KARYOTYPE CHARACTERIZATION OF ALFALFA COLLECTED FROM WESTERN DESERT OF EGYPT

R. M. KHALAF AND ABEER ELHALWAGI

National Gene Bank and Genetic Resources. Agricultural Research Center

Alfalfa (*Medicago sativa* L.) is widely planted in the Egyptian Western Desert Oases as forage and is most often harvest for hay, but can also be made into silage, grazed, or fed green. Alfalfa has the highest feeding value among common hay crops, being used less frequently as pasture. One of the major forage crops in the oases is alfalfa. A part from being an important forage crop, it has also medicinal uses. It is a perennial crop that resides in the soil from three to twelve years, depending on the variety and climate. Alfalfa has the ability to fix nitrogen, one feddan of alfalfa adds about go to 100 Kg of atmospheric nitrogen to the soil year, which would benefit other crops.

Cytogenetical research on alfalfa lagged far behind other crops mainly because, alfalfa chromosomes are very small, the chromosomes are morphologically very similar, cultivated alfalfa has relatively high number of chromosomes (2n= 4X= 32), and alfalfa is an autotetraploid (Bauchan and Hussain, 1997).

Bolton (1962) described difficulties involved in the mitotic study of Medicago sativa due to its small chromosomes and chromosome stickiness however, other authors believe that Medicago sativa meiosis can be studied successfully (Clement and Leman, 1962). The first karyotype was published by Buss and Cleveland (1968) and additional karyotypes have been developed by Agarwal and Gupta (1983) and Bauchan and Campbell (1994).

The objectives of this study were to determine the chromosome numbers karyotype and ideograms for 20 accessions of alfalfa. In this investigation an attempt was made to study Karyotype characterization will indicate the following:

1- Determining area of chromosome.

2- Determining length of chromosome.

3- Evaluation of long arm and short arm chromosome.

4- Determining Centromeric index and Centromeric position of chromosomes for the landraces alfalfa.

MATERIALS AND METHODS

The present study has been carried out on twenty accessions chosen for cytogenetically studies. These accessions were collected from the Western Desert Oases in Egypt. The name and pedigree of these genotypes are presented in Table (1).

Cytological analysis

Chromosomal studies are based on visible characteristics of the chromosomes. Karyotype analysis is a well established method. It is based on the morphological characteristics of chromosomes and is widely used in cytogenetic analysis (Fukui and Kakeda, 1994). Imaging by digital camera in the C-metaphase of dividing root tips cells, pretreated with 0.05% colchicine and analyzed using the video test karyotype software. Measurement of the total length of chromosome (µm), long arm, short arm of chromosome, area of chromosome (µm), arm ratio, Centromeric position and Centromeric index percentage (length of short arm/length of chromosome) were taken for every chromosome.

From the karyotype analysis of alfalfa genome, the four homologues (a, b, c and d) of each chromosome pair were judged according to similarities in length of short arm, long arm, total lengths, arm ratio, area and Centromeric index percentage. An average length, arm ratio, and Centromeric index percentage were calculated (a, b, c and d)/4 for each pair was determined and the chromosome pairs were arranged in descending order and were given the numbers from 1 to 8.

Samples preparation

Seeds were germinated on moisture filter paper in Petri dishes at 25 - 30°C in an incubator. Root tips were obtained 3 days after germination was initiated. The lateral roots were collected of about 1.5:2.0 cm length.

Colchicine treatment

The roots of 1-1.5 cm were placed in glass vials containing 2 ml of 0.05% colchicine for three hours at room temperature or ice water over night.

Fixation and slide preparation

1- Fixation was done using ethanol – glacial acetic acid (3:1 v/v) fixative. Then, samples were washed thoroughly with water.

2- Flamed by forceps and staining by the aceto orcein solution.

3- These stained samples were used for automatic scanning experiments.

4- Karyotype analysis was carried out using Image Process Analysis System (Video Test-Karyo). (Fukui and Kakeda, 1994)

5- The mean measurements in the c-metaphase of fifteen cells for each accession were used to construct the karyotype.

RESULTS AND DISCCUSSION

Investigate the karyotype analysis of alfalfa that can be utilized different breeding programs. Karyotype analysis showed that all of studied accessions were autotetraploid, with a chromosome number of 2n= 4x= 32, Fig. (1a, b, c and d). Karyotype analysis includes area of chromosome, length of chromosome, arm ratio

and centromeric index, as well as centromeric position of all accessions are recorded (Tables 3, 4, 5 and 6) and illustrated in Fig. (1a, b, c and d).

