Range Mgmt. & Agroforestry 31 (1) : 7-10, 2010

ISSN 0971-2070

Cytological comparison of two cultivars of Egyptian clover (*Trifolium alexandrinum* L)

Magda I. Soliman[1], E. M. Zayed [*2] and G. A. Ramadan [2]

Department of Botany, Faculty of Science, Mansoura University, Mansoura, Egypt.

*Corresponding author e-mail : ehabzayed31@hotmail.com

Received : 20th August, 2009 Accepted : 25th April, 2010

Abstract

Cytological studies including chromosome number and karyotype analysis have been carried out on two cultivars "Helaly" and "Fahl" in Egyptian clover *(Trifolium alexandrinum L.).* The somatic chromosome counts for two cultivars were 2n = 16. Karyotype analysis showed differences in chromosome morphology. Chromosomes nsm (+) were observed in cultivar Helaly. The karyotype formula for Helaly was 2 nsm (+) + 2 nsm (-) + 12 nm. For Fahl, 6 nsm (-) + 10 nm were recorded. Helaly had highest A1 and A2 where Fahl had highest TF %, S%, Syi index and Rec index. Karyotype analysis revealed that cultivar Helaly is advanced one whereas cultivar Fahl is primitive. Chromosomal abnormalities were observed at mitotic division, which was higher in cultivar Fahl.

Keywords : Chromosomal abnormalities, Chromosome number, Fahl, Fodder, Helaly, Karyotype analysis, *Trifolium alexandrinum*

Introduction

Egyptian clover (*Trifolium alexandrinum*) is one of the most important legumes of the Middle East and the Mediterranean because of its multicut nature. However, only single cut is possible from its ecotype Fahl. Cytological characters, including chromosome number and karyotype analysis have been considered important tool for taxonomic and evolutionary relationships (Moore, 1968). The number, size and shape of chromosomes were used to characterize the karyotype and define the taxonomic differences. The purpose of this study was to investigate chromosome number of two cultivars Helaly and Fahl, construct karyotypes and to reveal the types of mitotic irregularities if present and their frequencies.

Materials and Methods

Viable seeds of the two cultivars, Helaly and Fahl were obtained from Forage Crops Research Department (FCRD), Field Crops Research Department (FCRI), Agricultural Research Center (ARC), Giza, Egypt. Seeds were germinated and actively growing root tips were pretreated for 2-4 h in 0.002 M 8- hydroxyquinoline, fixed in 3:1 (absolute ethanol : acetic acid), hydrolyzed for 5 min in 1 N HCl at 60°C and stained in aceto-orcein according to Lacour (1941); Chattopadhyay and Sharma (1988). Well spread five metaphase plates were selected and photographed. Karyograms were drawn and lengths of long arm (L) and short arm (S) were measured for karyotype analysis. Karyotype analysis was carried out using "Micro Measure" Computer Program (Reeves, 2001). Mean chromosome length (MCL) in µ, the total chromosome volume (TCV) and the total chromosome length (TCL) were determined. To estimate karyotype asymmetry, two numerical parameters, A1 (intra-chromosomal asymmetry index) and A2 (inter-chromosomal asymmetry index) were used according to Zarco (1986). Symmetry percent (S%), Rec index (resemblance between chromosomes), Syi index (the symmetric indices) and the total form percent (TF %) *i.e.,* the average degree of symmetry over the whole karyotype were calculated according to Huziwara (1962).

Results and Discussion

The two cultivars of *Trifolium alexandrinum L.* in the study were found to be diploid (2n= 16) (Plate 1) which is in confirmation with previous reports of the same genus (Bir and Kumari, 1979). There were substantial differences in karyotype of the two cultivars. The dissimilarity was found in the morphology of chromosomes and also in the mean chromosome length (MCL), total chromosome length (TCL) and total chromosome volume (TCV). The values of MCL, TCL and TCV were higher in cultivar Helaly than in Fahl (Table 3). The first chromosome in Helaly was longer than that of its counterpart in Fahl (Table 1) whereas eighth pair was shorter in Fahl than that in Helaly (Table 2). These differences might be due to chromosome deletions or due to different levels of condensation and differential contraction of chromosomes as suggested by El-Nahas

