or Feulgen.

For nucleolar studies, air dried slides containing root tip tissue were stained in 50% aqueous solution of silver nitrate.

Observations

1. *T. clusiana* var. *chrysantha*

1.a. Karyotype: All well spread metaphase plates consist of 24 chromosomes. Except for the smallest two which are submetacentric all other chromosomes are of the subtelocentric type. Details of the karyotype have been presented in Table I. Figs. 1 and 2 reveal that eight chromosomes bear secondary constriction. In two of them, the secondary constriction exists in the middle of short arm and in the remaining six, it is located terminally in the long arm. The chromatin segments distal to the secondary constrictions in these six chromosomes are too minute and therefore elude detection. However, in Feulgen stained preparations, they appear

Table 1. Karyotype of *T. clusiana* var. *chrysantha*

Details	Chromosome type				
	A	B	C	D	E
No. of chromosomes	6	2	8	6	2
Length (μm)	4.74–5.54	5.08	3.29–4.27	2.68–3.30	2.61
r-index value*	5.90	3.14	4.70	3.40	1.40
Position of centromere**	St	St	St	St	Sm

* Long arm: short arm ratio

** St, subtelocentric; Sm, submetacentric

Figs. 1-3. 1, 2, photomicrograph (Fig. 1) and camera lucida drawing (Fig. 2) of a part of the root tip chromosome complement of diploid *Tulipa clusiana* var. *chrysantha* showing 8 nucleolar chromosomes (arrows). Scale 10 μm . 3, camera lucida drawings of nucleolar chromosomes from various cells of 2x *T. clusiana* var. *chrysantha* pretreated with HQ and PDB.

as tiny dots at the tips of long arms. Only in 20% of the total cells scored, were all the eight satellites visible; cells with five and six are most frequent. For detecting satellites, HQ and PDB pretreatments proved equally effective (Fig. 3). Of the eight SAT chromosomes, six match into homomorphic pairs but two are heteromorphic.

1.b. Nucleolus: Interphase nuclei of root meristem are mono- to octonucleolate (Figs. 4, 5). Cells with maximal (8) and minimal (1) number of nucleoli are least frequent. Frequency of cells with different nucleolar number has been presented in Table 2.

2. *T. clusiana* var. *stellata*.

2.a. Karyotype: Forty-eight chromosomes exist in all well spread metaphase plates of this taxon. These resemble those of diploid *T. clusiana* var. *chrysantha* in gross morphology (Table 3).

Table 2. Break-up of root tip cells of 2x *T. clusiana* var. *chrysantha* with different nucleolar numbers

	Number of nucleoli per nucleus							
	1	2	3	4	5	6	7	8
No. of cells	11	130	294	344	212	69	15	3
% frequency	1.02	12.05	27.27	31.91	19.66	6.40	1.39	0.27

Table 3. Karyotype of *T. clusiana* var. *stellata*

Details	Chromosome type				
	A	B	C	D	E
Number of chromosomes	12	4	16	12	4
Length (μ m)	8.0-10.7	8.2-9.5	5.7-7.9	4.3-5.6	3.6-4.3
r-index value	6.97	3.87	5.83	4.46	1.80
Position of centromere	St	St	St	St	Sm

Table 4. Percentage frequency of different nucleolar counts in root tip nuclei of tetraploid *T. clusiana* var. *stellata*

	Number of nucleoli per nucleus											
	1	2	3	4	5	6	7	8	9	10	11	12
Total no. of nuclei scored 1, 135	2.1	2.7	4.5	4.6	5.7	17.8	27.4	18.2	7.5	3.9	3.6	1.7

A maximum of eleven secondary constrictions could be detected per chromosome complement (Fig. 6), although in majority of cells scanned only five to seven could be detected with certainty (Fig. 7). Chromosomes bearing secondary constrictions are of four types: two correspond to those of var. *chrysantha*; of the remaining two types, one is represented by the smaller submedian chromosomes which have the secondary constrictions half-way within the short arm. The fourth type has two constrictions per chromosome in addition to the centromere; one is terminal in the long arm and another intercalary within the short arm.

2b. Nucleolus: Root tip cells possess 1 to 12 nucleoli per interphase nucleus; nuclei with 7 and 8 nucleoli are, however, most frequent (Figs. 8, 9). Table 4 depicts the break up of nuclei with different nucleolar counts.

Discussion

The most important task ascribed to secondary constriction is transcription of rRNA. On account of its potentiality, this chromosome segment is vital for the very survival of an organism. This is amply demonstrated by the fact that the anucleolate mutants of *Xenopus laevis* fail to differentiate beyond the tail bud stage (Fischberg and Wallace 1960). In the

Figs. 4-9. 4 and 5, root tip cells of 2x *T. clusiana* var. *chrysantha* showing 7 and 8 nucleoli respectively. Scale 10 μ m. 6, root tip chromosome complement of 4x *T. clusiana* var. *stellata* ($2n=4x=48$). Note 11 SAT chromosomes (arrows). 7, camera lucida drawing of nucleolar chromosomes from various cells of 4x *T. clusiana* var. *stellata*. 8 and 9, root tip cells of 4x *T. clusiana* var. *stellata* bearing 11 and 12 nucleoli respectively. Scale 10 μ m.

recent years, the second task ascribed to this region is of organizing the materials synthesized elsewhere on the chromosomes. In recognition of its dual role, the secondary constriction is aptly referred as "synthesizer-cum-organizer" (Phillips 1978).