1- Mean Karyotype of the area chromosome at the twenty landraces

The data obtained from the karyotype, expressed by area of chromosome in the twenty accessions are given in Table (2). This table showed that the variation in chromosome area among the different twenty studied accessions is observable. This variation ranged from the highest value of chromosome area in accession 7 (6.68 µm) to its lowest value (5.07 µm) in accession 3. Table (2) showed that all accessions have higher value of area chromosome than the accession 3. The maximum mean chromosome area was recorded in chromosome 1 of all accessions, while the minimum was recorded in chromosome 7 of the all accessions. The same result was recorded by Bauchan and Hossain (2001). The high level of chromosome area indicate that more DNA in the cells.

Identification of alfalfa chromosomes at pachytene has been conducted by Gillius (1970) and Ho and Kasha (1974). Using a computerized image analysis system it is possible to determine the homologous chromosomes and develop a karyotype based on chromosome arm length, arm ratio and total chromosome length (Bauchan and Campbell, 1994).

2- Mean karyotype of the length chromosome at twenty landraces

Figure (1a, b, c and d) and Table (3) illustrated the C-metaphase, karyotype and karyotype analysis for accessions. The data presented in table (3) showed that the chromosome length depended on twenty accessions. These differences ranged from the highest score of chromosome length in accession 8 (5.81 µm) to the lowest estimate in accession 3 (4.12 µm). However, all accessions exhibited highly frequencies of chromosome length than accession 3. The data of this table showed that differences in chromosome length could be attributed to the effects of environmental variation on studied strains. The maximum mean chromosome length was 8.55 µm recorded in chromosome 1 of accession 10, whereas the minimum was 3.07 µm recorded in chromosome 7 of accessions 3. The high level of chromosome length indicated that more crossing over. (Fayed *et al*., 1984)

Measuring chromosome from photomicrographs, Falistocco (1987) obtained much larger chromosome measurements than what have reported for the gene Medicago, for example, a total length ranging between 9 and 12 µm. Schlarbaum *et al*. (1988) karyotype tetraploid alfalfa from plants that had been regenerated from a single cell protoplast in tissue culture; however, it is known that plants regenerated from a tissue culture system potentially can have chromosomes which have been alerted (Nagarajan and Walton, 1987).

3- Mean karyotype of the long arm and short arm chromosome at twenty accessions

The mean karyotype of the long arm and short arm chromosome the twenty different accessions are given in Tables (4 and 5). The accessions varied in their chromosome long arm and short arm, showing different trends. The highest chromosome long arm and short arm was exhibited by the accession 8 while the Lowes on was displayed in accession 3. The maximum mean chromosome long arm and short arm were 5.06 µm and 3.49 µm, respectively, recorded in chromosome 1 of accession 10, while the minimum chromosome were 1.58 µm and 1.48 µm recorded in chromosomes 7 of accession 3, respectively.

Preliminary studies of six accessions of tetraploid *ssp*. falcata offer a surprising result. Most of the plants possess chromosomes which have C-bands in addition to the normal Centromeric bands. There is a range from an accession which contains the fewest number of additional bands, 4 pairs of chromosomes have an extra telomeric band on their short arms, whereas the rest of the chromosomes only have a centromeirc band, two accessions which have multiple bands on each chromosome and a banding pattern similar to doubled diploid *ssp*. coerulea. In general a majority of the heterochromatic bands appear in the short arms of the chromosomes with only chromosomes possessing interstitial bands on their long arms (Bauchan and Hossain, 2001).

4- Mean karyotype of the arm ratio, Centromeric index and Centromeric position at twenty accessions

The data of mean karyotype, expressed by the arm ratio, Centromeric index and Centromeric position in twenty accessions are given in Table (6). This table showed that the variation in arm ratio between the studied strains, this variation ranged from the highest score of arm ratio in accession 17 (1.96 µm) to its Lowes estimate (0.99 µm) in accession 11. However all accessions exhibited higher frequencies of arm ratio than accession 11. The maximum mean Centromeric index was 50.14% recorded in chromosome 5 of accession 11, while the minimum was 33.77% recorded in chromosome 2 of accession 7. The centromeirc position ranged between submetacentric and metacentric in all accessions. The same results recorded by (Bauchan and Campbell, 1994).

The somatic chromosome karyotype of this subspecies consists of one pair of satellite chromosome (chromosome 8), four pairs of sub metacentric chromosomes (chromosomes 1-4), and there pairs of short metacentric chromosomes (chromosomes 5-7). Chromosome 8 possesses the nucleolar organizer reigon (NOR) of the alfalfa genome. The satellite chromosomes are diagnostic feature of the karyotype. The large submetacentric chromosome pair 1 is easy to distinguish if the chromosomes have not spiralized too much during pretreatment. Occasionally a tertiary constriction can be found on

chromosome 4 (Bauchan and Campbell, 1994).

The karyotype of African population alfalfa germplasm consists of one set of chromosomes with satellites (chromosome 8), for sets of submetacentric chromosomes (chromosome 1-4), and three sets of metacentric chromosomes (chromosomes 5-7). All of the chromosomes have Centromeric bands and a terminal band on the short arm, with the exception of the satellites (Bauchan and Hossain, 2001). Measurement of the total lengths of chromosome (µm), area of chromosome (µm^{x}µm) and Centromeric index percentage (length of short arm/length of chromosome) were taken for every chromosome based on Hussein (2005).

