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CONTRIBUTION TO THE REVISION OF THE BARLEY KARYOTYPE

by

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Abstract: *The karyotype of another Hordeum vulgare L. cultivar, namely the «Georgia» barley, was established. Chromosome numbering followed the order of the standard karyotype, according to which 1-5 designate the non satellite members of the genome, and 6-7 represent the two SAT pairs. The results clarify the following ambiguous points: 1) Chromosome No. 1 has the most median centromere among the three longest pairs, but it is not significantly longer than chromosome No. 2. 2) Chromosome No. 5 is the shortest and the second anisobrachial member of the complement. 3) Satellited chromosome 7 is typically submetacentric, falling to the SM centromere class. The data, discussed in detail, comparatively to the information provided by other workers for different barley varieties, are in agreement with the revision proposed recently for barley karyotype as regards the arm ratio values of the two longest chromosomes. Besides, they give additional evidence concerning the morphology of the SAT pairs.*

INTRODUCTION

Chromosome morphology of common barley (*Hordeum vulgare* L.) has for long been studied by various workers (Tjio and Hagberg 1951, Morrison 1959, Tuleen 1973, Noda and Kasha 1978). Though the informative data provided so far are really substantial the identification of particular chromosome pairs remains partly unconfirmed. This is due to the differences revealed by the numerous cultivars used, at times, and the length overlapping constantly found between some of the pairs.

Undoubtedly the Giemsa C-banding techniques, applied by Linde-Laursen firstly in «Emir» barley (1975) and later in 20 diverse lines (1978) as well as by Vosa (1976) in four distinct barley varieties provided new insights towards identifying particular members of the genome. However, the problem could not, as yet be thoroughly elucidated, owing to the restricted amount of heterochromatin, the distribution of the banding patterns into small zones and the observed C-banding intervarietal polymorphism (Weimarck 1975, Vosa 1976).

Tuleen (1973), based on karyotype analysis of barley multiple translocation stocks, was the first to find some discrepancies relative to the normal karyotype, as designated by Tjio and Hagberg (1951). Subsequently, due to the application of the new techniques, a revision of the standard karyotype was proposed (Noda and Kasha 1978, Linde-Laursen 1978). It is at this revision that the present work aims for further evidence, by establishing the karyotype of the cultivar «Georgia» barley, and by comparing the resulting data with the information provided so far about the normal chromosome complement.

MATERIALS AND METHODS

The «Georgia» barley, introduced formerly into Greece, has for long been cultivated here, and it is well known as a flexible variety successfully adjustable to environmental changes. The seed material, used in the present study, was kindly provided by Dr. E. Skorda Scientific Director of the Cereal Institute (Thessaloniki).

Microscopic slides were prepared and the observation carried out on mitotic metaphase cells of root-tips. The usual Feulgen technique of staining and squashing was applied (Riley et all. 1958, Coucoli and Symeonidis 1980), supplemented by cold pretreatment of the seedlings, according to the method used by Kaltsikes et al. (1969).

Metaphase cells were selected, analysed and photographed and the karyotype subsequently defined, in accordance to the process applied and described by Coucoli and Symeonidis (1980). The measurements were made on 10 selected cells with similar degree of contraction and separately recognizable chromosomes.

In the idiogram, the chromosones have been put in the numbering order firstly proposed by Tjio and Hagberg and maintained by all subsequent workers. On this basis, chromosomes 1-5 designate the nonsatellite members of the genome, arranged in order of decreasing total length, chromosome 6 is the one having large satellite, and member 7 represents the chromosome pair with the small satellite. Relative length was used as a substitute for absolute values, in order to compensate for the intercell variation owing to different degree of contraction. A t-test was used to determine the differences in chromosome relative lengths.

RESULTS

The synoptic data of karyotypical analysis are presented in Table I.

TABLE I

Total chromosome relative length, arm relative length and arm ratio in barley cultivar «Georgia» (Percentage values)

Chrom. pair	Total Relative length	short arm length	long arm length	arm ratio	centromere class
1	15.67±1.42	7.34±0.47	8.33±0.56	0.88	M
2	15.53±0.85	6.79±0.24	8.74±0.59	0.78	MSM
3	14.59±0.43	6.61±0.36	7.98±0.39	0.83	MSM
4	13.55±0.97	6.45±0.40	7.10±0.76	0.90	M
5	12.41±0.67	5.26±0.41	7.15±0.40	0.73	SM
6	13.59±0.58	6.62±0.40 (4.62+2.00*)	6.97±0.35	0.94	M
7	14.66±0.49	5.60±0.27 (4.15+1.45*)	9.06±0.31	0.62	SM

*length of satellite

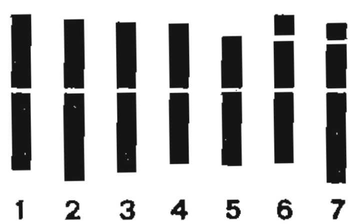
M = a.r. 0.86 - 1.00
MSM = a.r. 0.76 - 0.85
SM = a.r. 0.50 - 0.75
ST = a.r. <0.50

According to the above values, the variation of size ranges between 15.67 (chromosome 1) and 12.41 (chromosome 5), which means that the shortest pair is equal to 79.19% the length of the longest one (ca. 4:5) Between the two longest pairs (1 and 2) no significant difference was found, so their mean values overlap each other, due to the size variation of the same chromosome in different cells, chromosome 1 being more variable than chromosome 2. As regards the arm ratio, the two members, though broadly similar in centromere class, can be fairly distinguished

(a)

(b)

Fig. 1. The chromosome complement of the "Georgia" cultivar barley. a. Karyotype, b. mitotic chromosomes in a metaphase cell, c. the corresponding idiogram.



