

Karyotype analysis of some lines and varieties belonging to *Carthamus tinctorius* L. species

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Received : 22.11.2017
Accepted : 06.01.2018

Carthamus tinctorius L. türüne ait bazı hat ve çeşitlerin karyotip analizi

Abstract: In this paper, karyology of thirty one accessions of Safflower (*Carthamus tinctorius* L.) were investigated in terms of their chromosome numbers and karyomorphology. The chromosomal counts confirmed the results of previous reports, that the *Carthamus* has same basic chromosome numbers. According to our results discussed all accessions of chromosome numbers have been identified $2n = 24$, $x=12$ and also all have the diploid number of chromosomes. We found predominance of chromosomes being metacentric and sub-metacentric in karyotypes. Five quantitative asymmetry indices were used to evaluate our karyological results in all species and elucidate the chromosomal alterations of *Carthamus tinctorius* accessions. All karyotyping analyses were described for the first time in this report via KAMERAM programme. We hope that these findings would be contributed for *Carthamus* genetic and breeding studies.

Key words: Safflower, Karyomorphology, Asteraceae.

Özet: Bu çalışmada 31 Aspir (*Carthamus tinctorius* L.) çeşidine ait kromozom sayıları ve karyomorfolojileri araştırılmıştır. Kromozom sayıları, *Carthamus*'un aynı temel kromozom sayılarına sahip olduğunu gösteren daha önceki raporları doğrulamıştır. Elde edilen sonuçlara göre, kromozom sayıları tüm çeşitler için $2n = 24$, $x = 12$ olarak tanımlanmış ve aynı zamanda tümünün diploid kromozom sayısına sahip oldukları bulunmuştur. Karyotiplerde metasentrik ve submetasentrik olan kromozomların baskın olduğu tespit edilmiştir. Tüm çeşitleri karyolojik açıdan değerlendirmek ve *C. tinctorius* çeşitleri arasındaki kromozomal değişikliklerini aydınlatmak için beş adet kantitatif asimetri indeksi kullanılmıştır. Tüm karyotip analizleri ilk kez bu raporda KAMERAM programı ile tanımlanmıştır. Çalışmada elde edilen bulguların *Carthamus* türünün genetik ve ıslah çalışmalarına katkıda bulunmasını umuyoruz.

Anahtar Kelimeler: Aspir, Karyomorfoloji, Asteraceae

1. Introduction

Carthamus L. genus consists of about 25 main species in the world. *Carthamus tinctorius*, commonly called safflower, is the only cultivated species of this genus (Anjali and Srivastava, 2012). Safflower, *C. tinctorius* L., is a member of the family Compositae or Asteraceae, cultivated mainly for its seed, which is used as edible oil and as birdseed. Traditionally, the crop was grown for its flowers, used for colouring and flavouring foods and making dyes, especially before cheaper aniline dyes became available, and in medicines (Dajue and Hans-Henning, 1996). Safflower is one of humanity's oldest crops, but generally it has been grown on small plots for the grower's personal use and it remains a minor crop with world seed production around 800.000 t per year (Gyulai, 1996). Oil has been produced commercially and for export for about 50 years, first as an oil source for the paint industry, now for its edible oil for cooking, margarine and salad oil.

As far as we know *Carthamus* species have different chromosome numbers. Cassini (1819) and De Candolle (1838) classified the species of *Carthamus* into two genera, *Carthamus* and *Kentrophyllum* and after Knowles (1958) divided the genus into four taxonomic sections on the basis of chromosome numbers. Section I, II, III and IV contained taxa with $2n = 24$, $2n = 20$, $2n = 44$, and $2n = 64$, respectively (Sehgal et al., 2009). The species having 10 pairs of chromosomes are characterized by a

preponderance of purple, blue and pink flowers and include *C. boissieri*, *C. dentatus*, *C. glaucus*, *C. leucocaulos* and *C. tenuis* (López-González, 1989). The only species with 11 pairs of chromosomes is *C. divaricatus* which has a very restricted range in Libya (Knowles, 1988). *C. tinctorius*, *C. alexandrius*, *C. syriacus* and *C. tenuis* have diploid chromosome number as $2n = 24$. *C. lanatus* L., a tetraploid species with 22 pairs of chromosomes, occurs naturally in Portugal, Spain, Morocco, Greece and Turkey ($2n=44$). In addition a hexaploid member of *Carthamus* species, *C. baeticus* has $2n = 64$ chromosome number.