SUMMARY

In this investigation an attempt was made to study karyotype characterization of alfalfa. Another aim of this study was the determination of area chromosome, length chromosome, short and long arm chromosome, Centromeric index, and Centromeric position using twenty accessions. The studied accessions exhibited variation ranged from the highest score of area chromosome in accession 7 to its lowest estimate only in accession 3. The results exhibited differences ranging from the highest score of length chromosome in the accession 8 (5.81 µm) to the lowest in the accession 3 (4.12 µm). The Karyotype of accessions alfalfa consists of one set of chromosome with satellites (chromosome 8), four sets of submetacentric chromosomes (chromosome 1-4), and four sets of metacentric chromosomes (chromosomes 5-8). All of the landraces have the same number of chromosome 32 chromosome (2n= 4X= 32) with four nearly identical sets of chromosomes. Investigation the chromosomal maps of landraces that can be utilized in different breeding programs.

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REFERENCES

Agarwal, K. and P. K. Gupta (1983). Cytological studies in the genus *Medicago* L. Cytologia, 48: 781-793.

Bauchan, G. R. and M. A. Hossain (2001). Utilization of a computerized image analysis system to characterize small plant chromosomes. Microsc and Anal., 48: 57.

Bauchan, G. R. and M. A. Hossain (1997). Identification of alfalfa chromosomes using geimsa banding and image analysis techniques. Proceeding of the XVIII International Rangeland Congress, Canada, 4: 61-62.

Bauchan, G. R. and T. A. Campbell (1994). Use of an image analysis

system to Karyotype diploid alfalfa (*Medicago sativa* L.). J. Hered. 85: 18-22.

Bolton, J. L. (1962). Alfalfa. Inter-science publishers, Inc., New York.

Buss, G. R. and R. W. Cleveland (1968). Karyotype of a diploid *Medicago sativa* L. Analyzed from sporophyteand gametophyte mitosis.

Clement, W. M. and W. F. Leman (1962). Fertility and Cytological studies of diploid plant of alfalfa. *Medicago sativa* L. Crop. Sci., 2: 45-53.

Fayed, A. H., A. S. Mandour and M. A. Ismail (1984). Variation in chiasma frequency in various types of *Vicia faba* L. Indian J. Genet. Pl. Breed., 14: 640 -646.

Falistocco, E. (1987). Cytogenetic investigations and karyological relationships of two *Medicago M. sativa* L. (alfalfa) and *M. arboreal*. Caryologia, 40: 339-346.

Fukui, K. and K. Kakeda (1994). Dynamic changes in the morphology of barley chromosomes during the mitotic metaphase stage. The Japanese J. of Genetics, 69: 545-554.

Gillius, C. B. (1970). Alfalfa chromosomes. II. Pachytene Karyotype of tetraploid *Medicago sativa L*. and its relationship to *M. sativa*. Crop Sci., 10: 172-175.

Hussein, A. H. M. (2005). Identification of molecular markers for some morphological and biochemical characters in some medicine plants. MSc. Thesis Ain Shams Univ., Fac. Agric.

Ho, K. M. and K. J. Kasha (1974). Differential chromosome contraction at a pachytene stage of meiosis in alfalfa (*Medicago sativa* L.). Chromosome, 45: 163-172.

Nagarajan, P. and P. D. Walton (1987). A comparison of somatic chromosomal instability in tissue culture regenerates from *Medicago media*. Pres. Plant Cell Reports, 6: 109-113.

Schlsbaum, S. E, L. B. Johnson and D. L. Stuteville (1988). Characterization of somatic chromosome morphology in alfalfa, *Medicago sativa* L. Comparison of donor plants with regenerated protoclones. Cytologia, 54: 499-507.

Table (1): The names and pedigree of the twenty alfalfa accessions.

Code	Accession Number	Pedigree	Origin
1	K 1	Collection from El-Kharga	Egypt
2	K 2		
3	K 4		
4	K 14		
5	K 15		
6	D 20	Collection from El-Dakhla	Egypt
7	D 21		
8	D 22		
9	D 23		
10	D 25		
11	F 14	Collection from El-Farafra	Egypt
12	F 15		
13	F 16		
14	F 17		
15	F 18		
16	S 1	Collection from Siwa	Egypt
17	S 2		
18	S 4		
19	S 5		
20	S 6		

Table (2): Mean of chromosome area of the eight chromosomes for accessions alfalfa.

