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# Karyotypic analysis of different populations of *Carthamus tinctorius* Linnaeus (Asteraceae)

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**Abstract.** Somatic chromosomes of *Carthamus tinctorius* L. were analysed. A karyotype formula for each studied population was formulated. Although all samples have 2n = 24, they could be differentiated by their karyotype formula and quantitative parameters of the karyotypes. The chromosomes were assorted into different categories on the basis of arms ratio following Levan et al. (1964). These were further subdivided into different types, on the basis of total length of the chromosomes. Based on an evolutionary point of view, variation in total chromosome length without major changes in the karyotype formula suggests that changes in the amounts of genomic DNA are proportional to the relative length of an each chromosome arm. All samples possessed symmetrical or slightly asymmetrical karyotype. The samples belonging to the same species were showing intra-specific or inter-specific chromosome polymorphism. This finding may provide useful information for *Carthamus* evolutionary, genetic, and breeding studies.

**Key words:** Asteraceae, *Carthamus*, karyotype variation.

## Introduction

The genus *Carthamus* Linnaeus, a member of the family Asteraceae, comprises more than 20 species. *Carthamus tinctorius* Linnaeus commonly called as safflower is the only cultivated species of the genus; its corolla is known as an important crude drug in traditional Chinese medicine for promoting blood circulation and removing blood stasis. The genus *Carthamus* divided into 4 sections. Section one (2n=20) includes *C. oxycantha* and *C. palaestinus*, section two (2n=24) – *C. tinctorius*, *C. alexandrius*, *C. glaucus*, *C. syriacus* and *C. tenuis*, section three (2n=44) – *C. lanatus* and section four (2n=64) – *C. baeticus*. The first two sections are diploids, the third is a tetraploid and the fourth section includs hexaploid species (Khidir, 1969).

Safflower is an ancient crop with numerous manners of practical useing (Esendal, 2001; Corleto et al., 1997; Guangwei, Dayue, 1999). Traditionally safflower was grown for its flowers, which were used as a fabric dye, a food dye and medicinal purposes. Today, mainly seeds are used for different purposes: as a high quality edible, for receiving an industrial oil and as a bird feed (Knowles, 1989).

In plant taxonomy, genetic studies and breeding information about chromosome karyotypes can be useful in species identification and analysis of hybrid populations. The somatic chromosome analysis of the genus *Carthamus* is very difficult due to poor stainability, stickiness and tendency to overlap at metaphase and diffuse appearance of primary and secondary constrictions of the chromo-

somes. An efficient squash technique for resolving the somatic chromosomes of safflower was developed. It permitted the detailed analysis of the karyotype. Three dimensional somatic karyotype were analyzed for establishing the chromosome and karyotype polymorphism.

## Material and Methods

The present paper deals with the karyotypic analysis of different populations of Carthamus tinctorius. The samples that were used for somatic karyotype are listed in Table 1.

**Table 1.** Material used for somatic karyotype analysis.

| No. | Lab Code | Source Country |
|---|---|---|
| 1 | T-47 | India, Delhi |
| 2 | T-49 | India, Delhi |
| 3 | T-52 | Iran |
| 4 | T-78 | Portugal, Azores |
| 5 | T-81 | Spain, Cordoba |
| 6 | T-83 | Sudan |
| 7 | T-88 | USA |
| 8 | T-90 | USA, Arizona |

The root tips squash techniques used by early workers (Sikdar, De, 1967; Shrivastava, Joshi, 1972; Reddy 1973) gave unsatisfactory results. The squash technique developed by Pillai et al. (1981), however with minor modification was found suitable for the present study. For karyotype analysis, fresh and dry seeds were spread over in Petri dishes with moist filter paper for germination.

For investigating the somatic karyotype, roots were pre-treated with 0.05% colchicine solution during 3-4 hours at $10^{o}$ C in darkness. The roots were then washed thoroughly with water and fixed in the mixture of methanol and glacial acetic acid (1 : 1) during 24 h.

The above fixative was replaced with modified Pienar's solution (Isopropyl alcohol, Propionic acid, solvent ether and acetone, 6 : 3 : 1 : 1). These procedures were done at $10^{0}$ C. After 24 h the root tips were washed in water and hydrolysed in 1NHCL during 10 min. at $60^{o}$ C and stained with 2% aceto-orcein. Root tips were prepared in a drop of 1.5% aceto-orcein. The somatic chromosomes were analysed from the photomicrographs using computerized Nikon Image Capturing system with an oil immersion lens. For each seed source, chromosome evaluation was conducted from at least five root tips (five seeds). Chromosome measurements were made on the enlarged prints and converted to micrometers by relating measurements made in the microscope with a micrometer. Karyotype analysis was based on at least five high-quality metaphase cell plates. The following parameters were used for the somatic karyotype analyses.

(a) Length of long arms, short arms and the whole chromosome, (b) arm ratio, (c) total length of long arms (TLLA), all short arms (TLSA) and the whole chromosome (TLCC), (d) gradient index (GI), (e) symmetry index (SI), (f) radius of a chromatid of the chromosome of a complement and (g) total volume of long arms (TVLA), short arms (TVSA) and the whole chromosome of the complement (TVCC).