In plant taxonomy, chromosome karyotypes can be useful in genetic studies and breeding information about species identification and analysis of hybrid populations (Anjali and Srivastava, 2012). Further studies were therefore undertaken to elucidate chromosomal details with the following objectives: (i) to determining the karyotypes of the populations, according the shape, size and number of chromosomes, (ii) classification of the populations with regard to karyotype evolution, and (iii) determining the parents for intersections, to obtain maximum diversity for the next generation (Yazdani, 2013). As far as we know, there have been many studies about karyology of *Carthamus tinctorius* (Yenice and Bayraktar 1996; Anjali and Srivastava 2009a, 2009b, 2009c).

Within this study we aimed to carried out the chromosome number and calculate chromosomal indices of

economically important thirty one *Carthamus* lines and varieties.

2. Materials and Method

The research material, different lines and varieties of *C. tinctorius* L. were provided by Ass. Prof. Dr. Rahim ADA. Some varieties used in the study were derived from patented plant genetic resources. Thirty one samples were genotyped in this study. Code of some lines and

Sample number	Sample code	Sample ID
1	Tcar 1	Black sun 2
2	Tcar 2	J-41
3	Tcar 3	CB
4	Tcar 4	F4
5	Tcar 5	KS03
6	Tcar 6	KS07
7	Tcar 7	CT-8-3
8	Tcar 8	G8
9	Tcar 9	J-19
10	Tcar 10	A13
11	Tcar 11	A29
12	Tcar 12	H3
13	Tcar 13	E12
14	Tcar 14	C12
15	Tcar 15	J51
16	Tcar 16	G16
17	Tcar 17	Black sun 1
18	Tcar 18	F6
19	Tcar 19	C11
20	Tcar 20	Y 11-8-14-1
21	Tcar 21	A30
22	Tcar 22	F5
23	Tcar 23	J29
24	Tcar 24	C 2-8-1
25	Tcar 25	E5
26	Tcar 26	H7
27	Tcar 27	H14
28	Tcar 28	Dinçer
29	Tcar 29	Remzibey
30	Tcar 30	E1
31	Tcar 31	KS06

varieties of Safflower species are given in Table 1.

Table 1. The codes of *Carthamus* varieties and lines.

Particularly, mature seeds were selected and periodically germinated for chromosomal analyses. Somatic chromosomes were studied in root tips obtained from germinating seeds, which were pretreated in hydroxylamine for a while, then fixed in 3:1 absolute ethanol: glacial acetic acid overnight, stored in fixative and stained using the Feulgen technique, and squashed in 2% aceto-orcein. Slides were made permanent in Euparal by mean of Bowen's method (1956). Minimum of ten metaphase cells were selected for preparing the karyotype. The best metaphase plates were photographed (100 x) with a digital camera (Olympus DP-72) mounted on an Olympus BX53 microscope). Karyotyping analyses were carried out via KAMERAM chromosomal analyses software and to measure chromosome parameters such as Total haploid complement length (TCL), longest chromosome length (LC), shortest chromosome length (SC), mean length of the long short arm (p), mean length of the long arm (q), mean centromeric index (CI), karyotype asymmetry index (AI), coefficient of variation of chromosome length (CVCL) and coefficient of variation of centromeric index (CVCI). Chromosome nomenclature followed Levan et al.

(1964), the symbols mc, smc and st designating metacentric, submetacentric and subtelocentric chromosomes, respectively (Table 2 and 3). The chromosomes were assorted into different categories on the basis of arm's ratio following Levan et al (1964) ($m=1.0-1.7$, $sm=1.7-3.0$, $st=3.0-7.0$).

3. Results and Discussion

Chromosomes usually give very important informations about plant taxonomy. As a result of our cytogenetic examinations, mitotic metaphase chromosome numbers, karyotype analysis, idiograms and chromosomal indices were determined for 31 *Carthamus* varieties. The karyotype formulas, asymmetry index (AI) values and the other karyotype parameters are given in Table 2 and 3.

All of the studied varieties and lines have a chromosome number of $2X=2n=24$ as supported with the earlier studies (Knowles 1988, Anjali and Srivastava, 2012, Yazdani 2013). On the whole, karyotypes of the most analysed varieties had a predominance of metacentric (mc) chromosomes. The most common formula among analysed specimens was determined to be $18m+6sm$ (eleven species). The other karyotype formulas were various, followed in $20m+4sm$ (eight species), $22m+2sm$ (eight species) and $24m$ (three species) and $10m+14sm$ (one species).

The karyotype analysis revealed that there was no subtelocentric chromosome in any of the thirty one accessions of *C. tinctorius*. When we evaluate varieties in point of their chromosome sizes, it is seen that very small size chromosomes range from $1.14 \mu\text{m} - 1.9 \mu\text{m}$. Sheidai et al. (2009), were performed karyotype and meiotic studies in thirty-seven cultivars of *C. tinctorius* grown in Iran and their findings are suitable with our results in terms of the predominance of metacentric chromosomes but their chromosome sizes ranged between 1.55 to $4.63 \mu\text{m}$.