Code	Chromosome total area µm									
	1	2	3	4	5	6	7	8	Total area	Mean area
1	9.75	7.59	6.67	6.03	5.51	4.82	3.78	5.45	49.60	6.20
2	8.58	7.50	6.79	5.59	4.66	4.01	3.31	5.53	45.97	5.75
3	8.03	6.02	5.38	5.02	4.99	3.65	2.97	4.55	40.52	5.07
4	9.37	8.27	6.98	6.38	5.45	4.40	3.47	5.20	49.52	6.19
5	9.23	8.33	7.16	6.09	5.13	4.25	3.53	5.07	48.79	6.10
6	9.36	7.70	5.58	5.35	4.89	4.25	3.44	6.30	46.88	5.86
7	9.38	8.28	7.29	6.61	5.96	5.24	4.37	6.28	53.40	6.68
8	9.07	7.89	6.86	5.86	4.65	3.80	3.56	5.61	47.28	5.91
9	8.79	7.76	6.69	5.69	5.19	4.15	3.30	5.41	46.98	5.87
10	8.17	7.19	6.78	5.64	5.14	4.49	3.48	5.81	46.68	5.84
11	8.02	6.48	5.73	5.49	5.09	4.53	4.05	7.46	46.85	5.86
12	8.75	8.02	6.48	5.97	5.57	5.11	4.36	7.19	51.45	6.43
13	8.89	7.94	6.88	6.25	5.28	4.65	4.10	7.12	51.11	6.39
14	7.95	6.62	6.06	5.77	5.34	4.34	3.37	6.63	46.07	5.76
15	8.03	6.85	5.77	5.21	4.61	4.18	3.63	6.20	44.48	5.56
16	9.09	8.16	7.13	6.25	5.60	5.19	4.64	6.77	52.83	6.60
17	8.94	7.66	6.71	5.79	4.79	4.02	3.32	4.96	46.19	5.77
18	7.45	6.34	5.73	5.20	4.64	4.07	3.35	6.09	42.86	5.36
19	8.52	6.79	6.10	5.48	4.89	4.28	3.48	5.44	44.99	5.62
20	7.69	6.55	6.50	4.86	4.25	3.64	3.26	5.87	42.62	5.33

Table (3): Mean of chromosome length of the eight chromosomes for accessions alfalfa.

Code	Chromosome total length µm									
	1	2	3	4	5	6	7	8	Total length	Mean length
1	8.13	6.28	5.53	5.11	4.70	4.13	3.38	5.88	43.12	5.39
2	7.30	6.54	5.84	5.23	4.76	4.01	3.42	4.63	41.72	5.22
3	5.55	4.72	4.56	3.45	3.44	3.13	3.07	5.06	32.98	4.12
4	5.82	5.06	4.76	4.48	4.33	4.10	3.51	4.70	36.76	4.59
5	6.21	5.30	5.02	4.53	4.22	4.03	3.65	4.48	37.44	4.68
6	7.31	6.24	5.36	4.68	4.30	3.97	3.24	6.26	41.36	5.17
7	7.09	6.09	5.61	5.27	4.74	4.21	3.68	4.86	41.55	5.19
8	8.53	7.76	6.69	5.64	5.14	4.13	3.32	5.30	46.52	5.81
9	7.16	6.02	5.60	4.88	4.60	4.17	3.81	5.33	41.58	5.20
10	8.55	6.92	5.62	4.79	5.28	3.83	3.42	4.70	43.10	5.39
11	6.80	6.24	5.27	4.61	4.57	4.08	4.02	6.72	42.31	5.29
12	5.76	5.13	4.53	4.28	4.05	3.86	3.29	4.85	35.75	4.47
13	7.10	6.26	5.20	4.90	4.62	4.31	3.52	5.51	41.41	5.18
14	6.08	5.54	4.68	4.38	4.13	3.81	3.28	5.08	36.98	4.62
15	6.41	5.63	4.98	4.77	4.50	4.05	3.54	5.27	39.13	4.62
16	6.45	5.92	5.00	4.64	4.33	3.95	3.41	5.67	39.36	4.92
17	5.78	5.02	4.55	4.15	3.85	3.68	3.27	5.86	36.17	4.52
18	5.70	5.06	4.47	4.03	3.77	3.48	3.19	5.27	34.97	4.37
19	6.58	5.87	5.32	4.87	4.48	4.12	3.40	5.94	40.57	5.07
20	6.55	5.58	5.19	4.80	4.24	3.70	3.32	4.88	38.26	4.78

Table (4): Mean of chromosome long arm of the eight chromosomes for accessions alfalfa.

