Theor. Appl. Genet. 53, 229-231 (1978)

Revision of *SAT* Chromosome Number in *Triticum monococcum* with Respect to Nucleolar Organizers Activity

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Summary. Six varieties of *Triticum monococcum* were analysed by means of the nucleolar test; i.e., estimation of the maximum number of primary nucleoli per nucleus. All of the varieties exhibited 4 primary nucleoli in telophase and early interphase. Following detailed karyological analysis four *SAT* chromosomes in all six karyotypes were found in accordance with the maximum nucleolar number. Secondary constrictions and microsatellites were localised on the short arms of chromosome pairs 3 and 5. A new order of the chromosomes in the idiogram of *Tr. monococcum* is proposed.

Key words: *SAT* chromosome – *Triticum monococcum* – Nucleolar test

Introduction

The rule postulated by Heitz (1931) that 'the number of telophase nucleoli is constant, as well as the number of *SAT* chromosomes, for a given karyotype' acquires, at present, a new meaning and explanation since it has been determined that the genes coding for ribosomal RNA are located in the secondary constriction of *SAT* chromosomes. The evidence supporting this viewpoint is based on in situ hybridisation (see review in Wobus 1975), on experimental data (Beermann 1960; Nicoloff et al. 1977; Anastassova-Kristeva et al. 1977, 1978) and on electron microscopic findings (Scheer et al. 1977; Goessens 1976; Jordan and Luck 1976; Jordan 1977). As a result of the data obtained it can now be maintained that the nucleoli are formed *from*, but not *on*, the secondary constrictions. This leads to the conclusion that the maximum number of nucleoli per nucleus should correspond to the number of the synthesizing rRNA loci characteristic for a given karyotype. This rule has repeatedly been proved and confirmed (Anastassova-Kristeva 1977; Nicoloff et al. 1977).

Because of its presumable contribution to the genome of the hexaploid wheat *Triticum aestivum L.*, the diploid wheat species *Triticum monococcum* L. has often been studied genetically and cytologically (Smith 1946; Câmara 1943; Levan and Tjio 1950; Kihara and Yamashita 1954; Riley and al. 1958; Upadhya and Swaminathan 1963; Coucoli and Scorda 1966).

In the idiogram of *Tr. monococcum var.* 'Hornemanni' reported by Câmara (1943), two satellite chromosome pairs have been described, one of them being the shortest chromosome pair of the karyotype. This finding, however, has not been confirmed by more recent data in which only one satellite chromosome pair has been recorded (Riley et al. 1958; Upadhya and Swaminathan 1963; Coucoli and Scorda 1966; Patil and Deodicar 1967). In some cases no *SAT* chromosome pair whatever has been reported (Stapova 1969).

By means of the nucleolar test we aimed at establishing the number of primary nucleoli in *Tr. monococcum* and by suitable karyological analysis, to identify the *SAT* chromosome number so far as it corresponds to the maximum number of nucleoli produced.

Material and Methods

The following varieties of *Tr. monococcum* were studied: 1. var. 'vulgare', 2. var. 'flavescens', 3. var. 'Einkorn', 4. var. 'monococcum', 5. var. 'bestissimum', 6. var. 'Hornemanni'.

Nucleolar Test

Root tip meristems were fixed in ethanol, formaldehyde, glacial acetic acid (6:3:1) for 24h at 4° C. After rinsing in 70% ethanol and in tap water, a maceration with 4% pectinase for 90 min at 37° C was performed. Squashes, prepared by the dry ice method, were stained for 40 min at 56° C with methyl green pyronine (Unna) and differentiated with absolute ethanol or tertiary butanol. The preparations were clarified in xylene and mounted in Canada balsam. [The slides were viewed through a Zetopan

0040-5752/78/0053/0229/$ 1.00

Reichert microscope using a Hg-monochromate filter D and a yellow-green filter.]

It is worth mentioning that a selective nucleolar staining was obtained by the Ag-As method (Goodpasture and Bloom 1975) applied after the above mentioned manner of fixation (Fig. 1).

Chromosome Identification

The seeds were presoaked in distillated water for 2h and germinated at 24° C. The roots were fixed in ethanol-acetic acid (3:1) after pretreatment in a saturated solution of α-bromonaphthalene for 90 min. Squash preparations were prepared after Feulgen stain and maceration in Bistrin for 40 min. Ten metaphases were scored for each variety studied.

Results and Discussion

The maximum number of nucleoli in the cells of all six varieties was four. Two of the primary nucleoli were usually smaller: this difference being better expressed in var. 'Hornemanni' than in var. 'Einkorn' (Fig. 1). The differences in the nuclear size were more conspicuous in telophase, since in early interphase the nucleoli became consecutively uniform and began to fuse (Fig. 1). The percentage of cells containing the maximum number of nucleoli depends on the rate of association after mitosis and is different in the varieties studied.

Two *SAT* chromosome pairs for each of the six varieties were identified (Fig. 2). In the karyotype of var. 'Hornemanni', a definite difference in the size of the satellites was observed. Two of them are comparatively larger and belong to the longer *SAT* chromosome pair while the smaller satellites are situated on the short arm of the shorter *SAT* pair. In other cases the shorter *SAT* pair possesses larger satellites but further extensive quantitative studies for the exact estimation of these differences are warranted.