Total chromosome lengths (TCL) of the studied samples were between 27.304 and $45.534 \mu\text{m}$. Tcar 13 (E12) had larger chromosomes as well as a larger genome than other samples. Anjali and Srivastava (2012) investigated karyomorphological features of twelve accessions of *C. tinctorius*. Their total chromosome lengths range from $38.79 - 55.56 \mu\text{m}$. Their chromosome sizes were moderately larger than our samples. Among the different samples of *C. tinctorius* in the present study all the accessions having maximum number of metacentric chromosomes may be evaluated as the most primitive. In terms of chromosomal indices and analyses, Tcar 18 (F6), Tcar 21 (A30) and Tcar 29 (Remzibey) safflower types have the lowest chromosomal variation; all of them have only metacentric chromosomes. So, they considered as primitive types. At the same time these three varieties asymmetry index is quite low and in terms of selection they have high potential to be used as rootstock in the hybridization studies. Particularly, the commercial Remzibey variety may be evaluated a good rootstock for inbreedings. Both of them are quite close to each other because of the based on the same genetic source from Turkey.

On the other hand karyotype asymmetry is one of the important standards for evaluating evolutionary relationships (Li and Chen, 1985). Karyotype asymmetry is a good expression of the general morphology of plant

karyotypes, which has been frequently related to evolution AI values ranged from 0.443 to 2.401. When we evaluate in higher plants (Stebbins, 1971). According to our results,

Table 2. Karyotype formula according to Levan et al. (1964) and characteristic parameters of the studied varieties. R-range; SC-the shortest chromosome length; LC- the longest chromosome length; p-mean length of the short arm; q- mean length of the long arm; CL- mean length of the chromosome; TCL- the total chromosome length; CI-mean centromeric index; CF- chromosome formula; m-metacentric; sm-submetacentric; SD-standard deviation.