Code	Chromosome total long arm µm									
	1	2	3	4	5	6	7	8	Total long arm	Mean long arm
1	4.68	3.77	3.37	3.07	2.50	2.15	1.78	3.06	24.37	3.05
2	4.33	3.89	3.47	3.29	2.42	2.13	1.79	2.38	23.70	2.96
3	3.40	2.95	2.94	2.23	1.83	1.61	1.58	2.62	19.17	2.40
4	3.62	3.15	2.84	2.61	2.27	2.12	1.83	2.47	20.92	2.61
5	3.81	3.22	2.95	2.72	2.22	2.12	1.89	2.32	21.26	2.66
6	4.34	3.70	3.14	2.74	2.27	2.07	1.71	3.28	23.26	2.91
7	4.16	3.71	3.32	3.10	2.47	2.24	1.93	2.48	23.41	2.93
8	4.99	4.71	3.85	3.46	2.66	2.15	1.79	2.72	26.35	3.29
9	4.13	3.58	3.33	2.90	2.43	2.18	2.01	2.73	23.28	2.91
10	5.06	4.16	3.40	2.83	2.72	2.26	1.78	2.39	24.60	3.07
11	3.86	3.66	3.07	2.68	2.28	2.13	2.12	3.50	23.29	2.91
12	3.32	2.94	2.65	2.49	2.43	2.35	1.69	2.52	20.39	2.55
13	4.10	3.78	3.02	2.89	2.49	2.29	1.79	2.87	23.23	2.90
14	3.66	3.24	2.77	2.57	2.34	2.04	1.69	2.70	21.00	2.63
15	3.70	3.23	2.96	2.75	2.49	2.14	1.79	2.78	22.85	2.86
16	4.13	3.81	3.25	2.82	2.30	2.08	1.74	2.88	23.02	2.88
17	3.71	3.32	2.97	2.70	2.46	1.90	1.72	2.97	21.74	2.72
18	3.70	3.23	2.73	2.64	1.98	1.91	1.65	2.64	20.48	2.56
19	4.03	3.69	3.25	2.93	2.37	2.16	1.76	3.00	23.19	2.90
20	3.98	3.44	3.27	2.98	2.22	1.94	1.73	2.51	22.08	2.76

Table (5): Mean of chromosome short arm of the eight chromosomes for accessions alfalfa.

Code	Chromosome total short arm µm									
	1	2	3	4	5	6	7	8	Total short arm	Mean short arm
1	3.45	2.51	2.16	2.04	2.19	1.98	1.60	2.82	18.75	2.34
2	2.97	2.65	2.37	1.93	2.33	1.88	1.63	2.25	18.02	2.25
3	2.15	1.77	1.63	1.22	1.60	1.52	1.48	2.44	13.81	1.73
4	2.20	1.91	1.92	1.87	2.06	1.98	1.68	2.22	15.84	1.98
5	2.40	2.08	2.06	1.81	2.00	1.90	1.77	2.15	16.18	2.02
6	2.97	2.54	2.22	1.93	2.03	1.90	1.54	2.98	18.11	2.26
7	2.93	2.37	2.29	2.17	2.27	1.97	1.75	2.39	18.14	2.27
8	3.54	3.04	2.83	2.18	2.48	1.98	1.53	2.58	20.17	2.52
9	3.03	2.44	2.27	1.98	2.17	1.99	1.81	2.60	18.30	2.29
10	3.49	2.77	2.22	1.96	2.55	1.56	1.64	2.31	18.50	2.31
11	2.94	2.59	2.21	1.93	2.29	1.95	1.90	3.22	19.03	2.38
12	2.44	2.19	1.87	1.79	1.62	1.51	1.60	2.33	15.36	1.92
13	2.99	2.48	2.19	2.01	2.13	2.01	1.73	2.64	18.18	2.27
14	2.43	2.30	1.91	1.81	1.79	1.77	1.59	2.38	15.98	1.99
15	2.70	2.40	2.02	2.01	2.00	1.90	1.75	2.49	16.42	2.05
16	2.31	2.11	1.74	1.82	2.02	1.87	1.67	2.79	16.34	2.04
17	2.08	1.70	1.58	1.46	1.39	1.79	1.55	2.89	14.43	1.80
18	2.00	1.84	1.73	1.39	1.79	1.57	1.54	2.63	14.49	1.81
19	2.54	2.18	2.08	1.93	2.11	1.96	1.64	2.94	17.38	2.17
20	2.57	2.13	1.92	1.82	2.01	1.75	1.56	2.38	16.74	2.09

Table (6): Mean of chromosome arm ratio, Centromeric index and Centromeric position of eight chromosomes for accessions alfalfa.