The volume of each chromosome was measured assuming that it is made of two cylinders corresponding to two sister chromatids. The chromosomes were assorted in to different categories on the basis of arm's ratio following Levan et al (1964) (M=1.0, m=1.0-1.7, sm=1.7-3.0, st=3.0-7.0) and were classified in to four categories on the basis of total length, A-D (A= 4.5-3.5µm, B=3.5-2.5µm, C=2.5-1.5µm and D=1.5-0.50µm). The chromosome complements were also divided in to four types, W-Z using mean radius of the chroma-

tids of the complement (W= 0.10-0.15µm, X= 0.15-0.20µm, Y= 0.20-0.25µm, Z= 0.25-0.35µm). Further, the chromosome complements were divided, as per Stebbins (1958) in to different categories on the basis of arm ratio (Table 3).

## Results and discussion

Mitotic metaphase cell plates are shown in Figs 1-8. As previously reported (Knowles, 1988), chromosome number of *Carthamus tinctorius* is 2X=2n=24. For analysis of the soma-tic karyotype, the above listed parameters were used. The results of the analysis are presented in Table 2.

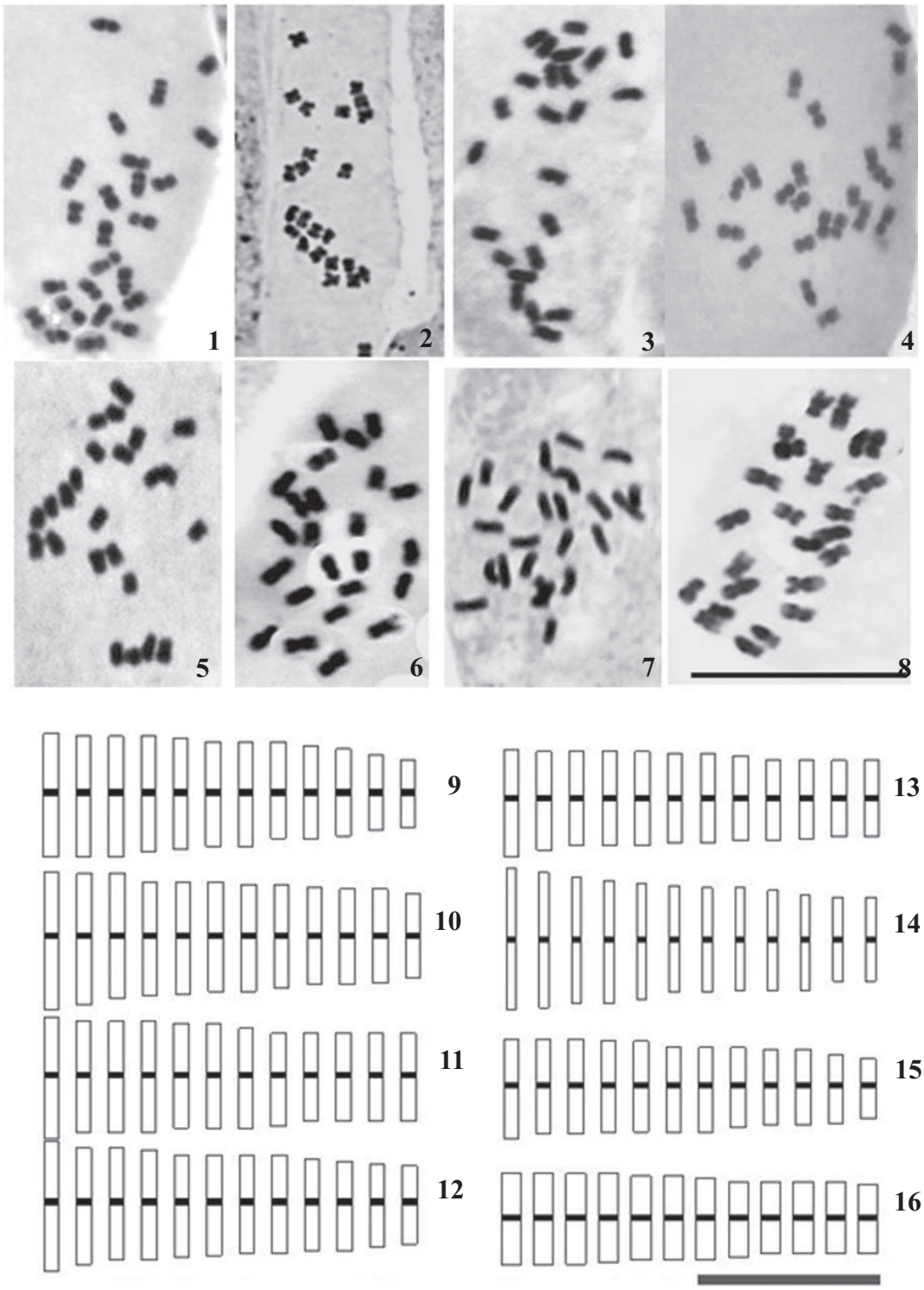
Table 2 also contains karyotypic formulae for somatic karyotypes of all analyzed samples. Three dimensional ideograms of the gametophytic set of somatic chromosomes are depicted in Figs 9-16.

