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Investigation of Cytogenetic and Karyological Characteristics of Sunflower

Mehdi Zohdi Aghdam*

Department of Agriculture and Plant Breeding, Khoy Branch, Azad Islamic University, Khoy, Iran.

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***Corresponding author:** Mehdi Zohdi Aghdam, Department of Agriculture and Plant Breeding, Khoy Branch, Azad Islamic University, Khoy, Iran.
Email: zohdiaghdammahdi@gmail.com

Abstract

In this research, cytogenetica characteristics of *H. tuberoses* and *H. annuus* including chromosomal morphology, shape, size, number of satellite were studied. Caryotypic analysis of *H. tuberosus* showed that this species with genome formula of 2n = 6x = 102 is a hexaploid. In these species, average length of a haploid chromosomes set was 230.415 ± 3.311 (µ). Out of 51 pairs of chromosomes 34 were identified metacentric, 14 pairs sub metacentric and 3 pairs sub acrocentric. Among them 3 pairs satellite chromosomes were observed. The karyotype analysis of *H. annuus* showed that it is a diploid species 2n = 2x = 34. Average length of haploid chromosome set was 75.682 ± 2.995 (µ). Out of 17 pairs of chromosomes, 11 pairs were metacentric, 2 pairs sub metacentric and 4 pairs were sub acrocentric. Among them, 2 pairs of satellite were observed. The karyotype analysis of these two species showed that there are some differences in the chromosomes morphology, number and kind of metacentric, sub metacentric and sub acrocentric, number of satellite, arm ratio index and chromosomal formula.

Keywords

Chromosome, Karyotype, Metacentric, Acrocentric

1. Introduction

Plants are the main source of food for the world and more than 50% of human food comes from plants [1]. In this regard, it seems necessary to make the most of the hereditary facilities of some plant species using modern technologies in breeding and farming programs [2]. Therefore, in order to meet their current and future food needs, humans should seek to find more and better diversity among crops and increase the number and types of usable crops by studying the species' genetic and cytotological studies. Metaphase and karyotypic analysis of different species and study of the number of chromosomes and genomic formulas of the species can be transferred to some of the favorable and resistant genes within a modified cultivar by conventional breeding or biotechnology. One of these species is *H. tuberosus* which is a suitable species for cytogenetic study, as a gene source in generating resistance to several chromosomes (meta-centric, sub-metasentric and sub-ecrocentric) with a large number of chromosomes and a variety of chromosomes. Wild species have also played an important role in the genetic modification of sunflower and the most prominent example of this is the transfer of sterile male cytoplasmic trait from *H. petiolaris* to crop species which needs further investigation and study.

2. Materials and methods

2.1. Cytogenetic study method

a) Seed germination: Two samples of 5 species of both species were selected and placed in a petri dish containing 5 annuus seeds between two filter papers and appropriate amount of distilled water was added and placed in a germinator at 20°C. Tuberosus tubers were also rooted in a pot (containing 5 tubers).

b) Pre-treatment: The first step in karyotype preparation is the treatment of dividing cells with substances that inhi-

bit microtubule web activity and cause chromosomes to remain in the metaphase phase with the highest degree of compression. In this study, 0.05% colchicine solution was used to cut the roots and place in room solution for 2.5 hours.

c) Consolidation: After pretreatment and washing, roots were placed in fixative solution. The fixator used in this study was the Levitsky Fixer, a mixture of chromic acid (1%) and formaldehyde (10%) in a ratio (1: 1). This solution prevents the chromosomes from becoming too short. Chromic acid oxidizes chromosome structural components and formaldehyde also tightens components and preserves chromosome structure [3]. This solution was prepared immediately before immobilization and poured into test tubes of 20 ml approximately to a height of 1.5 cm. The roots were cut 0.5 cm from the end and fixed in a fixation solution and kept in the refrigerator for 36 hours. They were then rinsed with water for 3 hours [3].