Code	1			2			3		
No. of Chro-mosomes	Arm ratio	Centromeric index	Centromeric position	Arm ratio	Centromeric index	Centromeric position	Arm ratio	Centromeric index	Centromeric position
1	1.36	42.44	S.M	1.46	40.68	S.M	1.58	38.70	S.M
2	1.50	39.94	S.M	1.47	40.52	S.M	1.67	37.50	S.M
3	1.56	39.04	S.M	1.46	40.64	S.M	1.80	35.66	S.M
4	1.50	39.96	S.M	1.70	37.00	S.M	1.82	35.41	S.M
5	1.14	46.67	M	1.04	49.04	M	1.15	46.58	M
6	1.08	48.02	M	1.13	46.97	M	1.06	48.55	M
7	1.12	47.27	M	1.10	47.66	M	1.07	48.33	M
8	1.00	50.00	M	1.06	48.55	M	1.08	48.18	M
Code	4			5			6		
No. of Chro-mosomes	Arm ratio	Centromeric index	Centromeric position	Arm ratio	Centromeric index	Centromeric position	Arm ratio	Centromeric index	Centromeric position
1	1.65	37.80	S.M	1.59	38.64	S.M	1.46	40.68	S.M
2	1.65	37.72	S.M	1.55	39.26	S.M	1.46	40.69	S.M
3	1.48	40.31	S.M	1.43	41.09	S.M	1.42	41.39	S.M
4	1.40	41.74	S.M	1.50	40.02	S.M	1.42	41.32	S.M
5	1.11	47.51	M	1.11	47.34	M	1.12	47.17	M
6	1.07	48.22	M	1.12	47.28	M	1.09	47.86	M
7	1.09	47.95	M	1.07	48.36	M	1.11	47.41	M
8	1.11	47.35	M	1.08	48.11	M	1.10	47.55	M
Code	7			8			9		
No. of Chro-mosomes	Arm ratio	Centromeric index	Centromeric position	Arm ratio	Centromeric index	Centromeric position	Arm ratio	Centromeric index	Centromeric position
1	1.42	41.31	S.M	1.41	41.50	S.M	1.36	42.36	S.M
2	1.56	39.02	S.M	1.55	39.23	S.M	1.47	40.51	S.M
3	1.45	40.83	S.M	1.36	42.39	S.M	1.47	40.52	S.M
4	1.43	41.14	S.M	1.59	38.60	S.M	1.46	40.63	S.M
5	1.09	47.89	M	1.07	48.22	M	1.12	47.18	M
6	1.14	46.73	M	1.09	47.92	M	1.09	47.76	M
7	1.10	47.58	M	1.17	46.10	M	1.11	47.39	M
8	1.04	49.10	M	1.05	48.71	M	1.05	48.85	M

Table (6): Cont'

Code	10			11			12		
No. of Chro-mosomes	Arm ratio	Centromeric index	Centromeric position	Arm ratio	Centromeric index	Centromeric position	Arm ratio	Centromeric index	Centromeric position
1	1.45	40.84	S.M	1.31	43.26	S.M	1.36	42.39	S.M
2	1.50	39.94	S.M	1.41	41.44	S.M	1.34	42.67	S.M
3	1.53	39.53	S.M	1.39	41.88	S.M	1.42	41.39	S.M
4	1.44	40.90	S.M	1.39	41.82	S.M	1.39	41.89	S.M
5	1.06	48.43	M	0.99	50.14	M	1.11	47.45	M
6	1.04	49.10	M	1.09	47.85	M	1.13	46.89	M
7	1.09	47.95	M	1.12	47.24	M	1.06	48.59	M
8	1.04	49.10	M	1.09	47.92	M	1.08	48.06	M
Code	13			14			15		
No. of Chro-mosomes	Arm ratio	Centromeric index	Centromeric position	Arm ratio	Centromeric index	Centromeric position	Arm ratio	Centromeric index	Centromeric position
1	1.37	42.19	S.M	1.51	39.91	S.M	1.37	42.18	S.M
2	1.53	39.58	S.M	1.41	41.55	S.M	1.38	42.28	S.M
3	1.38	42.02	S.M	1.45	40.78	S.M	1.47	40.50	S.M
4	1.44	41.06	S.M	1.42	41.30	S.M	1.37	42.27	S.M
5	1.17	46.14	M	1.07	48.24	M	1.10	47.69	M
6	1.14	46.76	M	1.15	46.42	M	1.12	47.08	M
7	1.03	49.16	M	1.06	48.59	M	1.03	49.38	M
8	1.09	47.91	M	1.13	46.88	M	1.12	47.23	M
Code	16			17			18		
No. of Chro-mosomes	Arm ratio	Centromeric index	Centromeric position	Arm ratio	Centromeric index	Centromeric position	Arm ratio	Centromeric index	Centromeric position
1	1.78	35.91	S.M	1.78	35.93	S.M	1.84	35.17	S.M
2	1.81	35.59	S.M	1.96	33.77	S.M	1.76	36.29	S.M
3	1.87	34.85	S.M	1.88	34.78	S.M	1.58	38.81	S.M
4	1.55	39.28	S.M	1.85	35.07	S.M	1.89	34.58	S.M
5	1.14	46.80	M	1.16	46.18	M	1.11	47.42	M
6	1.11	47.33	M	1.06	48.52	M	1.22	45.05	M
7	1.04	48.92	M	1.11	47.31	M	1.07	48.24	M
8	1.03	49.21	M	1.03	49.31	M	1.00	50.00	M

Table (6): Cont'