Comparison of the data related to length and volume of the gametophytic sets of the chromosomes of different samples revealed very clearly that a direct correlation between length and volume of the chromosome complement did not exist. For instance, the length of the chromosome complement of T-81 was greater (64.66 µm) over that of T-83 (50.65 µm) but the volume of the chromosome complement was higher for T-83 (10.16 µm) over that of T-81 (7.84 µm). The volume of a chromosome depends upon the length as well as thickness. Therefore, this set of observations indicated that the length of the chromosome complement should not be taken as the only parameter for deciding the variability in the amount of genetic material. In general, chromosomes of 'M' and 'm' were present in high frequency in chromosome complement. However, 'sm' was also observed in some samples.

**Table 2.** Data related to karyotype of *Carthamus*. TLCC=Total length of chromosome compliments, TLSA= Total length of short arm, TLLA = Total length of long arm, GI = Gradient index, SI = Symmetry Index, TVCC = Total volume of chromosome complement, TVSA = Total volume of short arm, TVLA = Total volume of long arm, KF = Karyotypic Formula.

| Lab Code | TLCC (µm) | TLSA (µm) | TLLA (µm) | GI | SI | TVCC | TVSA | TVLA | KF |
|---|---|---|---|---|---|---|---|---|---|
| T-47 | 48.33 | 23.82 | 24.51 | 49.21 | 97.19 | 7.30 | 3.62 | 3.68 | X[2B(m)+6C(m)+14C(M)+2D(M)] |
| T-49 | 43.69 | 21.67 | 22.02 | 70.00 | 98.44 | 6.60 | 3.29 | 3.31 | X[6C(m)+18C(M)] |
| T-52 | 57.28 | 27.00 | 30.27 | 58.33 | 89.20 | 8.62 | 4.03 | 4.59 | X[2B(M)+6B(m)+12C(m)+4C(M)] |
| T-78 | 44.38 | 21.59 | 22.79 | 67.92 | 94.72 | 6.66 | 3.25 | 3.42 | X[12B(m)+12B(M) |
| T-81 | 64.67 | 30.62 | 34.06 | 51.76 | 89.90 | 14.84 | 7.00 | 7.84 | Y[2A(m)+14B(m)+8C(m)] |
| T-83 | 50.65 | 23.91 | 26.75 | 59.38 | 89.39 | 19.17 | 9.02 | 10.16 | Z[4B(m)+16C(m)+4C(M)] |
| T-88 | 55.73 | 26.57 | 29.15 | 65.63 | 91.15 | 8.46 | 4.00 | 4.46 | X[2B(M)+20C(m)+2C(M)] |
| T-90 | 41.45 | 19.87 | 21.59 | 67.35 | 92.03 | 2.16 | 1.04 | 1.12 | W[18C(m)+4C(M)+2D(M)] |

The karyotype analysis revealed that there was no secondary constriction and sub-terminal chromosome in any of the eight analyzed samples. When the karyotype asymmetry is taken into consideration the asymmetrical karyotypes are supposed to be more advanced than the symmetrical ones (Stebbins, 1950). Among the different samples of *Carthamus tinctorius* in the present study all of them having a maximum number of metacentric chromosomes may be considered as the most primitive. But none of them showed sub-terminal




**Figs 1-16.** Karyotypes and ideograms of somatic chromosomes of different *Carthamus tinctorius* samples. **1, 9** - T-47. **2 , 10** - T-49. **3, 11** - T-52. **4 , 12** - T-78. **5 , 13** - T-81. **6 , 14** - T-83. **7 , 15** - T-88. **8 , 16** - T-90. Bars = 20 μm (for Figs 1-8) and 2.5 μm (for Figs 9-16).

chromosome, which is the characteristic of advanceness. However, on the basis of karyotype analysis in the present study along with the number of metacentric chromosomes and lacking of sub-terminal chromosomes as observed in all samples of *Carthamus tinctorius* may be considered them as primitive type. It has been suggested (Stebbins, 1950) that the changes in symmetry are usually associated with chromatin loss.

The parameters used for categorizing the somatic karyotypes are (I) proportion of chromosomes with arm's ratio: more then 2 : 1 and (II) ratio between the largest vs. smallest chromosome of the compliment. The chromosomes were belonging to 1A and 1B types (Table 3) only and therefore, could be considered symmetrical or slightly asymmetrical. Only three from eight samples (T-47, T-81 and T-83) are 1B and others are 1A type due to the chromosome polymorphism. Dageri et al. (2007) have reported three pairs of satellite chromosomes while we could not observe it; it probably connects with chromosome polymorphism of *C. tinctorius.*

| Samples | Type |
|---|---|
| T-47 | 1B |
| T-49 | 1A |
| T-52 | 1A |
| T-78 | 1A |
| T-81 | 1B |
| T-83 | 1B |
| T-88 | 1A |
| T-90 | 1A |

**Table 3.** Types of karyotypes according to Stebbins (1958).

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