Sample code	2n	R (SC – LC) (µm)	Ratio LC/SC	p (µm) Ort±SD	q (µm) Ort±SD	CL (µm) Ort±SD	TCL (µm)	CI Ort±SD	CF
TCAR1	24	1,19 - 1,86	1,556	0,63 (±0,10)	0,88 (±0,16)	1,51 (±0,22)	36,216	42 (±0,04)	20,m + 4,sm
TCAR2	24	1,12 - 1,51	1,345	0,56 (±0,04)	0,75 (±0,09)	1,31 (±0,10)	31,426	43 (±0,04)	20,m + 4,sm
TCAR3	24	1,11 - 1,83	1,641	0,57 (±0,08)	0,80 (±0,12)	1,37 (±0,17)	32,882	41 (±0,04)	18,m + 6,sm
TCAR4	24	0,94 - 1,89	2,005	0,58 (±0,07)	0,85 (±0,19)	1,43 (±0,25)	34,294	41 (±0,04)	18,m + 6,sm
TCAR5	24	0,97 - 1,41	1,465	0,49 (±0,06)	0,69 (±0,09)	1,18 (±0,13)	28,212	41 (±0,02)	22,m + 2,sm
TCAR6	24	1,11 - 1,73	1,561	0,58 (±0,07)	0,83 (±0,14)	1,42 (±0,16)	34,044	41 (±0,05)	18,m + 6,sm
TCAR7	24	0,95 - 1,86	1,952	0,62 (±0,14)	0,83 (±0,18)	1,45 (±0,25)	34,92	43 (±0,06)	20,m + 4,sm
TCAR8	24	1,17 - 1,70	1,455	0,60 (±0,07)	0,85 (±0,13)	1,45 (±0,15)	34,776	41 (±0,04)	20,m + 4,sm
TCAR9	24	1,00 - 1,54	1,537	0,54 (±0,08)	0,70 (±0,09)	1,24 (±0,16)	29,702	44 (±0,03)	22,m + 2,sm
TCAR10	24	0,90 - 1,42	1,57	0,50 (±0,08)	0,64 (±0,08)	1,14 (±0,14)	27,304	44 (±0,04)	22,m + 2,sm
TCAR11	24	1,19 - 1,59	1,338	0,57 (±0,07)	0,81 (±0,10)	1,38 (±0,12)	33,086	42 (±0,04)	18,m + 6,sm
TCAR12	24	1,35 - 1,96	1,454	0,66 (±0,09)	0,93 (±0,11)	1,60 (±0,17)	38,306	42 (±0,04)	20,m + 4,sm
TCAR13	24	1,56 - 2,33	1,487	0,73 (±0,09)	1,17 (±0,22)	1,90 (±0,24)	45,534	39 (±0,05)	10,m + 14,sm
TCAR14	24	1,26 - 1,82	1,44	0,66 (±0,09)	0,91 (±0,11)	1,57 (±0,17)	37,63	42 (±0,04)	20,m + 4,sm
TCAR15	24	1,37 - 2,08	1,525	0,71 (±0,11)	0,99 (±0,15)	1,69 (±0,21)	40,61	42 (±0,04)	22,m + 2,sm
TCAR16	24	0,99 - 1,61	1,617	0,53 (±0,08)	0,78 (±0,11)	1,31 (±0,16)	31,534	41 (±0,03)	18,m + 6,sm
TCAR17	24	1,11 - 1,50	1,358	0,55 (±0,09)	0,73 (±0,07)	1,28 (±0,13)	30,648	43 (±0,04)	18,m + 6,sm
TCAR18	24	0,96 - 1,47	1,539	0,51 (±0,08)	0,67 (±0,08)	1,19 (±0,14)	28,452	43 (±0,03)	24,m
TCAR19	24	1,06 - 1,86	1,76	0,64 (±0,12)	0,88 (±0,18)	1,52 (±0,25)	36,454	42 (±0,05)	18,m + 6,sm
TCAR20	24	1,56 - 2,20	1,413	0,76 (±0,11)	1,07 (±0,14)	1,83 (±0,19)	44,036	41 (±0,04)	18,m + 6,sm
TCAR21	24	1,04 - 1,51	1,456	0,57 (±0,06)	0,71 (±0,08)	1,28 (±0,14)	30,606	45 (±0,02)	24,m
TCAR22	24	1,18 - 1,74	1,47	0,61 (±0,10)	0,84 (±0,14)	1,45 (±0,15)	34,846	42 (±0,06)	20,m + 4,sm
TCAR23	24	0,91 - 1,82	2,002	0,62 (±0,12)	0,86 (±0,18)	1,48 (±0,22)	35,524	42 (±0,06)	18,m + 6,sm
TCAR24	24	1,02 - 1,59	1,566	0,55 (±0,06)	0,73 (±0,12)	1,28 (±0,15)	30,83	43 (±0,03)	22,m + 2,sm
TCAR25	24	1,03 - 1,58	1,537	0,55 (±0,06)	0,70 (±0,14)	1,25 (±0,16)	29,988	44 (±0,05)	22,m + 2,sm
TCAR26	24	1,16 - 2,14	1,836	0,71 (±0,11)	0,97 (±0,18)	1,68 (±0,26)	40,422	42 (±0,04)	22,m + 2,sm
TCAR27	24	1,12 - 1,61	1,437	0,60 (±0,07)	0,75 (±0,11)	1,35 (±0,14)	32,42	45 (±0,04)	22,m + 2,sm
TCAR28	24	1,36 - 2,12	1,563	0,71 (±0,13)	1,00 (±0,17)	1,71 (±0,24)	41,086	42 (±0,05)	18,m + 6,sm
TCAR29	24	1,02 - 1,59	1,555	0,55 (±0,07)	0,68 (±0,10)	1,24 (±0,17)	29,676	45 (±0,02)	24,m
TCAR30	24	1,22 - 2,12	1,73	0,65 (±0,12)	1,00 (±0,18)	1,65 (±0,24)	39,592	40 (±0,05)	18,m + 6,sm
TCAR31	24	1,07 - 2,06	1,932	0,67 (±0,10)	0,89 (±0,24)	1,55 (±0,30)	37,224	43 (±0,05)	20,m + 4,sm

Table 3. Karyotypes of *Carthamus* varieties using different methods of evaluating karyotype asymmetry. A1-intrachromosomal asymmetry index; A2-interchromosomal asymmetry index; CV_{CL}-relative variation in chromosome length; CV_{CI}-relative variation in centromeric index; AI-karyotype asymmetry index; DI-dispersion index; Stebbins' types-classification of karyotypes in relation to their degree of asymmetry according to Stebbins (1971).