Code	19			20		
No. of Chro-mosomes	Arm ratio	Centromeric index	Centromeric position	Arm ratio	Centromeric index	Centromeric position
1	1.59	38.66	S.M	1.55	39.23	S.M
2	1.69	37.15	S.M	1.61	38.25	S.M
3	1.56	39.02	S.M	1.70	37.07	S.M
4	1.52	39.73	S.M	1.64	37.89	S.M
5	1.12	47.10	M	1.10	47.53	M
6	1.10	47.58	M	1.11	47.45	M
7	1.07	48.35	M	1.09	47.89	M
8	1.02	49.44	M	1.06	48.54	M

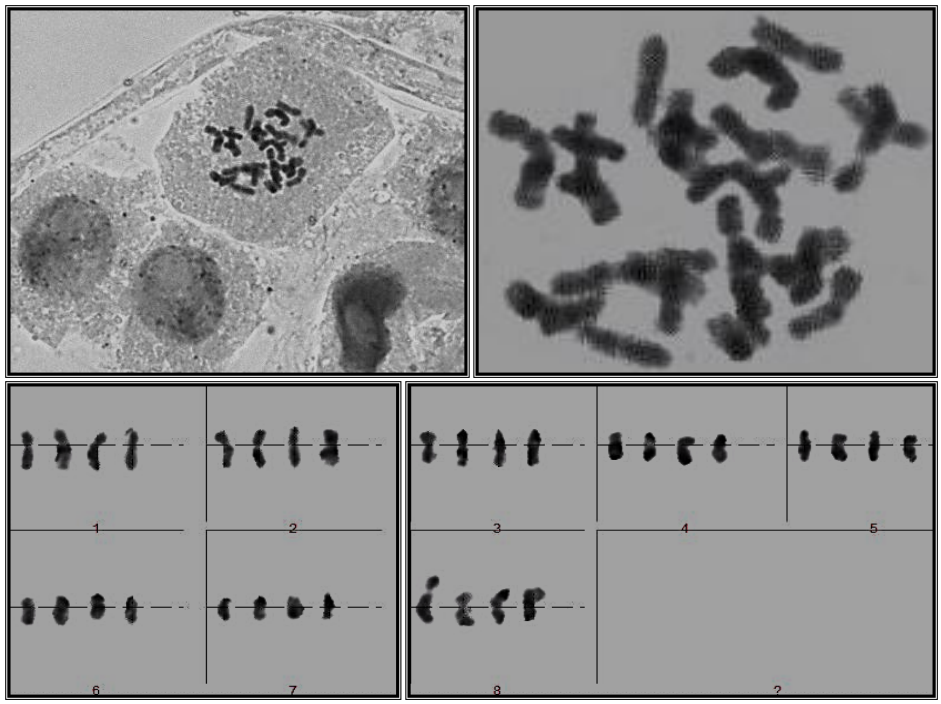

Fig. (1a): Mitotic metaphase and karyotype of chromosome of alfalfa at El-Kharga.

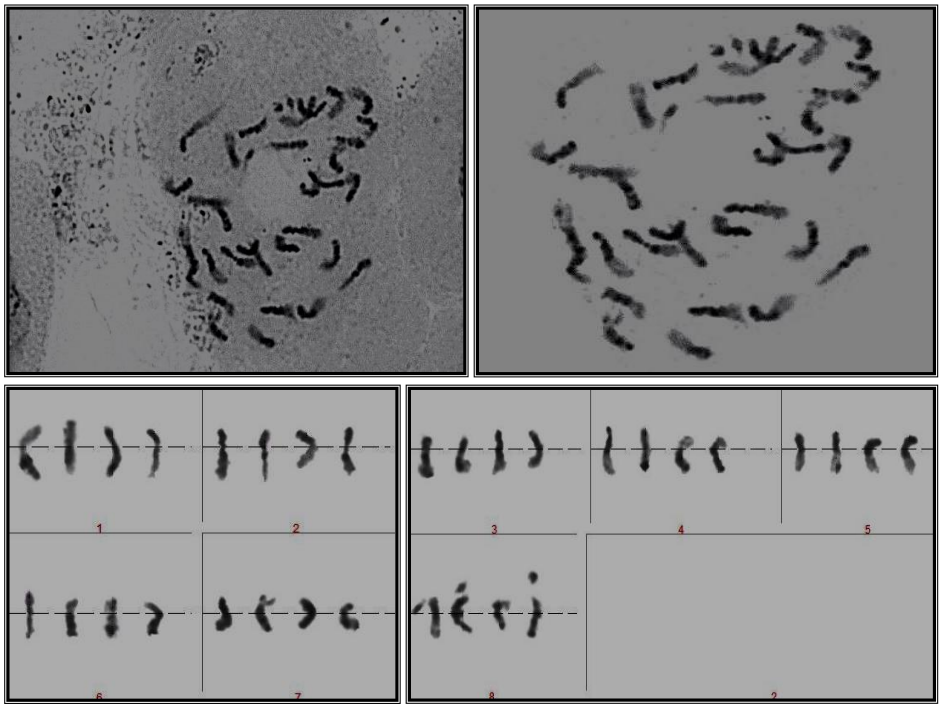

Fig. (1b): Mitotic metaphase and karyotype of chromosome of alfalfa at at El-Dakhla.