(c)

because chromosome 2 was constantly found less equal armed, whereas chromosome 1 appeared typically metacentric (Fig. 1).

Chromosome 3 seemed to be rather uncertain, resembling with 2 in arm ratio, but checked significantly shorter. Chromosome 4 can be identified by being the most metacentric non-satellite member of the genome (a.r. 0.90), yet significantly shorter than chromosome 1 (the same arm ratio). No. 5 chromosome is one among the most clearly recognizable chromosomes, being the shortest and most anisobrachial non-satellite member of the whole set (Fig. 1).

The two SAT chromosomes are also fairly identifiable in all cells studied. The pair No 6 has the larger satellite, which is equal or slightly smaller the half of the remaining adjacent proximal part of the short arm (2:4.5:7). The satellite of the chromosome 7 represents a length about the one third of the short arm. The long arm is the longest one of the whole complement, so this chromosome is apparently the most anisobrachial SM member (1.5:4:9).

TABLE 2

Comparative values of chromosome relative length (R.L) and arm ratio (A.R) taken from four barley varieties

Chromosome identity (linkage groups)	«Emir» Linde-Laursen 1975		«Tibet» Vosa 1976		«York» Noda and Kasha 1978		«Georgia» Coucoli et al. 1981	
	R.L.	A.R	R.L.	A.R	R.L.	A.R.	R.L.	A.R.
1	14.50	0.97	15.58	0.87	13.18	0.89	15.67	0.88
2	15.62	0.81	15.58	0.72	14.59	0.83	15.53	0.78
3	15.10	0.77	13.79	0.90	13.23	0.65	14.59	0.83
4	13.68	0.77	13.79	0.77	12.82	0.88	13.55	0.90
5	12.18	0.75	12.61	0.75	10.29	0.69	12.41	0.73
6	14.20	0.98	13.79	0.90	10.00	0.59	13.59	0.94
7	14.72	0.64	14.86	0.64	12.89	0.46	14.66	0.62

In Table II comparative data are given, concerning the corresponding values of relative length and arm ratios of four barley varieties. In other words, the values determined from the karyotype study of «Georgia» have been put in common with the respective data referred or inferred from recent papers on three other known barley varieties, namely «Emir» (Linde-Laursen 1975), «Tibet» (Vosa 1976) and «York» (Noda and Kasha 1978).

The information provided by Table II is pointing to the following clarification.

1) In all four cases, the two longest pairs, not always significantly different in size, can be distinguished by arm ratio, chromosome 1 being constantly more metacentric.

2) The distinction between pairs 3 and 4 used to be not a definite one. Concretely, in «Emir» the two chromosomes are of the same centromere class, but of significantly different size. «Tibet» is characterized by chromosome 3 and 4 of the same size, but the one (3) is fairly more metacentric. In «York» the condition is reversed, chromosome 4 being much more equal armed. The same holds for «Georgia», in which chromosome 4 was recognized more metacentric.

3) The chromosome No 5 and the two SAT chromosomes were in all instances clearly and similarly identifiable as regarding both size range and arm ratio.

DISCUSSION

The standard karyotype of *Hordeum vulgare* L., first established by Tjio and Hagberg (1951) was associated with the linkage groups and adopted by the North American Barley Research Workers Conference (Ramage et al., 1961).

The general acceptance of the above relationships was later questioned because the identity of chromosomes 1-4 proved not an easy task for the cytologists and cytogeneticists. Moreover, possible identifications subsequent to rearrangements such as interchanges, might have increased the uncertainties (Noda and Kasha 1978).

Thus the objections, at first presented by Tuleen (1973) and later corroborated by Künzel (1976), imposed the necessity of a partial revision of the karyotype, as finally proposed by Kasha and Noda (1979).

Our results in barley cultivar «Georgia» seem to be in closer agreement with the revised, more recent findings, in particular approximating the arm ratio values given by Noda and Kasha (1978), having precisely worked with the cultivars «Betzes» (a 2-rowed barley of European origin) and «York» (a 6-rowed barley from Canada). Next to this, comes the similarity with «Emir» according to the data presented in Table 2. The resemblance is almost complete for the chromosomes 1, 2, 4 and 5. With regards to chromosome No 3, the a. r. value found in «Georgia» (0.83) lies in between the corresponding findings presented by Linde-Laursen (0.77) and Vosa (0.90). All results are different from the standard karyotype in the reversed sequence between 1 and 2 chromosomes, as well as between 3 and 4 (apart from Vosa's). Thus the correct position of the revised karyotype appears prominent.

