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Chromatin organisation and computer aided karyotyping of *Triticum durum* Desf. cv. Timilia

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Abstract - The tribe Triticeae includes three cereal genera *Secale*, *Hordeum* and *Triticum* and because of their economical and agronomical importance, the relationships among species on the tribe have been extensively investigated. The wild wheat relatives are an important source of genetic variation for cultivated species and wheat is an important crop of the mediterranean region. Banding pattern of metaphase chromosomes and nuclear DNA content in root meristematic cells of an old sicilian landrace "Timilia" were determined. Microdensitometric evaluation of nuclear absorption at different thresholds of optical density indicates the organization of chromatin in the interphase nuclei. Chromosome morphometric data, karyotype simmetry, the TF% values and Syi indices were determined. The results are compared with the data of other durum wheat varieties as Capeiti and Simeto.

Key words: chromosome banding, chromatin organization, image analysis, plant chromosomes, *Triticum*, cv. Capeiti, cv. Simeto, cv. Timilia, wheat.

INTRODUCTION

Wheat is an important crop of the Mediterranean region since the very beginning of agriculture in all its forms and species, hulled or naked, diploid or polyploid. For South Europe, Middle East, and North Africa, *durum* wheat, the tetraploid form traditionally used to prepare pasta, cous-cous, burgul, and a variety of traditional dishes of the Mediterranean culture, has had peculiar importance. In Sicily, until 60 years ago, many landraces were grown, accounting for a tremendous amount of genetic variation, and possessing many genetic characters well adapted to the local environments (DE CILLIS 1942; PORCEDDU and PIGNONE 1977; BELAY *et al.* 1997).

Unfortunately, most of them have now been replaced by modern cultivars. Some trials using foreign landraces in Sicily (PECETTI *et al.* 1996; PERRINO and MARTIGNANO 1973) have demonstrated the possible good performance of landarces respect to improved cultivars**.** Timilia is a group of old Sicilian autochthonous landraces which were cultivated in the past. Their characteristic was to be sown in spring (DE CILLIS 1927, 1942; PORCEDDU and BENNET 1971; GALLO *et al.* 1998; PIGNONE *et al.* 2000).

In this paper we report new experimental evidence on cytological and karyological parameters of Timilia. The results are compared to other old (Capeiti) and modern (Simeto) *durum* wheat varieties.

MATERIALS AND METHODS

Plant materials

Capeiti is a variety registered in 1958 obtained by Cappelli x Eiti (CASALE 1961), Simeto, registered in 1988, obtained by Capeiti x Valnova (CALCAGNO *et al.* 1985) and Timilia is an old spring Sicilian land races (DE CILLIS 1927, 1942). Timila is a part of the Stazione Sperimentale seed bank.

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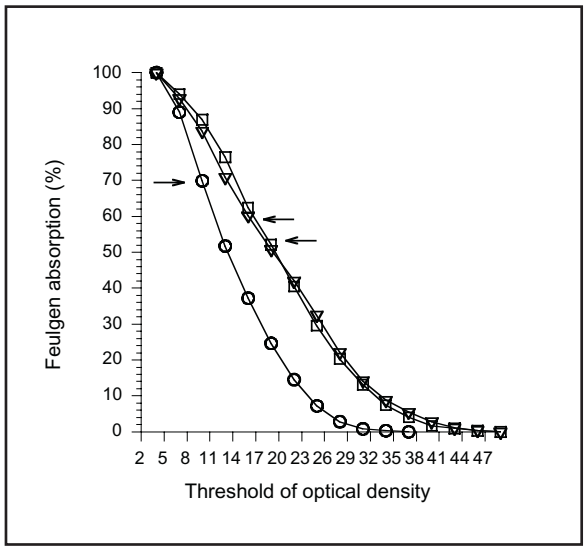

Fig. 1 – Percentage of Feulgen absorption at different thresholds of optical density of 4C intephase nuclei in *Triticum durum* Desf.: cvs. Capeiti (□), Simeto (▽) and Timilia (○). The arrows indicate the inflection points.