Designating the seven chromosomes by their relative length, the *SAT* chromosome pairs in our idiogram (Fig. 3A) appear to be number 3 and number 5. They are clearly submetacentric, the S/L ratio being 1,98 and 2,33; in all other chromosomes this ratio ranges about 1 to 1,50, so that they are rather metacentric. This difference allows for an easy distinction between chromosome pairs 3 and 4 and between 5 and 6, whose total length is nearly identical. We propose to place the two *SAT* chromosome pairs at the end of the idiogram, which makes it more clear (Fig. 3B). Such an order is accepted for barley karyotype and shown to be suitable for wheat karyotype as well. According to this manner of arrangement, the *SAT* chromosome pairs of *Tr. monococcum* shift to number 6 and number 7. In some cases chromosome pair number 1 appears to be heteromorphic, the S/L ratio being approximately 1,00 in the one chromosome and 1,50 in the other.

The present studies reveal without question the presence of two *SAT* chromosome pairs in the karyotype of all the six varieties of *Tr. monococcum* analysed. This finding corresponds to the maximum number of four nucleoli found in these species. Our results concerning the *SAT* chromosome number are in accordance with the findings of Câmara (1943), although they differ in the identification of the *SAT* chromosomes in the karyotype. In Câmara's idiogram *SAT* chromosome pairs are number 2 and number 7, while according to our estimation they are number 3 and number 5. This means that the second *SAT* chromosome pair is not the shortest in the karyotype as claimed by Câmara. Moreover, according to Câmara, the larger satellite in var. 'Hornemanni' belongs to the shortest chromosome pair, while in our studies it belongs to the longer *SAT* chromosome pair number 3.

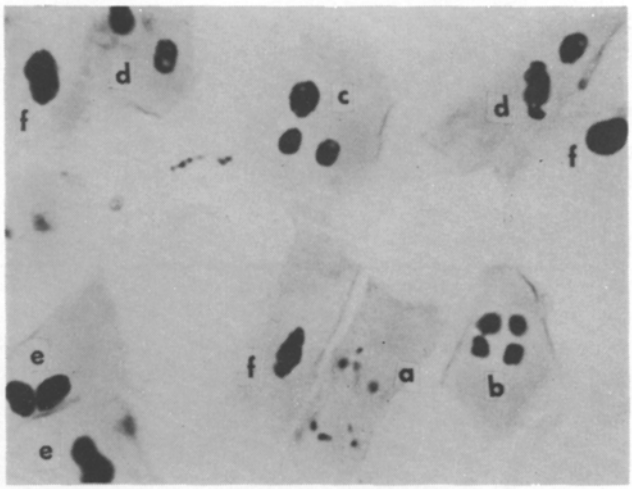

Fig. 1. *Triticum monococcum* var. Einkorn. Ag-As stain of cells fixed according to Serra.a) Early telophase cells containing four primary nucleoli.b) The four nucleoli grow bigger and two of them are already connected with a thin bridge.c) The first fusion is already realized.d) Two further nucleoli are associated.e) The two double nucleoli fusing together.f) All nucleoli are associated, forming a single nucleolus. 800x

Fig. 2. Four *SAT* chromosomes are well visible in the metaphase plate. 2000x

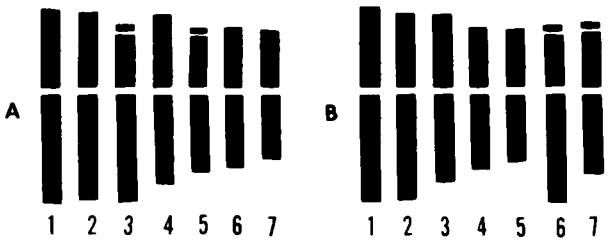

Fig. 3. A-B. Idiogram of *Tr. monococcum.* **A** order according the chromosome length; *SAT* chromosomes are number 3 and number 5; **B** the *SAT* chromosomes ordered at the end of the idiogram are now number 6 and number 7 (our proposal)

Chromosome N° according to B	1	2	3	4	5	6	7
Short arm	50	49	46	42	40	30	32
Long arm	75	67	54	53	47	70	63
Total length (relative)	125	116	100	95	87	100	95
S/L ratio	1,50	1,37	1,11	1,26	1,18	2,33	1,98

(The share (in per cent) of individual chromosome arms is in relation to the longer *SAT* chromosome pair taken as 100)

The attachment of two bivalents (Georgiev 1977) to the nucleolus in meiosis should be additional evidence for the participation of two *SAT* pairs in nucleolar formation in *Tr. monococcum*. However, only one or no bivalent may be attached to the nucleolus in diakinesis. Our investigations (Anastassova-Kristeva 1975, 1976) have shown that during late diplotene the necleolus does not incorporate tritiated uridine and is synthetically inactive. Therefore, the connection between nucleolar organizers (chromosome secondary constrictions) and a persistent diplotene nucleolus (nucleolar granular part) in diakinesis is only spatial and not functional. The estimation of the *SAT* chromosome number in *T. monococcum*, according to the nucleolar organizers activity by means of the nucleolar test, indicates that this approach may be very useful for examination of the nucleolar pattern and *SAT* chromosome number of tetra- and hexaploid wheat as well. A similar revision is obviously necessary for all the karyotypes to which *Tr. monococcum* is supposed to be the donor of the *AA* genome.

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Received July 5, 1978
Communicated by F. Mechelke

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