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Karyotype Analysis of Three Wild Species of Safflower from West Azerbaijan, Iran

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ABSTRACT

Wild relatives of the cultivated plant species are valuable genetic resource for breeding crop plants. An introductory step for obtaining genetic and evolutionary information from a plant species is the characterization of its chromosome complement which has an obvious value in cytotaxonomic studies. Studies conducted on three wild species *Carthamus oxyacantha* M. Bieb., *C. dentatus* Vahl and *C. lanatus* subsp.*turkestanicus* Raym.-Hamet from West Azerbaijan presented here. Seeds were germinated in petri dishes and the squash technique applied for chromosome studies. Results revealed that *C. oxyacantha* (2n=24) consisted of nine pairs of metacentric (m) and three pairs of submetacentric (sm) chromosomes in which the longest two pairs had satellites. One set of its chromosomes had a total length of 10.03µm. Among the 10 pairs of chromosomes of *C. dentatus*, five pairs were metacentric and three submetacentric, with two pairs of satellites. The remaining two pairs were subacrocentric (st). The total chromosome length of haploid complement in *C. dentatus* was 10.63µm. In *C. lanatus* subsp.*turkestanicus* (2n=64) the total chromosome length of one set was 20.60µm. Complement length comparison of the species confirm the allopolyploid or amphidiploid origin of *C. lanatus* subsp.*turkestanicus* from the diploid species.

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INTRODUCTION

The genus *Carthamus* L. is a member of the Compositae family and consists of both annual and perennial species. There are more than 20 species in this genus of which only *C. tinctorius* L. (safflower) is cultivated with 2n=24 [11]. The wild species of *Carthamus* are represented by diploid, tetraploid and hexaploid forms with 2n numbers of 20, 22, 24, 44 and 64. Six of these species have been reported from Iran [12].

Knowledge of the cytogenetic and taxonomic relationships among species of *Carthamus* provides a basis for the effective utilization of characteristics in wild and weedy relatives of cultivated safflower in future breeding programs. Kumar [8] provided an appraisal of past cytogenetic research in the *Carthamus*. Hybridization and chromosome pairing studies have to some extent revealed the species relationship and the progenitors of some of the polyploidy species have been determined. *C. oxyacantha* has been documented to be one of the closest relatives of cultivated safflower, carrying the same genome (BB). It is a densely branched and very spiny, annual weed adapted to habitats associated with people and crop cultivation [1]. The seed of this species contains about 28% oil and in some areas it is used for culinary and as lighting fuel [15].

Karyotypic studies of *Carthamus* species has been very limited as it is difficult to resolve the somatic chromosomes because of the poor stain ability, stickiness of chromosome, tendency of chromosome to overlap at metaphase and diffused appearance of primary and secondary constriction [4]. The oldest report encountered is that of *C. tinctorius* and *C. nitidus*, both with n=12. Each one of these species is reported to have one pair of satellited chromosome [7]. However, one significant study is that of Pillai *et al*., [13] in which the karyotype of cultivated safflower has been described. Malik and Srivastava [11] studied karyotypic analysis of different populations of *C. tinctorius*. They differentiated populations by their karyotype formula and quantitative parameters. In addition, the karyotype of *C. oxyacantha* in two Indian races has been reported [5].

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Advances in Environmental Biology, 9(2) January 2015, Pages: 117-122

MATERIALS AND METHODS

Plant materials were collected from three regions of West Azerbaijan province in Iran as follows: *C. oxyacantha* from Khoy, *C. dentatus* from Sardasht and *C.lanatus* subsp.*turkestanicus* from Shahindej. Fifty seeds from each species were germinated in Petri dishes lined with moist filter paper and placed in an oven at 25°C. Twenty seedlings from each species having a root length of 15-20 mm were selected and placed in a 0.002 M pretreatment solution of 8-hydroxyquinoline for three hours at room temperature. Seedlings were then washed thoroughly in distilled water and fixed in ethanol-acetic acid (3:1) for 12 h. About 2-3 mm terminal end of each root tip was then dissected and directly used for chromosome analysis or transferred into 70% ethyl alcohol and stored at 4°C for later investigation.

Root tips were transferred into a 1:1 solution of 1N HCl and 70% ethyl alcohol for 5 min for softening and separation of cells followed by their transfer into 70% ethyl alcohol to wash away the acid. A 2% solution of aceto-orcein for 15-30 min was used for the staining of root tips followed by their transfer into a solution of 45% acetic acid. Root tips were squashed in a drop of 45% acetic acid on a glass slide and the best chromosome spreads were observed and photographed under the microscope at 1000X. All measurements were taken from the photographic prints using a slide-caliper with an accuracy of 0.01 mm.