Cytophotometric analysis

Seeds of the three cultivars from seed bank collection of Stazione Sperimentale di Granicoltura, were soaked in running tap water over night and germinated in petri dishes at 22°C in darkness for two days. Root tips were fixed in ethanol- acetic acid (3:1, v-v). Squashes were made under a coverslip in a drop of 45% acetic acid after treatment with a 5% aqueous solution of pectinasae (Sigma) for 1 h at 37°C with the addition of 0.001 M. EDTA in order to annul the activity of DNAse if present (BERLYN *et al.* 1979). The coverslip were removed by the dry-ice method, and the squashes were idrolised in 5 N HCl at room temperature for 30 min and stained by Feulgen reagent. The slides were subjected, after staining, to three 10 min washes in SO_2 water prior to dehydration and mounting. Squashes of the root tips of *Vicia faba* cv. Aguadulce were stained for each group of slides and used as internal standard; DNA content was estimated by a Leitz MPV3 integrating microdensitometer equipped with HP computer at a wavelength of 550 nm in individual cell nuclei in post synthetic condition (G_2 phase, 4C). Absorptions measured in *V. faba* preparations were also used to convert relative Feulgen arbitrary units into picograms of DNA. With the same instrument and at the same wavelength, the Feulgen DNA absorptions of chromatin fractions with different condensation level were measured in the same nucleus after selecting different thresholds of optical density. The value of the thresholds of the optical density were mathematically elaborated in order to obtain the exact position of the inflection point in the curve. The residual Feulgen absorption at the inflection point represent the cytophotometrically determined heterochromatin. In order to asses whether some chromatin fractions were involved in cytophotometric differences among the cultivar, measurements were taken on interphase nuclei having Feulgen absorption values corresponding to 4C content and the same surphace.

Karyomorphometry

Slide were prepared and analysed according to VENORA *et al.* (1991). Karyotype morphometry was assessed by the automated image analysis system IKAROS 3.40 software package (Metasystem). The classification of STEBBINS (1971), the TF% index (HUZIWARA 1962) and the Rec and Syi indices (GREILHUBER and SPETA 1976) were used to perform the analysis. The classification of STEBBINS (1971) is based on the relative frequency of chromosomes with a long arm ratio greater than 2 and on the ratio between the lengths of the longest and the shortest chromosome in the complement. The TF% index is expressed by the ratio between the sum of the lengths of the short arms of individual chromosomes and the total length of the complement. The Rec index expresses the average of the ratios between the length of each chromosome and that of the longest one. The Syi value indicates the ratio between the average length of the short arms and the average length of the long arms. Cluster analysis was applied for grouping chromosome pairs (SCOTT and KNOTT 1974). The LEVAN (1964) nomenclature was followed excluding the satellite length in computing the arm ratio.

Table 1 – Feulgen absorption (a.u., mean ± S.E.), surface of interphase nuclei (μm², mean ± S.E.), nuclear DNA content (pg, mean ± S.E.), in T. durum cvs. Each Feulgen absorption value is the mean of 100 determinations carried out in root meristems: twenty prophases for each of five seedilings. *Vicia faba* cv. Aguadulce nuclear DNA content (53.12 pg*) was used as internal standard.

Triticum cultivars	Feulgen absorption	Interphase nuclei surface	DNA amount (4C)
Timilia	4244 ± 47.9	39.61 ± 0.7	44.9 ± 0.6 b
Capeiti	4545 ± 79.4	37.86 ± 0.10	48.1 ± 0.9 a
Simeto	4587 ± 72.5	38.54 ± 0.07	48.6 ± 0.8 a

Values followed by the same letter are not significantly different, according to the Cluster analysis of SCOTT AND KNOTT, (1974, capital letter P=0.01, small letters P=0.05). * CECCARELLI *et al.* 1995.