Sample code	A ₁	A ₂	CV _{CL}	CV _{CI}	AI
TCAR1	0,27	0,147	14,656	10,375	1,52
TCAR2	0,244	0,08	8,001	8,367	0,669
TCAR3	0,285	0,126	12,575	9,117	1,147
TCAR4	0,286	0,172	17,239	9,614	1,657
TCAR5	0,294	0,114	11,383	5,537	0,63
TCAR6	0,283	0,113	11,304	10,872	1,229
TCAR7	0,226	0,172	17,244	13,924	2,401
TCAR8	0,29	0,105	10,52	9,445	0,994
TCAR9	0,222	0,129	12,947	6,529	0,845
TCAR10	0,208	0,125	12,498	7,975	0,997
TCAR11	0,281	0,091	9,063	10,241	0,928
TCAR12	0,283	0,107	10,74	8,696	0,934
TCAR13	0,355	0,127	12,733	13,67	1,741
TCAR14	0,272	0,108	10,76	9,089	0,978
TCAR15	0,272	0,124	12,382	10,414	1,289
TCAR16	0,312	0,123	12,289	7,497	0,921
TCAR17	0,243	0,101	10,132	10,038	1,017
TCAR18	0,232	0,119	11,931	7,068	0,843
TCAR19	0,26	0,164	16,376	12,236	2,004
TCAR20	0,283	0,103	10,31	10,563	1,089
TCAR21	0,191	0,108	10,767	4,117	0,443
TCAR22	0,241	0,106	10,586	13,595	1,439
TCAR23	0,252	0,15	14,96	14,787	2,212
TCAR24	0,241	0,121	12,052	6,795	0,819
TCAR25	0,192	0,129	12,883	10,914	1,406
TCAR26	0,257	0,154	15,408	9,155	1,411
TCAR27	0,186	0,101	10,065	9,926	0,999
TCAR28	0,278	0,143	14,262	11,493	1,639
TCAR29	0,183	0,137	13,719	3,366	0,462
TCAR30	0,334	0,143	14,312	13,858	1,983
TCAR31	0,216	0,194	19,385	11,74	2,276

the varieties in terms of AI, Tcar 7 (CT-8-3) had the most asymmetric chromosomes. This asymmetry index of wild accession is 2.401 and higher than patented types. High asymmetry index values indicate the high levels of karyotypic heterogeneity sourced from extensive chromosomal alterations and it correlates with the potential for yield. A similar situation applies in Tcar 31

(KS06) and Tcar 23 (J29), they have high asymmetry indices 2.276 and 2.212 respectively and their karyotype formulas contain various types of chromosomes. These two varieties could be evaluated as having high chromosomal variation but certainly they are not possible to use more for hybridisations or rootstock.

In addition, Tcar 13 (E12) has a unique karyotype formula with $10m + 14sm$ chromosomes. This type's asymmetry index is 1.741. Cross-cultivations can be made between varieties to make this variety most efficient in terms of all features.

According to the A1 index, while 28 species with symmetric karyotypes had A1 values ranging from 0.183 to 0.29, the remaining three species with asymmetric karyotypes had higher A1 values, varying from 0.312 to 0.355. When we consider the A2 values of studied samples, they displayed a low variation level ranging from 0.08 to 0.194. Our results compatible with the other studies related with karyomorphology of *C. tinctorius*. Yazdani et al., (2013) studied seven *C. tinctorius* populations and their A1 values, varying from 0.36 to 0.46 and A2 values ranging from 0.13 to 0.18 respectively.

The CV_{CI} index evaluates differences in centromere position for each chromosome in the karyotype and provides a measure of intrachromosomal asymmetry. In contrast the CV_{CL} gives a measure of interchromosomal asymmetry as it reflects how variable the chromosome sizes are in the karyotype (Peruzzi, 2009). Our CV_{CI} values are between 3.366- 14.787. CV_{CL} values range from 8.001 to 19.385. In both situations, the larger value indicate the greater the asymmetry in the karyotype. So

when we evaluated our specimens in terms of these values, they consist of mostly symmetric chromosomes.

Population diversity existed with regards to the number of satellites and their positions on the chromosomes (Yazdani et al., 2013). In this study, satellites were observed at seventeen samples (Tcar 1, 7, 8, 10, 12, 14, 15, 19, 20, 22, 23, 25, 26, 27, 28, 30, 31) at various chromosome pairs (Table 4).

When we evaluate satellite locations of the studied species, Tcar 7, 23 and 30 have satellite at fourth chromosome pairs. They collected from same locality and asymmetry indices are close to each other. Therefore, we can say easily that these accessions are belonging to same origin or one species. On the other hand, Tcar 1 and 31 are American originated varieties and they share same chromosome formula. They also have satellites but at different chromosome pairs. We concluded that satellites are valuable chromosomal markers; however, they are not always to be held responsible in determining the chromosomal origin.

According to our karyological and karyotype analyses of *Carthamus* types and varieties we have been found cytotypes have a very high potential in terms of selection and hybridization studies.

Table 4. Satellite locations of the chromosomes.