d) Hydrolysis: For smoothing of roots and smooth crushing of sub-lamellae and better contrast, after staining, the roots were hydrolyzed for 70 minutes and rinsed with distilled water for 70 minutes. For normalization of hydrolysis, a normal NaOH was used at 60 ° C for 8 minutes. Immediately after hydrolysis, the samples were rinsed with distilled water for 30 min.

e) Chromosome staining: The dye was removed from the refrigerator 30 minutes before staining and filtered into small portions of approximately 3 mL in small bushes. The hydrolyzed samples were then incubated in the dye at 30 ° C after washing with minimal excess water and placed on porcelain bushes. The shelf life of the samples varied from 20 to 24 hours. The number of specimens laid in the color shall be such that it does not occupy more than 50% of the color volume and does not dilute the color. In this study aceto-iron-hematoxylin dye was used. For each population, a number of chromosomal features including small and large arm length, chromosome length, large to small arm ratio (arm ratio index) in 5 and 10 metaphase cells were determined for both types of karyologic features using Micromeasure software, respectively. Identification of homologous chromosomes was done based on centromere location.

2.2. Measurement and statistical analysis for data from cytogenetic studies

For each population, a number of chromosomal characteristics including small and large arm length, chromosome length, large to small arm ratio (arm ratio index) in 5 and 10 metaphase cells were determined for both types of cardiac characteristics using Micromeasure software, respectively. Identification of peer chromosomes was performed based on the location of the centromere and different karyograms and idiograms were prepared. The mean standard error was calculated from the formula $x \pm s_x$ for chromosomes of different samples, where x is the average length of chromosomes in microns and s_x is the standard error.

3. Results and Discussion

3.1. Cytogenetic study of wild and domesticated *Helianthus* species

In this study, two species of *Helianthus*, including *H. tuberosus* and *H. annuus*, were studied cytogenetically. The first species is a wild species of sunflower, which in Persian is also called pickled potato, and the second species, Sunflower is a crop that is considered as an oilseed. In this study, to determine the type of chromosome according to the location of the centromere, the method of [4] was used. In this method, the type of chromosome is determined based on the ratio of long arm to short arm (AR). Thus, chromosomes with an AR ratio between 1 and 1.67 are called metacentric (M). The ratio is between 1.68 and 3. The corresponding chromosome is called sub-metacentric (SM) chromosomes with AR between 3 and 7 are sub-acrocentric (SA) and chromosomes with AR greater than 7 are acrocentric (A). If there is no short arm and the centromere is at the end of the chromosome, the chromosome will be telocentric.

3.2. Cytogenetic study of *H. tuberosus*

In the present study, using the squash method, the chromosomes were well dispersed and metaphase domains were found suitable for karyotype preparation (Figure 1and3). The largest chromosome number 1 was 5.767± 0.019microns and its relative length was 2.503±0.008 % and was subacrocentric (SA). The smallest chromosome with meta centric type of 3.48±0.032 microns had chromosome number 51. In the study of arm ratio index, the highest arm ratio index was related to chromosome number 33, 3.265±0.327, and the lowest arm ratio index was related to chromosome number 11, 1.084±0.068 metacentric type. Also, in terms of homogeneity of arm ratio index, it was observed that arm ratio in chromosomes 16 and 47 were completely identical (1.236) and were metacentric. Overall comparison of chromosomes showed that chromosomes 9, 10 and 13 and 15 were identical in terms of chromosome length, relative length, short arm length, long arm length and arm ratio index and chromosome type (Table 1). Out of 51 pairs of chromosomes, 34 pairs were metacentric, 14 were sub-metacentric and 3 were subaccrocentric (SA) (Table 1). In the study of the number of satellites, it was found that this species has three pairs of satellite chromosomes containing chromosomes 4,21,38 which are sub-metacentric (chromosome 4) and metasentric. [4] also observed that hexaploid species of *H. tuberosus* has 3 pairs of satellite chromosomes by examining different species of hexaploid.