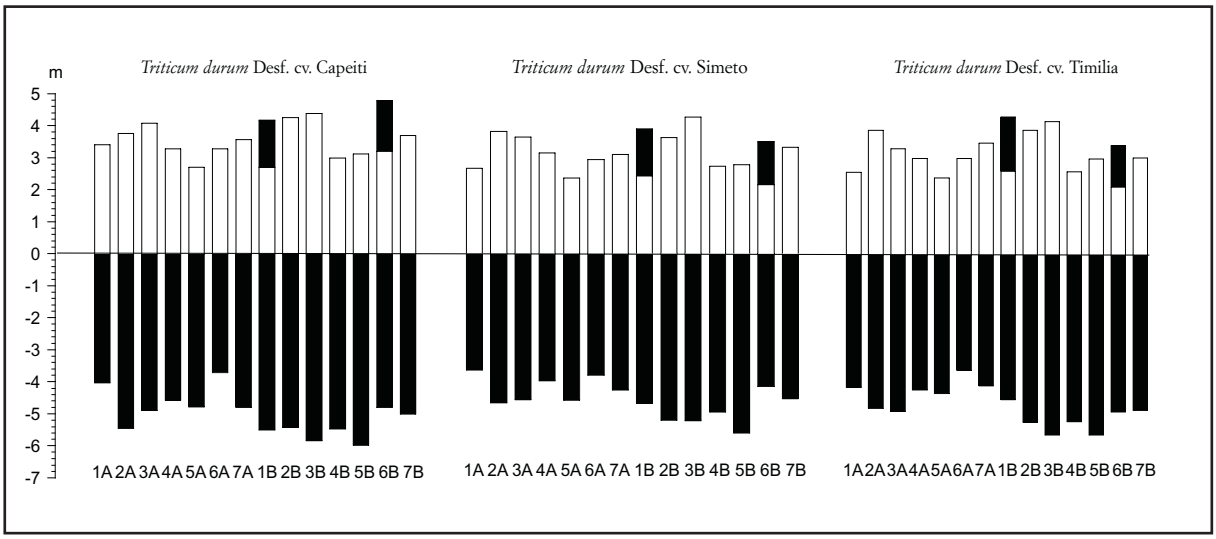

Fig. 2 – Idiogrammatic representation of the karyotipes of *Triticum durum* Desf. cvs. Capeiti, Simeto and Timilia.

Chromosome banding

Actively growing roots were excised and treated overnight with ice cold distilled water in order to accumulate metaphases. After fixation in ethanol acetic acid (3:1 v-v) for 24 h the root tips were squashed in 45% acetic acid and coverslips removed by the dry–ice method. For the C-banding the technique of GILARDEZ *et al.* (1979) was utilized. Slides were examined under high magnification and picture taken on Kodak high contrast film or acquired using a a Sony XC 75 b/w CCD camera; digital images, with 720 x 512 x 8 bit resolution, were processed running the Leica QWIN or Adobe Photoshop 5.5 softwares, using algorithms applying to the whole image at the same time, and were printed with a Mitsubishi CP-D1E photo printer.

RESULTS

Cytophotometric analysis

The DNA content estimated in cv Timilia was 45.87 pg in 4C interphase nuclei (Table 1). Results of analysis, on interphase nuclei (G_2) at different thresholds density are summarised in Table 2 and Fig. 1. The Feulgen absorptions are reduced to 0 at different threshold: 35 for Timilia and 47 for Simeto and Capeiti respectively, and the residual absorptions at the inflection point is 69.9% at optical density threshold 8 for Timilia, 60.2% at optical density threshold 14 for Simeto and 51.2% at optical density threshold 17 for Capeiti.

Table 2 – Percentages of Feulgen absorption (mean ± S.E.) at different thresholds of optical density of 4C interphase nuclei in the root meristems of five seedlings for each sample. 20 nuclei for each seedling were measured. (* indicate the inflexion point positions).

Thresholds of optical density	Feulgen absorption % Timilia	Simeto	Capeiti
2	100	100	100
5	89.0 ± 0.9	92.8 ± 1.0	94.1 ± 1.8
8	* 69.9 ± 1.2	83.7 ± 1.1	86.9 ± 2.0
11	51.7 ± 1.6	70.9 ± 1.4	76.5 ± 1.9
14	37.2 ± 1.9	* 60.2 ± 1.7	62.4 ± 1.1
17	24.6 ± 1.9	50.8 ± 1.8	* 51.2 ± 1.4
20	14.5 ± 1.6	42.0 ± 1.9	40.5 ± 1.6
23	7.2 ± 1.2	32.5 ± 2.2	29.6 ± 1.8
26	2.8 ± 0.7	22.1 ± 2.3	20.3 ± 1.7
29	0.8 ± 0.2	14.2 ± 2.3	13.1 ± 1.4
32	0.3 ± 0.1	8.8 ± 2.1	7.4 ± 1.1
35	———	5.5 ± 1.7	4.1 ± 0.8
38		2.8 ± 1.1	1.7 ± 0.2
41		1.1 ± 0.3	0.9 ± 0.1
44		0.4 ± 0.1	0.3 ± 0.1
47		———	———

Table 3 – Chromosome morfometric data of the three cultivars of *Triticum durum*. Each data is the mean of 50 determination carried out in metaphases chromosomes, ten metaphase plates for each cultivar.