Sample code	Sample ID	Satellite numbers	Satellite position	Chromosome location of satellite
Tcar 1	Black sun 2	1	Third chromosome	m
Tcar 7	CT-8-3	1	Fourth chromosome	m
Tcar 8	G8	1	Sixth chromosome	m
Tcar 10	A13	1	Seventh chromosome	m
Tcar 12	H3	1	First chromosome	m
Tcar 14	C12	2	First and sixth chromosomes	m
Tcar 15	J51	2	First and sixth chromosomes	m
Tcar 19	C11	1	Second chromosome	m
Tcar 20	Y 11-8-14-1	2	First and fifth chromosomes	m
Tcar 22	F5	1	Fifth chromosome	m
Tcar 23	J29	1	Fourth chromosome	m
Tcar 25	E5	2	Fourth and seventh chromosomes	m
Tcar 26	H7	1	Second chromosome	m
Tcar 27	H14	1	Third chromosome	m
Tcar 28	Dinçer	1	Third chromosome	m
Tcar 30	E1	1	Fourth chromosome	m
Tcar 31	KS06	1	Fifth chromosome	m

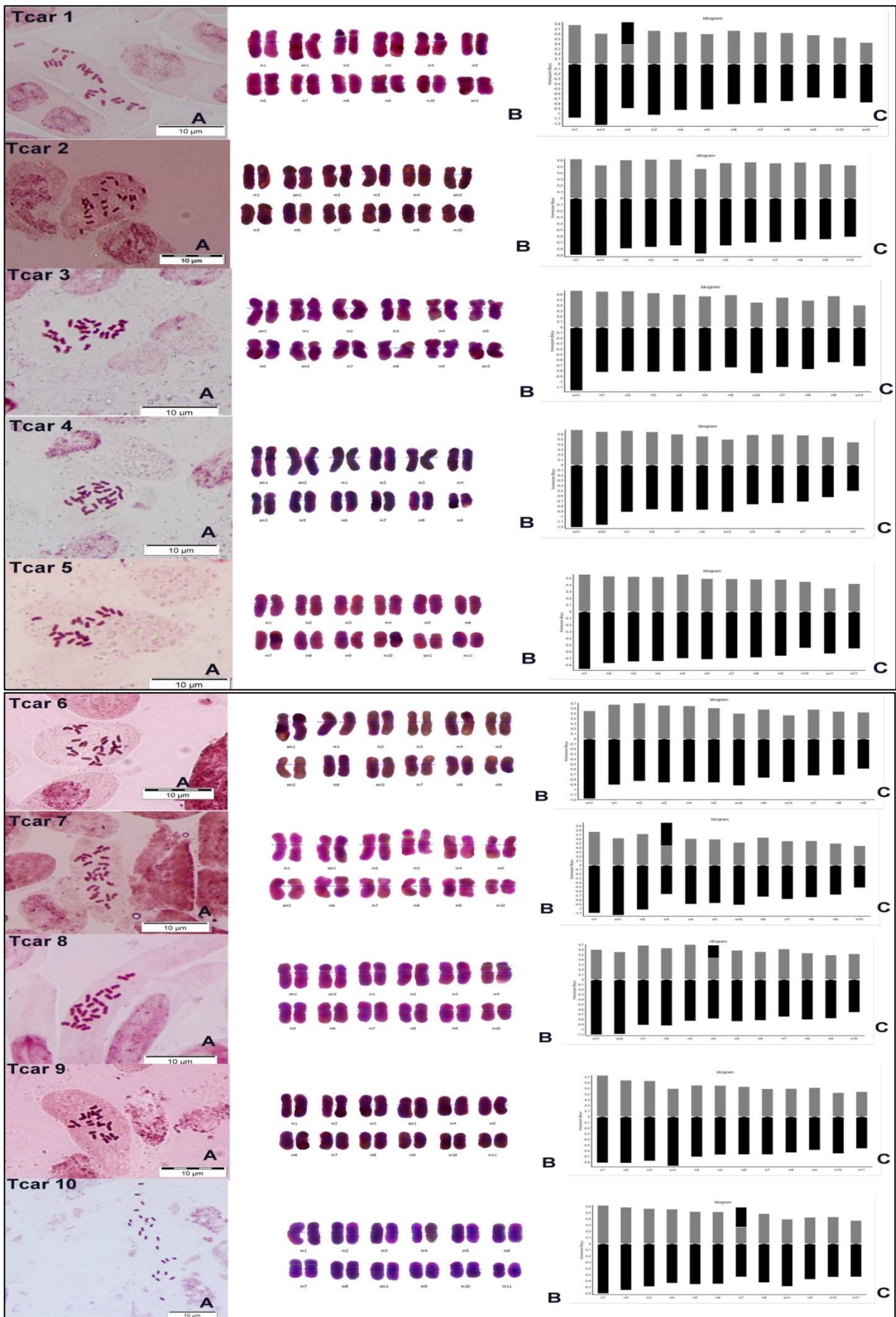


Figure 1. The metaphase plates (A), karyograms (B) and idiograms (C) of studied samples