In the study of genomes in terms of being the same, two genomes of the three genomes of the mentioned species were the same but the third genome was not the same, and therefore it was not possible to prepare a cardiogram of the species according to the number of genomes and required banding pattern. Kostoff (1939) argued that *H. tuberosus* has the AAB genomic formula in which the B genome is very close to the *H. annuus* B genome and shows that it is derived from the ancestor *H. annuus* or a close relative.

3.3. Cytogenetic study of H. annuus

In this species, the squash method was used to cause the chromosomes to be well dispersed and good metaphase ranges to be obtained to prepare the karyotype (Figure 2and4).The study of karyotypes of different specimens showed that this species has chromosomes 2x=2n=34 and diploid. The average length of one haploid chromosome (n) was estimated to be 75.682±2.995 microns. The largest chromosome, chromosome 1, was 6.033±0.341 microns and its relative length was 7.939±0.285 and metasentric (M). The smallest chromosome with a length of 3.563 0 0.208 microns was sub-metacentric. In the study of arm ratio index, the highest arm ratio index related to chromosome 15, 5.634±0.544 sub-ecocentric (SA) and the lowest arm ratio index related to chromosome number 1, 1.144±0.034 that satellites It was metacentric (M). Also, in terms of homogeneity of arm ratio index, it was observed that arm ratio in chromosomes 9 and 12 were completely identical (5.544) and were subcranocentric (Table 2). Out of 17 pairs of chromosomes, 11 pairs of metacentric, 2 sub-metacentric and 4 subaccranent (SA) were identified.

Examination of the number of satellite chromosome pairs revealed that this species had two pairs of satellite chromosomes containing chromosomes 1 and 3 that were metasentric (M). [5] produced the mitotic karyotype of 4 varieties of sunflower crops. Each of these varieties had 4 pairs of metasentric chromosomes, 8 sub-metacentric pairs with two satellite pairs, and 5 subcranocular pairs with one pair. [6] produced karyotypes of 12 species belonging to the genus Helianthus, including *H. annuus*. They observed chromosomes with 5 pairs of metacentric, 10 sub-metacentric and 3 subaccranent pairs of different basal groups. [7] examined 11 diploid species, each of which had only one pair of satellite chromosomes.

[8] has drawn the chromosomes and idiograms of the common sunflower H. annuus based on a series of studies. The 17 existing chromosomes are divided into 4 groups based on the centromere state and the presence or absence of chromosomes with satellites. The first group had 2 pairs of satellite chromosomes of metacentric type. The second group consists of 5 pairs of metacentric or sub-metacentric chromosomes. The third group has 6 pairs of sub-metacentric chromosomes and the fourth group has 4 pairs of sub-acrocentric chromosomes. Chromosome lengths vary from 3.5 to 6.2 microns.

4. Conclusions

Comparison of arm ratio index in two species showed that chromosome 15 *H. annuus* has the highest arm ratio index, 5.634± 0.544. However, the highest arm ratio in *H. tuberosus* was related to chromosome 33, 3.265 ± 0.327 and both chromosomes were subacroscentric. In comparison, in terms of which chromosomes *H. tuberosus* received from *H. annuus*, by examining the chromosome type and arm ratio index in both species, it was shown that the ratio of arm ratio of chromosome 33 of *H. tuberosus* to chromosome 16 of *H. annuus* is almost identical and is subacrocentric, and it is possible that the chromosome received from *H. annuus* is subacrocentric, but this view is not conclusive and it is necessary to better compare the banding pattern and study the meiosis.