Some features concerning the SAT chromosomes should be also discussed here. According to our measurements chromosome 6 is a typical metacentric (0.94) and the same holds for «Emir» (0.98) and «Tibet» (0.90). On the other hand chromosome 7 was measured as typically submetacentric, but fairly heterobrachial (0.62) whereas the corresponding values for «Emir» and «Tibet» are in both 0.64. Somehow different are the data offered by Noda and Kasha (6/0.59, 7/0.46), that is chromosome 6 has a submedian and chromosome 7 a subterminal centromere. These findings are in accordance witb the standard karyotype, (Tjio and Hagberg 1951), (0.61/0.41) and, in addition, with the data provided by Künzel (1976) (0.59/0.47).

Unless there have been differences owing to the various handlings of the different materials resulting in different degree of contraction and affecting the morphology of mitotic chromosomes, the alternative explanation could be that there are two sets of SAT chromosomes in the pool of the diverse *Hordeum vulgare* genotypes. The one includes one metacentric and one submetacentric members while the other, one submetacentric and one acrocentric chromosomes. The first case is the situation which occurs more frequently and it covers the cultivars «Georgia», «Emir», and «Tibet», as well as the ten different materials studied by Vosa (1976). The same condition is also represented by the two SAT pairs observed in *H. spontaneum* (Vosa 1976). We should add that the wild barleys studied so far by different workers (Morrison 1959, Richards and Booth 1976, Coucoli and Symeonidis 1980) containing either one (mostly) or two SAT pairs, showed constantly the presence of SM satellited members. However, the occurrence of acrocentric (ST) satellite chromosomes in *Hordeum vulgare* remains an undoubtful fact, since it was clearly defined for both the standard karyotype, as well as for the cultivars studied by Künzel and Noda and Kasha. Similar acrocentric (ST) satellite types have been frequently observed in other taxa of Triticeae (*Agropyron*, *Elymus*) by several workers (Heneen and Runemark 1962, 1972, Runemark and Heneen 1968, Schulz-Schaeffer and Jurasits 1962, 1967). Therefore, it cannot be excluded that the two types of satellited chromosomes have been in parallel stabilized in the different genotypes of *H. vulgare.*

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ΠΕΡΙΛΗΨΗ

ΣΥΜΒΟΛΗ ΣΤΗΝ ΑΝΑΘΕΩΡΗΣΗ ΤΟΥ ΚΑΡΥΟΤΥΠΟΥ ΤΗΣ ΚΡΙΘΗΣ

ὑπὸ

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Καθορίσθηκε ὁ καρυότυπος τῆς καλλιεργούμενης ποικιλίας κριθαριοῦ «Georgia». 'Η ἀρίθμηση τῶν χρωμοσωμάτων ἀκολούθησε τὴ σειρὰ τοῦ πρότυπου καρυοτύπου, ποὺ ταυτοποιεῖ τὰ μὴ δορυφορικὰ μέλη τοῦ γενώματος ὡς χρωμοσώματα 1-5 καὶ τὰ δύο SAT ζεύγη ὡς 6 καὶ 7. Τὰ ἀποτελέσματα διευκρινίζουν τὰ ἀκόλουθα, ἀμφισβητούμενα στοιχεῖα: 1) Τὸ χρωμόσωμα 1 εἶναι τὸ πιὸ μετακεντρικὸ ἀπὸ τὰ τρία μεγαλύτερα μέλη τοῦ καρυοτύπου, ἀλλὰ δὲν εἶναι στατιστικὰ μεγαλύτερο ἀπὸ τὸ χρωμόσωμα 2. 2) Τὸ ζευγάρι 5 ἀντιπροσωπεύει τὸ πιὸ βραχὺ μέλος τοῦ γενώματος, καὶ τὸ πιὸ ἀνισοβραχιονικὸ ἀπὸ τὰ μὴ δορυφορικὰ χρωμοσώματα. 3) Τὸ χρωμόσωμα 7 ἀναγνωρίσθηκε ὡς τυπικὸ ὑπομετακεντρικὸ (SM κεντρομερικὴ κλάση).

Τὰ δεδομένα, ποὺ συγκρίνονται ἀναλυτικὰ μὲ βιβλιογραφικὰ στοιχεῖα, ἀναφορικὰ μὲ ἄλλες ποικιλίες κριθαριοῦ, συμφωνοῦν μὲ τὶς πρόσφατες προτάσεις γιὰ μερικὴ ἀναθεώρηση τοῦ πρότυπου καρυοτύπου ἀναφορικὰ μὲ τὸν δείκτη βραχιόνων τῶν δύο μεγαλύτερων χρωμοσωμάτων. 'Επιπρόσθετα, δίνονται νέα στοιχεῖα σχετικὰ μὲ τὰ δύο SAT ζευγάρια.