Measurements taken from each chromosome consisted of the long arm, short arm and the length of the satellite, where present. Based on these measurements, the ratio between the long and short arms (Pa-Values) excluding the satellite (Blixt, 1958) was calculated for classifying the different types of chromosomes. These categories are M, m, sm, st, t and T for the respective Pa-Values of 1.00, 1.01-1.69, 1.70-2.99, 3.00-6.99, 7.00-36.99 and >37 [9]. Wherever necessary, for the confirmation of the exact centromic position, the relevant slide was reobserved under the microscope by focusing up and down and comparing the observation with the photographic print. The L% was calculated as the percentage of the length of each chromosome in the haploid genome. After detection of chromosome pairs, karyotype was prepared and photographed. For displaying the number and form of chromosomes in each species, the karyotype formula which characterizes one set of chromosomes was used [13]. In this formulae, group A chromosomes have a satellite and group B chromosomes are without a satellite. In addition, chromosomes of relatively similar length were combined accordingly.

RESULTS AND DISCUSSION

A: Carthamus oxyacantha:

A representative plant of this species is depicted in Fig. 1. It is annual, plant height varies 30-50 cm, it is extensively branched and spiny and bear sulfur yellow flowers.

Fig. 1: *Carthamus oxyacantha.*

Karyotype analysis revealed that its complement has 2n=24 chromosomes (Fig. 2) in which nine pairs were metacentric and the remaining three pairs were submetacentric (Table 1). Chromosomal length ranged from 0.54 to 1.10 µm, the average length being 0.83 µm. Based on the total relative length, chromosomes were arranged from 1 to 12 in descending order (Fig. 2).

Two pairs of the chromosomes consisting of No.1 and 2 each had a satellite on its short arm. In our measurements, the length of the long arms ranged between 0.33 and 0.65 µm and this range extended between 0.21 and 0.43 µm for the short arms. The total length of the chromosomes in one set of 12 was 10.03 µm. the Pa-values and L% of the non-satellited chromosomes (Class B), varied from 1.00 to 2.12 and 5.38 to 10.97, respectively (Table 1).

Advances in Environmental Biology, 9(2) January 2015, Pages: 117-122

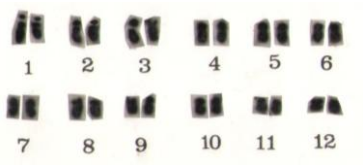

Fig. 2: Karyogram of *Carthamus oxyacantha*.

Table 1: Morphometric data on chromosomes of the diploid species *Carthamus oxyacantha* (2n=24).

Chromosome No.	Long chromosome arm(µ)	Short chromosome arm(µ)	Total chromosome length(µ)	Pa-value	Satellite length(µ)	L%	
1	0.63	0.24	1.10	2.62	0.23	10.97	sm-sat
2	0.45	0.31	1.02	1.45	0.26	10.17	m-sat
3	0.65	0.37	1.02	1.75		10.17	sm
4	0.53	0.43	0.96	1.23		9.57	m
5	0.48	0.40	0.88	1.20		8.77	m
6	0.52	0.33	0.85	1.57		8.47	m
7	0.40	0.40	0.80	1.00		7.98	M
8	0.53	0.25	0.78	2.12		7.78	sm
9	0.43	0.30	0.73	1.43		7.28	m
10	0.36	0.35	0.71	1.03		7.08	m
11	0.33	0.31	0.64	1.06		6.38	m
12	0.33	0.21	0.54	1.57		5.38	m
Total	5.64	3.9	10.03				

Since there are more metacentric than submetacentric chromosomes in this species, *C. oxyacantha* is considered to have a symmetrical karyotype and it is a generalized primitive species [14]. Because of the similarity in the length of chromosomes No. 5 and 6 and also chromosomes No. 9 and 10 (Table 1), only 10 groups of chromosomes can be observed in the karyotype formulae of this species as below:

$K(n=12)= 1A_1^{sm} + 1A_2^{m} + 1B_1^{sm} + 1B_2^{m} + 2B_3^{m} + 1B_4^{M} + 1B_5^{sm} + 2B_6^{m} + 1B_7^{m} + 1B_8^{m}$

In a separate report of karyotypic study in two races of this species, only one chromosome pair was identified to have a satellite [5] which is discrepant with our observations. On the other hand, in the cultivated species of safflower, *C. tinctorius* (2n=24), three pairs of the chromosomes have been reported to have a satellite [13]. *C. oxyacantha* is considered to be one of the parental species of cultivated safflower [1] and they cross easily with each other. These two species are placed in the same section in the reclassification of the genus *Carthamus* [10]. Although *C. oxyacantha* blooms earlier than the cultivated species under natural conditions, introgression of the weedy and cultivated species may still take place [16].

B: Carthamus dentatus:

This species has not been reported from the West Azerbaijan province before and we have not been able to find it in any place other than the Sardasht area in this province. A representative plant of this annual species having purple flowers and its karyotype is presented in Fig. 3.

Fig. 3: *Carthamus dentatus.*

Advances in Environmental Biology, 9(2) January 2015, Pages: 117-122

As can be seen, karyotype analysis has revealed 2n=20 chromosomes in which five pairs were metacentric, three pairs submetacentric and the remaining two pairs were subacrocentric (Table 2 and Fig. 4). The longest chromosome, No. 1, had a length of 1.78µm and it was submetacentric and the shortest chromosome, No. 10, was 0.67 µm and it was subacrocentric.