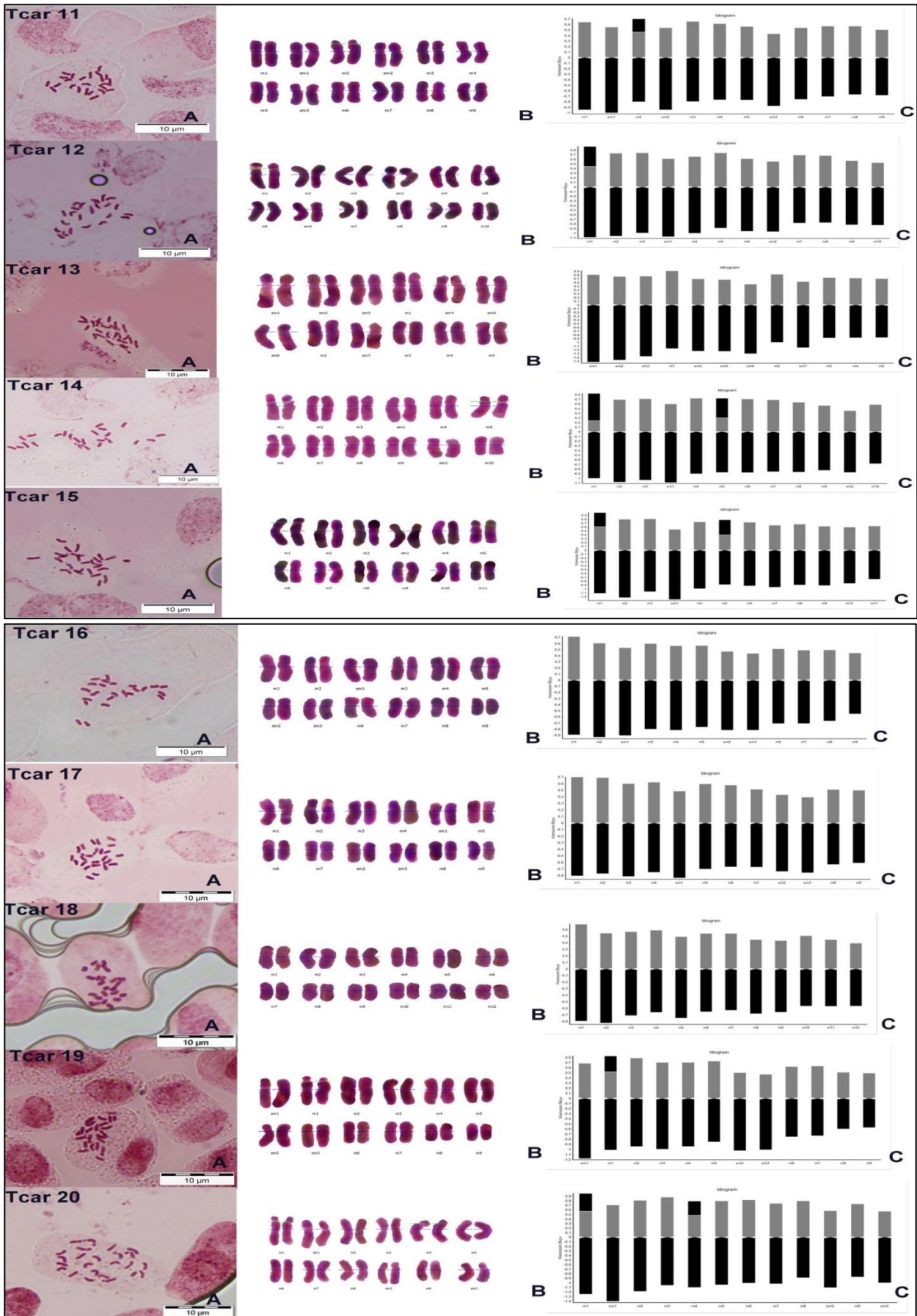


Figure 1(cont.). The metaphasis plates (A), karyograms (B) and idiograms (C) of studied samples