Chrom. No.	Relative length %	Chromosome length (µm)	Long arm (µm)	Short arm (µm)	Satellite (µm)	Arm Ratio long/short	Chromosome type
			Triticum durum Desf. cv. Timilia				
1A	5.99	6.80 lG	4.20 ± 0.35	2.60 ± 0.33		1.61 eE	m
2A	7.74	8.78 dC	4.85 ± 0.29	3.93 ± 0.27		1.23 hG	m
3A	7.33	8.32 eE	4.96 ± 0.11	3.36 ± 0.30		1.48 fF	m
4A	6.45	7.32 hG	4.27 ± 0.36	3.05 ± 0.18		1.40 gG	m
5A	6.01	6.82 iG	4.38 ± 0.59	2.44 ± 0.25		1.80 dC	sm
6A	5.90	6.69 lG	3.65 ± 0.23	3.04 ± 0.23		1.20 hG	m
7A	6.76	7.67 gF	4.14 ± 0.41	3.53 ± 0.25		1.17 hG	m
1B	7.87	8.93 cC	4.58 ± 0.38	2.64 ± 0.32	1.71 ± 0.10	1.73 dD	sm
2B	8.14	9.23 bB	5.29 ± 0.37	3.94 ± 0.28		1.34 hG	m
3B	8.73	9.92 aA	5.70 ± 0.36	4.22 ± 0.28		1.35 hG	m
4B	6.96	7.90 fF	5.27 ± 0.26	2.63 ± 0.24		2.01 bB	sm
5B	7.67	8.71 dC	5.69 ± 0.70	3.02 ± 0.23		1.88 cD	sm
6B	7.41	8.42 eD	4.97 ± 0.43	2.14 ± 0.24	1.31 ± 0.10	2.32 aA	sm
7B	7.04	7.99 fF	4.92 ± 0.28	3.07 ± 0.40		1.60 eE	m
			Triticum durum Desf. cv. Simeto				
1A	5.75	6.35 gG	3.68 ± 0.60	2.67 ± 0.35		1.38 bB	m
2A	7.73	8.53 cD	4.70 ± 0.61	3.83 ± 0.56		1.23 bB	m
3A	7.49	8.27 bB	4.61 ± 0.38	3.66 ± 0.37		1.26 bB	m
4A	6.48	7.15 gG	4.00 ± 0.70	3.15 ± 0.26		1.27 bB	m
5A	6.34	6.99 gG	4.62 ± 0.50	2.37 ± 0.33		1.95 aA	sm
6A	6.15	6.79 gG	3.84 ± 0.51	2.95 ± 0.45		1.30 bB	m
7A	6.72	7.41 fG	4.30 ± 0.36	3.11 ± 0.36		1.38 bB	m
1B	7.82	8.63 cC	4.72 ± 0.89	2.44 ± 0.30	1.47 ± 0.18	1.94 aA	sm
2B	8.06	8.89 bB	5.24 ± 0.53	3.65 ± 0.58		1.44 bB	m
3B	8.65	9.54 aA	5.26 ± 0.75	4.28 ± 0.69		1.23 bB	m
4B	7.01	7.73 fG	4.99 ± 0.59	2.74 ± 0.38		1.82 fG	sm
5B	7.64	8.43 dD	5.64 ± 0.81	2.79 ± 0.38		2.02 aA	sm
6B	6.98	7.70 fG	4.19 ± 0.61	2.16 ± 0.40	1.35 ± 0.21	1.94 aA	sm
7B	7.16	7.90 eF	4.57 ± 0.59	3.33 ± 0.38		1.37 bB	m
			Triticum durum Desf. cv. Capeiti				
1A	5.58	6.76 fD	4.03 ± 0.87	2.73 ± 0.49		1.48 dD	m
2A	7.61	9.21 dC	5.45 ± 0.88	3.76 ± 0.64		1.45 dD	m
3A	7.42	8.98 dD	4.90 ± 0.58	4.08 ± 0.84		1.20 fE	m
4A	6.49	7.86 fD	4.58 ± 0.63	3.28 ± 0.42		1.40 eD	m
5A	6.19	7.48 fD	4.78 ± 0.86	2.70 ± 0.22		1.77 bB	sm
6A	5.77	6.99 fD	3.71 ± 0.62	3.28 ± 0.32		1.13 fE	m
7A	6.91	8.36 fD	4.79 ± 0.69	3.57 ± 0.73		1.34 eE	m
1B	8.00	9.69 bB	5.51 ± 0.97	2.70 ± 0.32	1.48 ± 0.27	2.04 aA	sm
2B	8.00	9.69 bB	5.43 ± 0.73	4.26 ± 0.69		1.27 fE	m
3B	8.44	10.23 aA	5.85 ± 0.89	4.38 ± 0.60		1.34 eE	m
4B	6.99	8.46 eD	5.48 ± 0.75	2.98 ± 0.56		1.84 bB	sm
5B	7.51	9.09 dC	5.98 ± 1.02	3.11 ± 0.51		1.92 bB	sm
6B	7.91	9.58 cB	4.80 ± 0.57	3.20 ± 0.56	1.58 ± 0.12	1.50 cC	m
7B	7.19	8.70 dD	5.01 ± 1.05	3.69 ± 0.42		1.36 eE	m