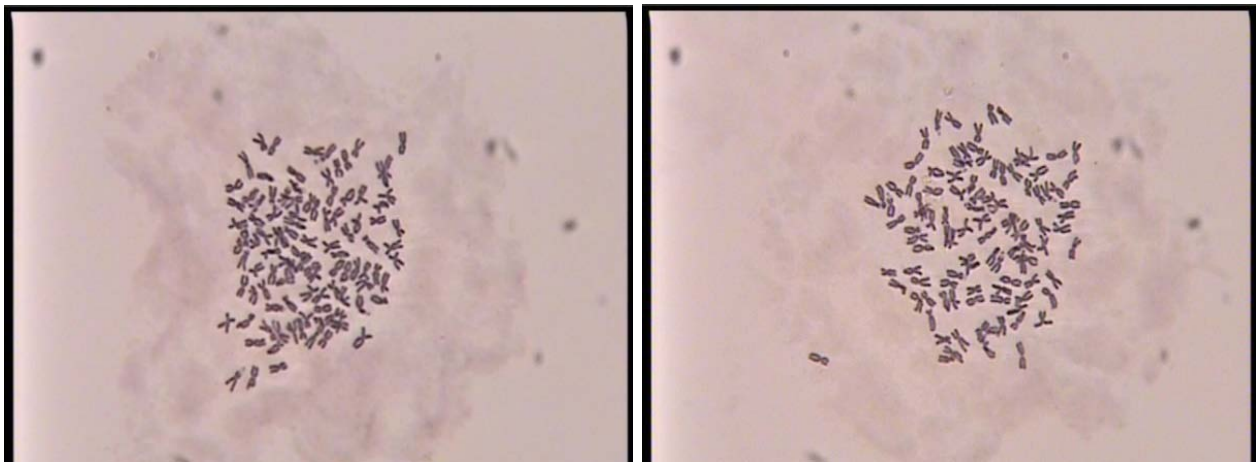

Figure 1. Microscopic photo sample of *H.tuberosus*.

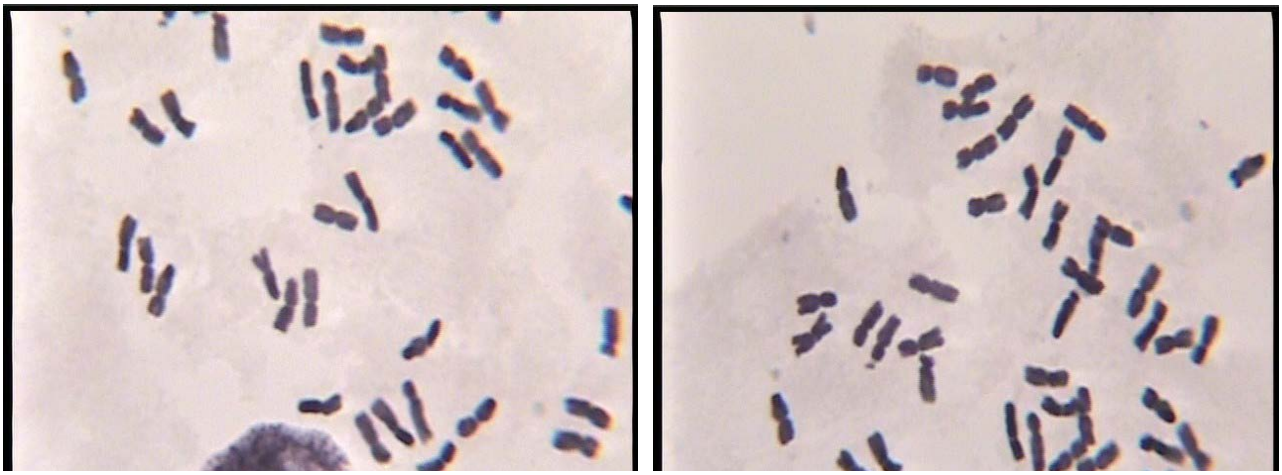

Figure 1. Microscopic photo sample of *H. annuus*.

Figure 3. Karyotype *H. tuberosus*.