2. Forage Crops Research Department, Field Crops research Institute, Agriculture Research Center, Giza- 12619, Egypt.

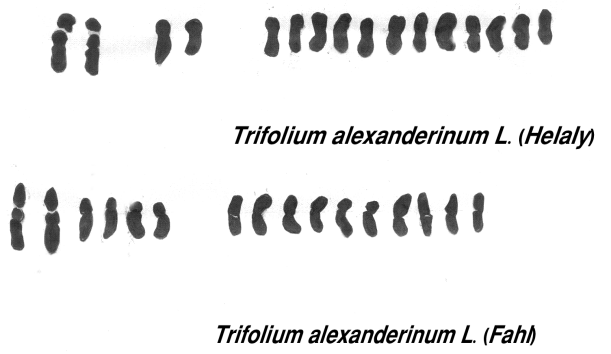

Plate 1: Karyogram of the two varieties of *T. alexandrinum L.* (A) Helaly and (B) Fahl.

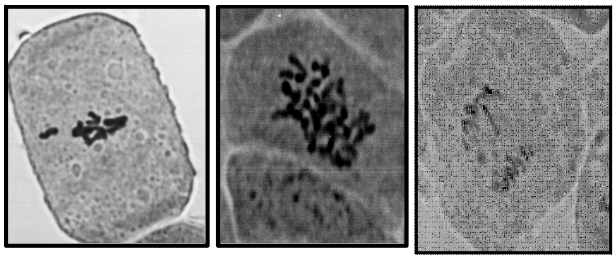

Plate 2 : Chromosomal abnormalities of the two varieties of *T. alexandrinum L.* (A): Non congression at metaphase, (B): Polyploidy and (C): Late separation at anaphase (X=1000).

(2000). The study confirmed the remarkable degree of chromosome variability between two cultivars. Near sub-metacentric and near metacentric chromosomes were common in both karyotypes (Table 3, Plate 1). Idiogram of the haploid complements is shown in Fig. 1 and 2 for Helaly and Fahl respectively. The evolution of karyotype is estimated by indices of symmetry. These values range theoretically from 0 to 100 for Rec and Syi indices (Greilhuber and Septa, 1976) from 0 to 50 for TF% as well as symmetry (S%). A karyotype with high values of these indices is considered as low evolved. According to these parameters, the higher values of S%, TF%, Rec index and Syi index were recorded in cultivar Fahl (Table 3). Thus, this cultivar may be considered as less evolved one.

According to Zarco (1986) the intra-chromosomal and inter-chromosomal asymmetry indices (A_1 and A_2 respectively) define differences between cultivars and the cultivar with high A_1 and A_2 values are considered more advanced than others. In general, high A_1 and A_2 values are scored in cultivar with higher degrees of variation in chromosome length (Kamel, 1999). Cultivar Helaly had the higher values of A_1 and A_2. Thus, this cultivar may be considered as the advanced one than Helaly.

The preliminary study of chromosomal aberration indicates that the type of spontaneous aberration found in the cells resembles those induced by agricultural chemicals, environmental pollutants and aging. The presence of chromosomal abnormalities may result from the effectiveness of many environmental pollutants. In recent years, countless number of chemicals is applied for plant protection as herbicides, insecticides and fungicides. Several reports have shown that many of these chemicals affect cell division and induce aberrations similar to those produced by radiations and chemicals used in mutagenesis (El-Sadek, 1972; Hamoud and Bodr. 1985; Soliman and Ghoneam, 2004). In the present study a variety of chromosomal abnormalities were observed in the studied cultivars such as polyploid cells, late separation and non congression (Table 4; Plate 2). Most species of *Trifolium* are diploid (2n=16) and only 16% of the 248 species are polyploid (Majumdar *et al*., 2004). 70% of the known polyploids occur in the subgenus *Amoria*, which is considered to be most primitive and unspecialized subgenera. Polyploid cells were recorded in cultivar Fahl cultivar only (Table 4; Plate 2). Gupta and Gupta (1978) pointed out that the reduction in the chromosome length is often associated with increasing evolution and that the occurrence of polyploidy is an

Table 1: The average measurements and arm ratios of somatic chromosomes of *T. alexandrinum L.* cv Helaly.