Values followed by the same letter are not significantly different, according to the Cluster analysis of SCOTT and KNOTT 1974 (capital letters P=0.01, small letters P=0.05).

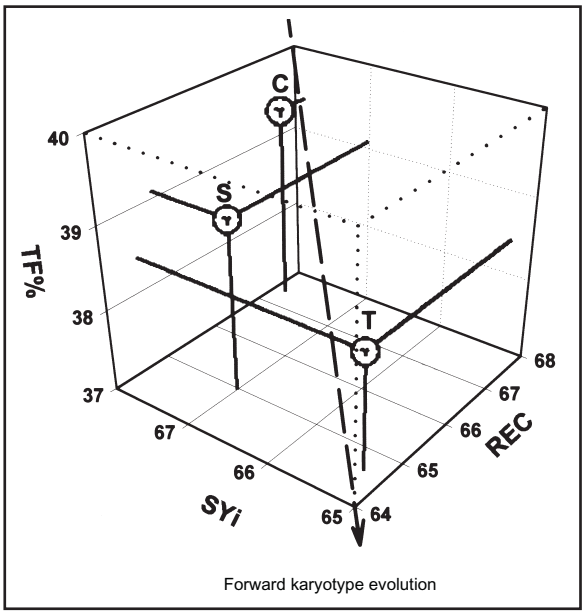

Fig. 3 – Karyotype simmetry of *Triticum durum* Desf.: cvs. Capeiti (C), Simeto (S) and Timilia (T) with Rec, Syi and TF% indices.

Karyomorphometry

All karyological data from the analysed cultivars are reported in Table 3 and Fig. 2, the longest chromosome pair is 3B in all examined cultivars, the shortest is 1A in Simeto and Capeiti and 6A in Timilia. The comparisons among the cultivars of each chromosomes length, arm ratio and total haploid set length are reported in Table 4.

As concern chromosomal length, Capeiti differs from the others for the chromosome 6B. As regards arm ratio Capeiti is different from the other two cultivar for chromosomes 2A and 6B, Simeto for 6A, 2B and 6B and Timilia for 1A, 3A, 7A. 4B 6B and 7B. Capeiti showed the longest haploid complement whereas Simeto the shortest one.

Fig. 3 illustrates the spatial representation of karyological indices of cultivars. The spatial representation highlights that Timilia is more karyologically evolved slightly.

Chromosome banding

Fig. 4 shows a metaphase plate with C-banding of Timilia, and Fig. 5 shows the idiogrammatic representation of the C-banding pattern.