Figure 4. Karyotype *H. annuus.*

Table 1. Characteristics of basal chromosomes of *Helianthus tuberosus*

Chromosome number	Chromosome length (µ)	Relative chromosome length (%)	Short arm length (µ)	Long arm length (µ)	Arm ratio index	Satellite Length (µ)	Chromosome type
1	5.767± 0.019	2.503±0.008	1.393±0.019	4.375±0.001	3.142±0.043		SA
2	5.735± 0.06	2.489±0.026	2.728±0.02	3.007±0.08	1.103±.037		M
3	5.466± 0.06	2.372±0.026	1.713±0.06	3.753±0.002	2.193±0.077		SM
4	5.373± 0.026	2.332±0.011	1.666±0.016	3.707±0.042	2.226±0.046	0.872± 0.016	SM
5	5.189± 0.027	2.252±0.012	2.431±0.049	2.758±0.022	1.135±.032		M
6	5.188± 0.08	2.252±0.035	1.965±0.05	3.223±0.13	1.643±0.109		M
7	5.155± 0.123	2.237±0.054	2.179±0.142	2.976±0.019	1.372±0.098		M
8	5.143± 0.197	2.232±0.085	1.833±0.196	3.31±0.001	1.826±0.195		SM
9	5.075± 0.046	2.202±0.02	2.075±0.002	3±0.044	1.445±0.02		M
10	5.053± 0.065	2.193±0.028	2.096±0.066	2.957±0.001	1.412±0.045		M
11	4.924± 0.016	2.139±0.07	2.367±0.069	2.562±0.085	1.084±0.068		M
12	4.89± 0.006	2.122±0.003	1.913±0.06	2.978±0.066	1.559±0.083		M
13	4.819±0.126	2.092±0.055	2.011±0.022	2.808±0.148	1.397±0.089		M
14	4.786±0.022	2.077±0.01	1.588±0.001	3.198±0.022	014/0±013/2		SM
15	4.726±0.04	2.052±0.017	044/0±989/1	2.739±0.004	1.378±0.033		M
16	4.713±0.088	2.046±0.038	2.11±0.044	2.6.3±0.132	088/0±236/1		M
17	4.7±0.033	2.04±0.014	1.564±0.01	3.136±0.043	2.005±0.041		SM
18	4.682± 0.085	2.032±0.037	1.836±0.019	2.846±0.067	1.55±0.02		M
19	4.655±0.021	2.02±0.009	2.052±0.021	2.603±0.001	1.269±0.014		M
20	4.653±0.058	2.02±0.025	1.715±0.01	2.939±0.069	1.714±0.051		SM
21	4.608±0.007	2±0.003	2.063±0.01	2.545±0.017	1.234±0.014	0.686±0.025	M
22	4.571±0.02	1.984±0.009	1.35±0.02	3.221±0.001	2.387±0.036		SM
23	4.544±0.221	1.972±0.096	1.817±0.126	2.728±0.095	1.505±0.052		M
24	4.511±0.039	1.958±0.017	1.967±0.018	2.544±0.02	1.293±0.002		M
25	4.478±0.021	1.943±0.009	1.898±0.089	2.58±0.11	1.366±0.122		M
26	4.475±0.001	1.942±0.001	1.762±0.109	2.713±0.11	1.549±0.158		M
27	4.465±0.18	1.937±0.078	1.989±0.04	22/0±476/2	1.248±0.136		M
28	4.464±0.049	1.937±0.021	1.812±0.001	2.652±0.048	1.463±0.025		M
29	4.396±0.023	1.908±0.01	1.526±0.152	2.87±0.13	1.908±0.275		SM
30	4.393±0.11	1.907±0.048	2.121±0.001	2.272±0.11	1.071±0.052		M
31	4.39±0.078	1.905±0.034	1.838±0.028	2.552±0.05	1.388±0.007		M
32	4.357±0.004	1.897±0.002	1.745±0.069	2.626±0.065	1.509±0.096		M
33	4.357±0.098	1.891±0.043	1.026±0.055	3.331±0.154	3.265±0.327		SA
34	4.305±0.109	1.868±0.047	1.525±0.019	2.78±0.09	1.823±0.036		SM
35	4.292±0134	1.863±0.058	1.444±0.153	2.848±0.019	1.995±0.225		SM
36	4.288±0.005	1.861±0.002	1.04±0.022	3.247±0.027	3.124±0.092		SA
37	4.239±0.092	1.84±0.04	1.456±0.044	2.783±0.136	1.917±0.152		SM
38	4.229±0.291	1.835±0.126	2.032±0.267	2.197±0.024	1.133±0.098	1.015±0.039	M
39	4.216±0.026	1.83±0.011	1.721±0.045	2.495±0.019	1.451±0.049		M
40	4.063±0.004	1.763±0.017	1.106±0.048	2.956±0.088	2.68±0.195		SM
41	4.026±0.036	1.747±0.016	1.905±0.04	2.121±0.003	1.114±0.025		M
42	4.014±0.009	1.742±0.004	1.445±0.104	2.569±0.095	1.791±0.195		SM
43	3.999±0.063	1.735±0.027	1.593±0.049	2.406±0.112	1.514±0.116		M
44	3.991±0.055	1.732±0.024	1.814±0.135	2.177±0.08	1.21±0.134		M
45	3.914±0.117	1.699±0.051	1.655±0.111	2.259±0.006	1.371±0.089		M
46	3.82±0.064	1.658±0.028	1.546±0.088	2.273±0.024	1.476±0.1		M
47	3.761±0.042	1.632±0.018	1.682±0.003	2.079±0.038	1.236±0.02		M
48	3.723±0.013	1.616±0.006	1.1±0.077	2.623±0.09	2.102±0.25		SM
49	3.668±0.049	1.592±0.021	1.522±0.022	2.146±0.071	1.41±0.067		M
50	3.665±0.085	1.591±0.037	1.481±0.019	2.184±0.065	1.475±0.025		M
51	3.48±0.032	1.51±0.014	1.464±0.046	2.017±0.014	1.379±0.053		M
The length of the whole genome	230.415±3.311	100					