Table 2: Morphometric data on chromosomes of the diploid species *Carthamus dentatus* (2n=20).

Chromosome No.	Long chromosome arm(µ)	Short chromosome arm(µ)	Total chromosome length(µ)	Pa-value	Satellite length(µ)	L%	
1	1.05	0.46	1.78	2.28	0.27	16.75	sm-sat
2	0.59	0.33	1.18	1.79	0.26	11.10	sm-sat
3	0.59	0.57	1.16	1.03		10.91	m
4	0.70	0.40	1.10	1.75		10.35	sm
5	0.53	0.49	1.02	1.08		9.60	m
6	0.60	0.41	1.01	1.46		9.50	m
7	0.62	0.39	1.01	1.59		9.50	m
8	0.47	0.41	0.88	1.14		8.28	m
9	0.65	0.17	0.82	3.82		7.71	st
10	0.51	0.16	0.67	3.18		6.30	st
Total	6.31	3.79	10.63				

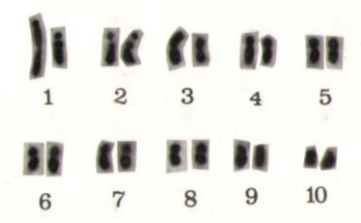

Fig. 4: Karyogram of *Carthamus dentatus.*

The average length of a chromosome in this species was calculated to be 1.06µm which is higher when compared with *C. oxyacantha*. However, similar to *C. oxyacantha*, two pairs of the chromosomes (No.1 and 2) had a satellite on the short arm, measuring 0.27 and 0.26µm in length, respectively. The total length of the chromosomes in one set of 10 was 10.63µm. Because of the similarity in the length of chromosomes No.5, 6 and 7 (Table 2), only eight groups of chromosomes can be observed in the karyotype formulae of this species as below:

$K(n=10)= 1A_1^{sm} + 1A_2^{sm} + 1B_1^{m} + 1B_2^{sm} + 3B_3^{m} + 1B_4^{m} + 1B_5^{st} + 1B_6^{st}$

C: Carthamus lanatus subsp.turkestanicus:

The annual species having yellow flowers may reach a height of 90 cm (Fig.5).

Fig. 5: *Carthamus lanatus* subsp.*turkestanicus.*

Mitotic spreads revealed its 2n=64 chromosomes which is an allohexaploid species with a genome formulae of $A_1A_1B_1B_1AA$. The detection of the centromeric position and the measurement of arm lengths was found to be

Advances in Environmental Biology, 9(2) January 2015, Pages: 117-122

relatively difficult for some of the chromosomes and for this reason only the total length of the chromosomes are being reported here (Table 3).

Table 3: Chromosome measurements of the allohexaploid species *Carthamus lanatus* subsp.*turkestanicus* (2n=64).

Chromosome No.	Total chromosome length(µ)	Chromosome No.	Total chromosome length(µ)
1	0.94	18	0.62
2	0.86	19	0.61
3	0.84	20	0.58
4	0.80	21	0.57
5	0.77	22	0.57
6	0.73	23	0.56
7	0.71	24	0.56
8	0.71	25	0.55
9	0.70	26	0.54
10	0.70	27	0.54
11	0.68	28	0.53
12	0.68	29	0.53
13	0.67	30	0.51
14	0.67	31	0.49
15	0.65	32	0.48
16	0.63	total	20.60
17	0.62		

However, the detection of chromosomal pairs was still possible and it is shown in order of diminishing length in Fig. 6.

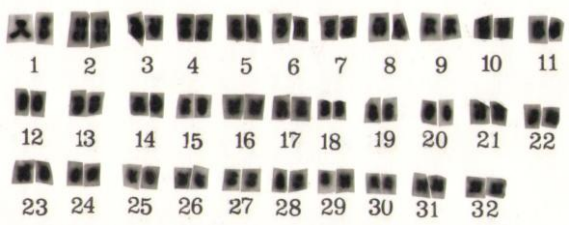

Fig. 6: Karyogram of *Carthamus lanatus* subsp.*turkestanicus.*

The chromosomes in this species were in general smaller and ranged between 0.48 and 0.94µm having a total length of 20.60µm. The greater total length of the chromosomes in this species is obviously due to its being a polyploidy. Chromosome pairing studies has disclosed the progenitors of *C. lanatus* subsp.*turkestanicus* to be the allotetraploid *C. lanatus* subsp.*lanatus* (2n=44)($A_1A_1B_1B_1$) and the diploid *C. glaucus* Bieb. (2n=20)(AA) [3,6].

However, no karyotypic analysis has been reported for this species. Both of the species *C. dentatus* of this study and *C. glaucus* having identical chromosome numbers have been classified in the section *Odonthagnathius* whereas *C. lanatus* ssp. *turkestanicus* of this study and *C. creticus*, another allohexaploid species (2n=64; $A_1A_1B_1B_1A_2A_2$) appear in a separate Section of *Atractylis*. These two sections are now considered to be distinct when compared with the section *Carthamus* which consists of only species with 2n=24 chromosomes [10].

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