DISCUSSION

The determination of the DNA amount shows lower values in cv. Timilia. The differences obtained in the Feulgen absorption values reflect real differences in the nuclear DNA content, since we have analysed nuclei in the same postsynthet-

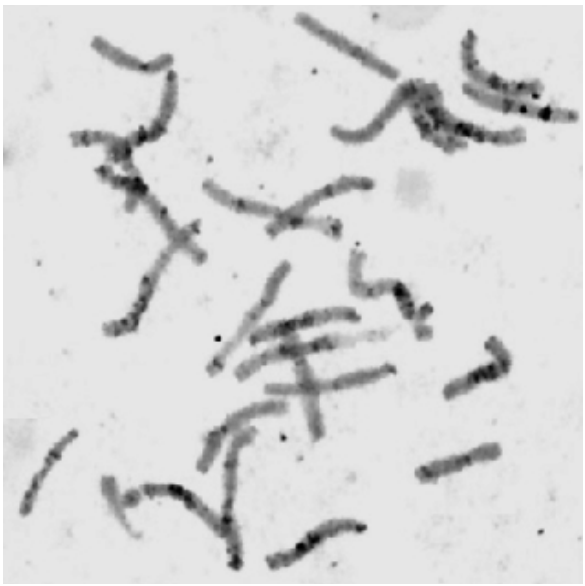

Fig. 4 – C-Banding metaphase chromosomes of *Triticum durum* Desf. cv. Timilia.

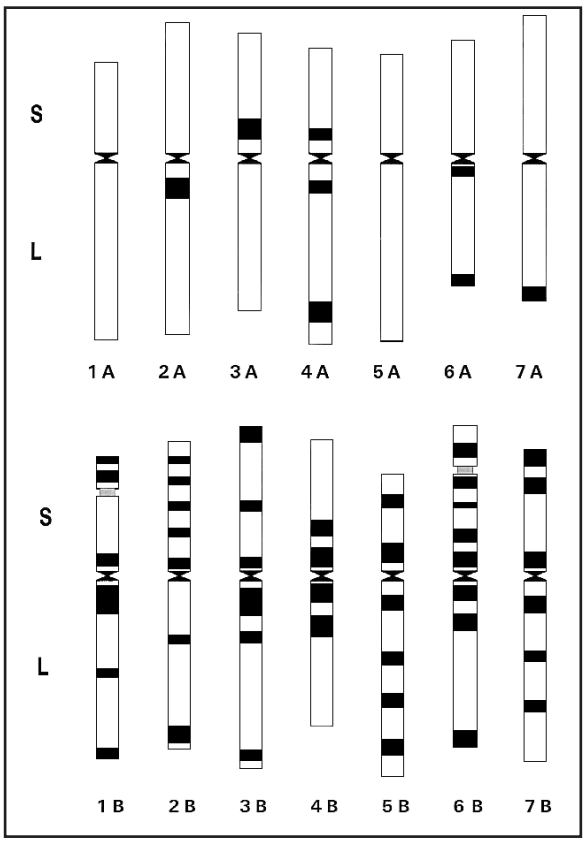

Fig. 5 – Idiogrammatic representation of C-Banding pattern of *Triticum durum* Desf. cv. Timilia.

ic condition from tissues at the same stage of development.

The determination of the nuclear chromatin fractions with different condensation levels by means of cytophotometry is used as an alternative method for the determination of the heterochromatin fractions.

The residual Feulgen absorption at the inflexion point represents the cytophotometrically determined heterochromatin component in interphase nuclei (CREMONINI *et al.* 1993). In our materials a clear difference is present among the cultivars attesting a different chromatin organisation in addition to different DNA amounts; on the contrary, karyotype morphometry does not evidence significant differences as well as no correlation is evidenced between nuclear DNA content and complement length.

The lower DNA content in cv. Timilia supports the relationship between lower content and ancestrality of a species and/or cultivar; this character is also confirmed by the higher content of condensed chromatin which is answerable of capability of adaptability in a natural plant species (BENNETT 1976, 1987).