In view of the paramount importance of NOR to the life of a cell, reports of the existence of chromosome complements devoid of NORs are intriguing. Should these be correct, where and how is the rRNA synthesized and nucleolus organized in these taxa. Materials where past work on karyology is silent about the secondary constriction are obviously in need of re-investigation. Do they represent a naive system with the site of nucleolar organisation lying somewhere other than the secondary constriction or have their NORs escaped observation in past? In tulips, secondary constrictions have been reported in very few species. In majority (cultivated as well as wild) secondary constrictions are unknown. Even where they have been reported, there is great deal of disagreement regarding their number and morphology (see Table 5).

Table 5. Number of nucleoli and nucleolar chromosomes in diploid tulips ($2n=2x=24$)

Species	No. of SAT chromosomes	Maximum no. of nucleoli	Authority
<i>T. stellata</i>	2	—	Upcott and Lacour (1936) Woods and Bamford (1937)
	6-8	6-9	Woods (1937)
<i>T. clusiana</i> var. <i>chrysantha</i>	—	8	Wafai and Koul (1981)
(<i>T. stellata</i> var. <i>chrysantha</i>)	8	8	Present work
<i>T. aitchisonii</i>	—	8	Wafai and Koul (1981)

Present results indicate that in tulips, at least the two worked out now, secondary constrictions are too minute and therefore elude visibility but the interphase nuclei bear varying numbers (1-8 or 12) of well organised nucleoli. Woods (1937) too noted a high number of nucleoli per nucleus. Based on his meiotic data, he pointed towards the nucleolar chromosome plurality, which for want of further reports, received no confirmation through karyology. Recently, Sen (1977) undertook karyotypic studies in the genus and detected only two secondary constrictions; those located midway in the short arms of long subterminal chromosomes. She attributed their detection to the hydroxy-quinoline pretreatment. Present findings on the contrary, clearly demonstrate that the material under reference responds equally well to both HQ as well as PBD. It is the minute size of satellite that accounts for their having remained unnoticed so far. This is probably the cause which prompted Woods (1937) to confess that, "determination of exact number of SAT chromosomes through karyology in *Tulipa* is rather difficult." The distal satellites on the long arms of six chromosomes are so small that their detection in aceto-orcein preparations is well high impossible. However, being DNA specific, Feulgen staining succeeds in bringing out these segments despite the disadvantage of their small size.

Existence of as many constrictions is confirmed by their activity estimated in terms of nucleolus organization. Based on present observations on two varieties of *Tulipa clusiana* and earlier work on *Allium consanguineum* (Langer and Koul 1977) we are inclined to state that the presence of specific NOR is imperative in eukaryotes and therefore, all those cases where nucleolus organizers have not been reported thus far, represent materials where these regions are not amenable to conventional schedules and have therefore eluded detection. Langer (1980) stressed that in cases, where NORs are located at chromosome termini, an extreme reduction of chromatic segment distal to secondary constriction may lead to the obscurity

of these regions. Similarly, interstitial secondary constrictions are likely to be overlooked when they are located very close to the centromere as in *Allium consanguinum*.

Abstract

In tulips secondary constrictions have been reported in very few species and these reports are highly controversial. Diploid, var. *chrysantha* and tetraploid, var. *stellata* of *Tulipa clusiana* respectively have 8 and 11 NORs per complement which is confirmed by the formation of as many nucleoli in root tip nuclei. The disparity in the reports made so far is on account of the terminal position (in long arm) and minute size of the satellites. They can conveniently be revealed by Feulgen staining.

References

- Fischberg, M. and Wallace, H. 1960. A mutation which reduces nucleolar number in *Xenopus laevis*. In: Mitchell, J. S. (ed.) The Cell Nucleus. London.
- Langer, A. 1980. Studies on the nucleolar organisation in some angiosperms. Ph. D. Theiss, University of Jammu.
- and Koul, A. K. 1977. Studies on nucleolus and nucleolar chromosomes in angiosperms I. Nucleolar organization in *Allium consanguinum* Kunth. *CIS* 27: 25–26.
- Phillips, R. L. 1978. Molecular cytogenetics of the nucleolus organizer region. In: Walden, B. (ed.) Maize Genetics & Breeding. John Wiley and Sons.
- Sen, S. 1977. Chromosomes and evolution in species of *Tulipa*. *Acta. Bot. Ind.* 5: 128–132.
- Upcott, M. and LA Cour, L. F. 1936. The genetic structure of *Tulipa* I. A chromosome survey. *J. Genet.* 33: 237–254.
- Wafai, B. A. and Koul, A. K. 1981. Chromosome complement in relation to species interrelationships within section Clusianae (genus *Tulipa* L.). In Manna, G. K. and Sinha, U. Perspectives in Cytology and Genetics 3: 73–79.
- Woods, M. W. 1937. The nucleolus in *Tulipa*. *Amer. Jour. Bot.* 24: 522–536.
- and Bamford, R. 1937. Chromosome morphology and number in *Tulipa*. *Amer. J. Bot.* 24: 175–184.
-