Chr. pairs no.	Chromosome length in um			Arm ratio (L/S)	Cent. index	Chromosome type
	Long arm (L)	Short arm (S)	Total chr. length			
1	24.860	8.030	34.860	3.100	0.230	nsm(+)
2	15.076	8.309	23.384	1.815	0.356	nsm(-)
3	11.623	11.085	22.707	1.049	0.488	nm
4	11.867	10.181	22.047	1.166	0.462	nm
5	11.001	10.272	21.273	1.072	0.483	nm
6	11.122	9.744	20.866	1.142	0.467	nm
7	11.516	8.873	20.385	1.298	0.435	nm
8	10.639	8.922	19.561	1.193	0.456	nm

nsm = nearly submetacentric; nm = nearly metacentric

Table 2 : The average measurements and arm ratios of somatic chromosomes of *T. alexandrinum L.* cv Fahl.

Chr. pairs no.	Chromosome length in um			Arm ratio (L/S)	Cent. index	Chromosome type
	Long arm (L)	Short arm (S)	Total chr. length			
7	11.516	8.873	20.385	1.298	0.435	nm
1	20.895	11.105	32.004	1.883	0.347	nsm(-)
2	11.800	6.920	18.671	1.716	0.368	nsm(-)
3	11.275	6.465	17.737	1.746	0.364	nsm(-)
4	10.910	10.220	21.129	1.068	0.484	nm
5	10.325	9.685	20.013	1.066	0.484	nm
6	9.170	8.315	17.490	1.103	0.476	nm
7	9.346	7.405	16.748	1.263	0.442	nm
8	8.342	7.535	15.879	1.108	0.475	nm

nsm = nearly submetacentric; nm = nearly metacentric

Table 3 : Karyotype parameters of somatic chromosomes of two varieties of *Trifolium alexandrinum L.*

Varities	TCL	MCL	TCV	S%	TF%	A1	A2	Syi index ± SE	Rec index ± SE	Karyotype formula
Helaly	370.170	23.136	31267.902	0.303	40.744	0.575	0.250	0.422 ±0.79	0.623 ±1.19	2nsm(+), 2nsm (-)+12 nm
Fahl	319.337	19.959	8921.359	0.331	42.340	0.568	0.205	0.430 ±1.29	0.682 ±1.35	6 nsm(-) +10 nm

evidence for its advanced state. Moscone (1992) also pointed out that polyploidy is an important event during the evolution.

Table 4 : Mitotic abnormalities percentage in two cultivars of *T. alexandrinum L.*

Varieties	% of normal cells	% of abnormal cells			Total abnormalities
		Non-congression	Poly-ploidy	Late separation anaphase	
Helaly	96.4	3.50	0.00	0.309	1.855
Fahl	95.8	0.00	1.61	1.196	2.127

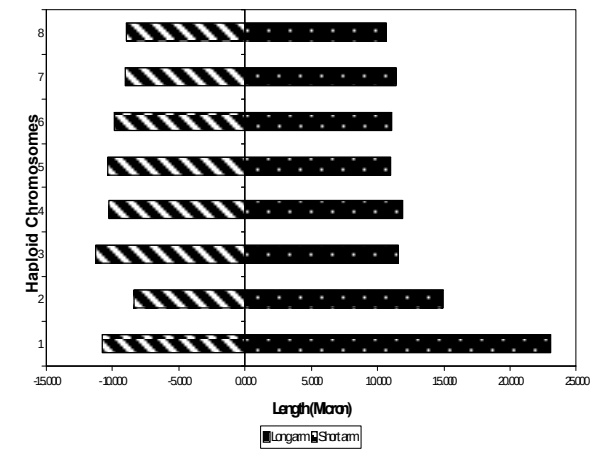

Fig 1: Idiogrammatic representation of the karyotype of *Trifolium alexandrinum* cv Helaly