The large variation in nuclear DNA content of durum wheat cultivars (CARROZZA *et al.* 1980) may find an explanation in the origins of the analysed cultivars which involved different genetic procedures (mutation induction, combination breeding…). In our material the cultivars Simeto and Capeiti are very close and the plant breeding programs have not induced variation in the total complement length and have induced only chromosomal rearrangements in the centromere position which is absent in cv. Timilia attesting its ancestrality

Landgdon and Cappelli were chosen as reference standards for C-banding, since these are considered standard for cytogenetics work previously described by PIGNONE *et al.* (1989) and SIMEONE *et al.* (1988).

The karyotype of Langdon has been assembled on the basis of double-ditelosomic lines, thus accounting for an improved precision in the assignment of each chromosome to a given homology group. Recently DEVOS *et al.* (1995) have demonstrated that the structural evolution of some chromosomes and errors in the previous assignments. For this reason it was necessary to re-assemble the C-banded karyotypes, the present paper takes into account the suggestions of DEVOS *et al.* (1995).

From a comparison of the C-banded karyotypes of Timila, Langdon and Cappelli it is possible to observe: 1) the A-genome always shows little amounts of heterochromatin and variations among the three varieties are marginal; 2) the B-genome is characterized by a greater amount of heterochromatin and differences among the three varieties are quite evident, even though the overall structure of each chromosome is conserved;

Table 4 – Comparison of each chromosome length (mm, L) and arm ratio (l/c) among the cultivars and total length of haploid set.

Chromosomes	*Triticum durum* Desf. cultivars					
	Capeiti		Simeto		Timilia	
	L	l/c	L	l/c	L	l/c
1A	6.76 a	1.48 b	6.35 a	1.38 b	6.80 a	1.61 a
2A	9.21 a	1.45 a	8.53 a	1.23 b	8.78 a	1.23 b
3A	8.98 a	1.20 b	8.27 a	1.26 b	8.31 a	1.48 a
4A	7.86 a	1.40 a	7.15 a	1.27 a	7.32 a	1.40 a
5A	7.49 a	1.77 a	6.99 a	1.95 a	6.82 a	1.80 a
6A	6.99 a	1.13 b	6.79 a	1.30 a	6.70 a	1.20 b
7A	8.36 a	1.34 a	7.41 a	1.38 a	7.67 a	1.17 b
1B	9.68 a	2.04 a	8.63 a	1.94 a	8.93 a	1.73 a
2B	9.69 a	1.27 b	8.89 a	1.44 a	9.23 a	1.34 b
3B	10.22 a	1.34 a	9.55 a	1.23 a	9.91 a	1.35 a
4B	8.46 a	1.84 b	7.74 a	1.82 b	7.90 a	2.01 a
5B	9.09 a	1.92 a	8.43 a	2.02 a	8.71 a	1.88 a
6B	9.58 a	1.50 c	7.70 b	1.94 b	8.42 b	2.32 a
7B	8.70 a	1.36 b	7.90 a	1.37 b	7.99 a	1.60 a
Total length of haploid set	121.07		110.33		113.49	

Values followed by the same letter are not significantly different in each rows, according to the Cluster analysis of SCOTT AND KNOTT (1974, small letters P=0.05).

3) Langdon shows the lesser amount of heterochromatin and Timilia the highest one. In detail, Timila shows more bands in the satellite of chromosome 1B and on the short arm of chromosome 2B; chromosomes 3B and 4B have one more band on the short and long arm respectively; chromosome 5B has a different band distribution and chromosomes 6B and 7B are richer in heterochromatin, respect to Langdom. With respect to Cappelli in Timilia the telomeric band on the short arm of chromosome 7A is absent; chromosome 1B has an extra telomeric band on the long arm, 3B is more heterochromatic, 6B more interstilial heterochromatin and an extra telomeric band on the long arm and, finally the telomeric band on the long arm of chromosome 7B is absent.

As regards C-banding distribution in Capeiti and Simeto, it is very similar to that of Cappelli but with a lesser overall heterochromatin content (data not shown). It has been hypothesized that in maize heterochromatin content is in relation to adaptation and cultivation: higher heterochromatin contents are correlated to difficult environments, while low heterochromatin trais is related to high yielding ability (CHUGHTAI *et al.* 1995; POGGIO *et al*. 1998). In this light Capeiti and Simeto since improved high yielding varieties show less heterochromatin than Timila. Conversely, Timila, a landrace, shows higher heterochromatin content due to lower selective pressure and indicating a higher adaptation ability.

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