M= metacentric, SM= sub metacentric, SA= sub acrocentric

Table 2. Characteristics of the basic chromosomes of *Helianthus annuus*

Chromosome number	Chromosome length	Relative chromosome length	Short arm length (µ)	Long arm length	Arm ratio index	Satellite length (µ)	Chromosome type
1	6.033±0.341	7.939±0.285	2.83±0.183	3.202±0.166	1.144±0.034	1.334±0.211	M
2	5.145±0.144	6.816±0.128	2.336±0.098	2.808±0.075	1.218±0.054		M
3	5.264±0.228	6.988±0.333	2.22±0.125	3.04±0.138	1.393±0.071	1.239±0.138	M
4	5.027±0.194	6.636±0.119	2.148±0.07	2.879±0.13	1.338±0.034		M
5	4.847±0.134	6.418±.0.095	2.142±0.074	2.704±0.076	1.27±0.039		M
6	4.639±0.154	6.129±0.061	2.022±0.074	2.616±0.087	1.297±0.026		M
7	4.471±0.16	5.88±0.172	1.939±0.094	2.517±0.143	078/0±311/1		M
8	4.456±0.208	5.904±0.063	1.687±0.047	2.784±0.147	1.658±0.089		M
9	4.375±0.123	5.793±0.093	0.692±0.042	3.682±0.135	5.544±0.047		SA
10	4.167±0.224	5.486±0.168	1.845±0.123	2.322±0.111	1.279±0.055		M
11	4.229±0.179	5.587±0.152	1.77±0.069	2.459±0.126	1.393±0.056		M
12	4.137±0.166	5.46±0.091	0.645±0.039	3.491±0.14	5.543±0.289		SA
13	4.021±0.182	5.299±0.125	1.43±0.088	2.591±0.119	1.847±0.104		SM
14	3.92±0.145	5.18±0.098	1.576±0.075	2.343±0.151	1.532±0.141		M
15	3.788±0.08	5.029±0.116	0.604±0.048	3.183±0.092	5.634±0.544		SA
16	3.603±0.125	4.686±0.176	0.808±0.104	2.755±0.178	3.909±0.506		SA
17	3.563±0.208	4.763±0.835	1.186±0.062	2.417±0.075	2.069±0.086		SM
The total length of the genome	75.682±2.995	100					

M= metacentric, SM= sub metacentric, SA= sub acrocentric

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