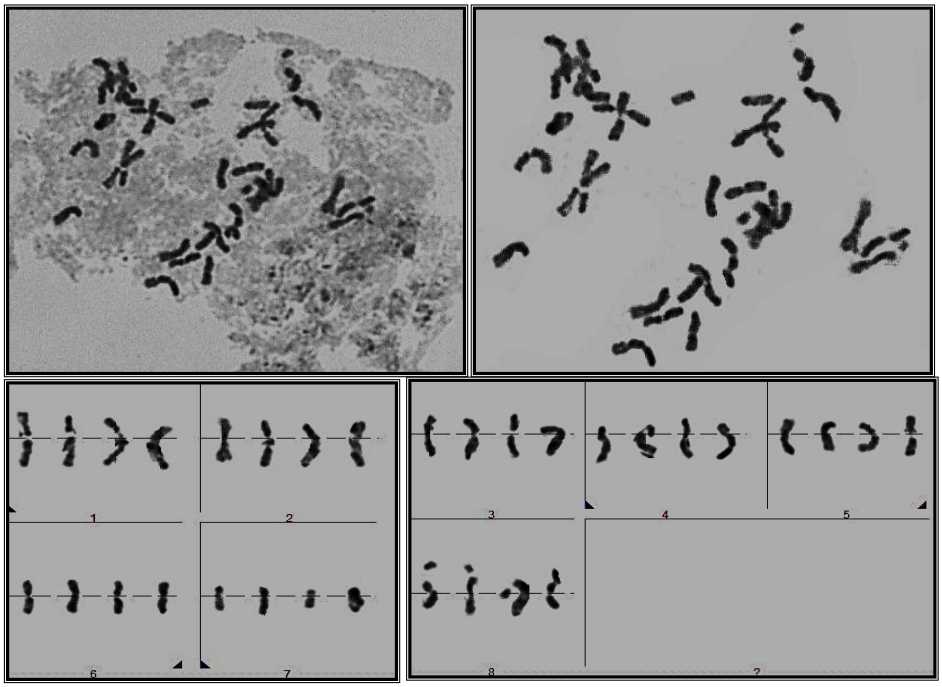

Fig. (1c): Mitotic metaphase and karyotype of chromosome of alfalfa at at El-Farafra.

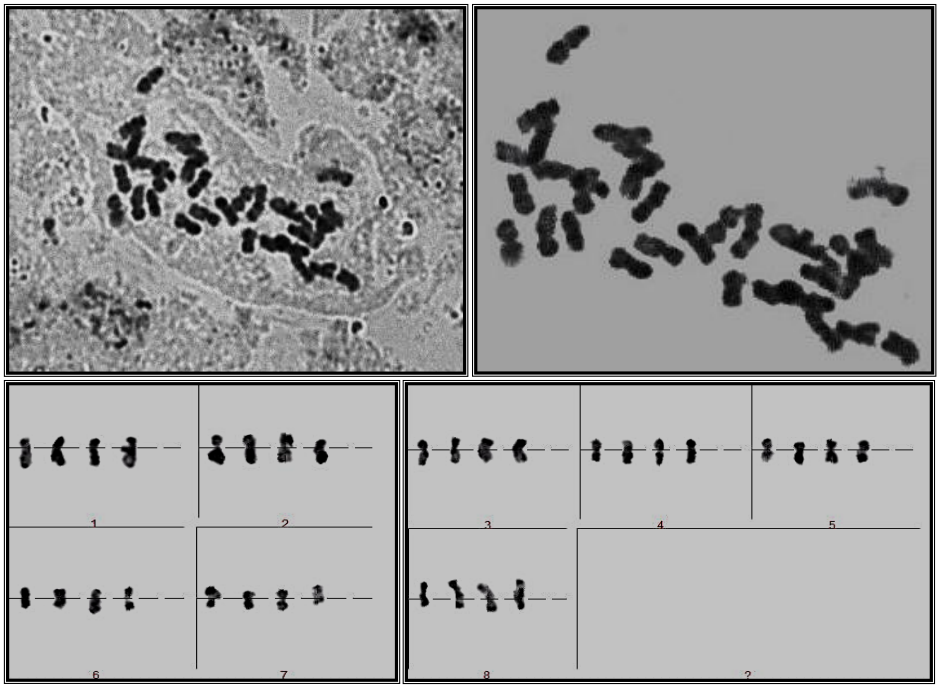

Fig. (1d): Mitotic metaphase and karyotype of chromosome of alfalfa at at Siwa.