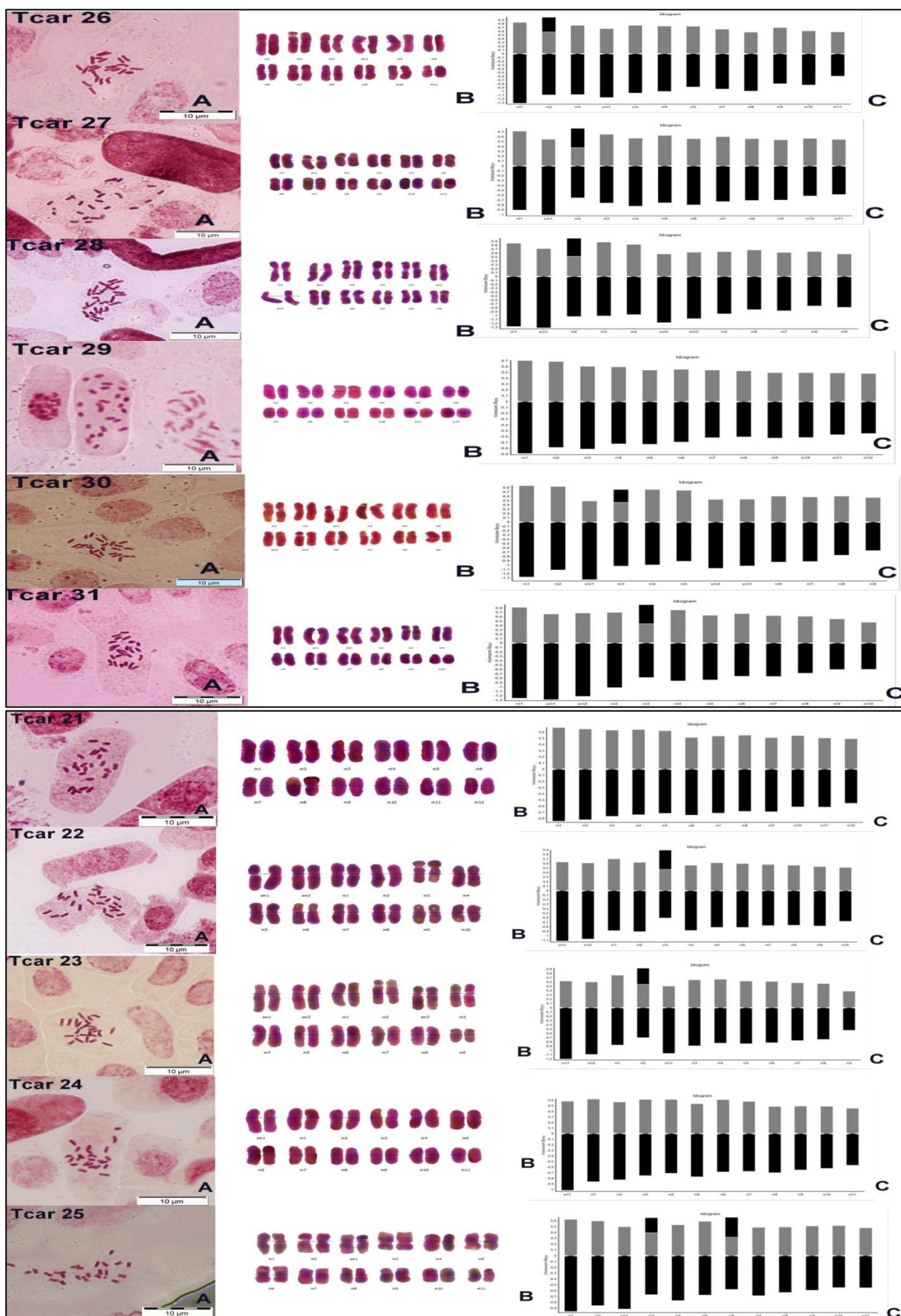


Figure 1 (cont.). The metaphase plates (A), karyograms (B) and idiograms (C) of studied samples

4. Conclusion

The present study emphasizes very important information in terms of chromosomal variation and, in particular for improvement of new offspring's or genotypes. The findings coming from these 31 accessions indicate the presence of significant differences between their karyotypes, in terms of chromosome formula, total length and symmetry indices. From this information we can declared that the karyotyping has a big and important potential for breeding and selection studies.

When we consider the karyological results obtained as a whole, we think that some of the varieties of ours can be

evaluated commercially because they have close-karyological values with the patented types.

Finally, we can conclude that it is also thought that molecular markers will be important for the selection of *Carthamus* varieties with similar characteristics to be made and will contribute to breeding varieties in terms of increase seed yield. These studies are important sources for plant breeding and rehabilitation.

Acknowledgments

We would like to thank the Scientific Investigation Project Coordinator of Selçuk University [project number: 13201053] for its financial support.

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Cite this article: Uysal T, Tekkanat BS, Şimşek Sezer EN, Ada M, Bozkurt M (2018). Karyotype analysis of some lines and varieties belonging to *Carthamus tinctorius* L. species. *Anatolian Journal of Botany* 2(1): 1-9.