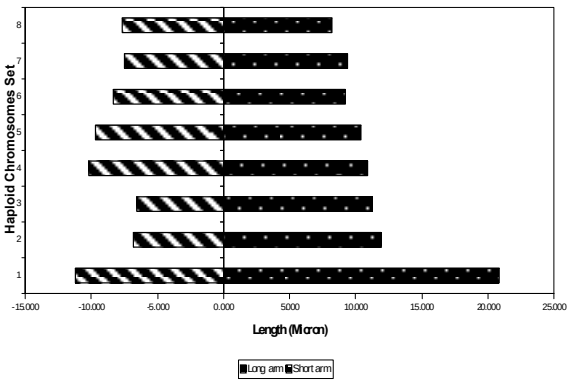

Fig 2 : Idiogrammatic representation of the karyotype of *Trifolium alexandrinum* cv Fahl

Lagging chromosomes were observed in cultivar Helaly only (Table 4, Plate 2) which might have resulted from the loose spindle apparatus or centromeres. It may also result from hindrance of pro-metaphase movement of chromosomes which cause unequal distribution of chromosomes into the poles or aneuploidy. Lagging chromosomes or chromatids may be due to irregular orientation of chromosomes. Delay of separation (late separation) could be due to stickiness. Delay of separation has been recorded in anaphase in two cultivars but the higher value was recorded in cultivar Fahl. In conclusion, the Egyptian clover cultivar Fahl (monocut) differed from cultivar Helaly (multicut) with respect to chromosome morphology and karyotype.

References

Bir, S. S. and S. Kumari. 1979. Cytological evolution of the leguminous flora of the Punjab plain. *Recent Res. Pl. Sci* (New Delhi) 7:252-260.

Chattopadhyay, D. and A. K. Sharma. 1988.A new technique for orcein banding with acid treatment. *Stain Technol.* 63: 283-287.

El-Nahas, A. I. 2000. Chromosomal criteria in relation to seed protein of some Egyptian cultivars of *Vicia faba. Egypt. J. Biotechnol* 7: 276-292.

El-Sadek, L. M. 1972. Mitotic inhibition and chromosomal aberrations induced by some arylasonic acids and its compounds in root tips of Maize. *Egypt. J. Genet. Cytol.* 1:218-224.

Greilhuber, J. and F. Septa. 1976. C-banded karyotypes in the Scilla hohenackeri group *S. persica and Puschkinia* (Liliaceae) *Plant Syst. Evol.* 126:149-188.

Gupta, R. and P. K. Gupta. 1978. Karyotypic studies in the genus *Crotalaria* linn. *Cytologia* 43:357-369.

Hamoud, M. A. and A. Badr. 1985. Cytotoxic effects of the insecticide Birlane in root meristem of *Vicia faba* and *Zea mays. Proc.6th Arab Pesticide Conf.,* Tanta Univ. 1: 435-444.

Huziwara, Y. 1962. Karyotypic analysis in some genera of compositae, VIII. Further studies on the chromosomes of Aster. *Amer. J. Bot.* 49: 116-119.

Kamel, E. A. 1999. Karyological studies on some taxa of the Asteraceae in Egypt. *Comp. News* I 33: 1-18.

LaCour, L. 1941 Acetic orcein. *Stain Technol.* 16:169-174.

Majumdar, S., S. Banerjee and K. Kumar. 2004. Meiotic behaviour of chromosomes in PMCs and karyotype of *Trifolium repens* L. from Darjeeling Himalaya. *Acta Biologica Cracoviensia Series Botanica* 46: 217-220.

Moore, D. M. 1968. The karyotype in taxonomy. In: *Modern methods of plant taxonomy* Heywood, V. H. (Ed.) Academic Press, London and New York. P.61-75.

Moscone, E. A. 1992. Meiotic chromosome studies in Solanaceae from Argentina. *Darwiniana* 31:261-297.

Reeves, A. 2001. MicoMeasure: A new computer program for the collection and analysis of cytogenetic data. *Genome* 4:439-443.

Soliman, M. I. and G. T. Ghoneam. 2004. The Mutagenecity potentialities of some herbicides using *Vicia faba* as a biological system. *Biotechnology* 3:140-154.

Zarco, C. R. 1986. A new method for estimating karyotype asymmetry. *Taxon* 35: 526-530.