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Karyotype Analysis and Chromosome Evolution in Three Species of Genus *Plantago* L. in Egypt

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ABSTRACT: The karyotypic characters, mitotic metaphase chromosomes and karyograms of ten populations of three species of *Plantago* L. (*P. lagopus* L., *P. major* L. and *P. squarrosa* Murr.) in Egypt were determined and compared with each other. Analysis of somatic metaphase showed that the chromosome numbers were 2n = 12. Although all the species had the same chromosome number, they could be differentiated by their karyotype formula and quantitative parameters of the karyotypes. Ten different methods of karyotype asymmetry (TF%, As K%, Rec and Syi, A_1 and A_2, DI, A, AI and M_{CA}) were determined for the elucidation of phylogenetic relationships and taxonomic treatments within a particular group or taxon. The highest value of Syi index was found in population of *P. lagopus* (*P. lag*1) collected from Abu AL-Matamir District (66.682). Population of *P. squarrosa* (*P.sq*1) collected from Qalabshu-bilqas District had the highest values of A_1 (0.543), where the highest value of A_2 (0.29) was recorded in population of *P. lagopus* (*P. lag* 2) collected from Ganaklis District. The highest positive correlation was observed between TF % and Syi index (0.997). *P. lagopus* population collected from Abu AL-Matamir District (*P. lag*1) was the most symmetrical karyotype may be considered as less evolved, while *P. squarrosa* population collected from Qalabshu- Bilqas District (*P. sq*1) was the most asymmetrical karyotype and may be considered as more advanced than the rest of populations. Populations of *P. lagopus* delimitated in a separate group from the rest of studied populations. The center of rest populations was occupied by *P. major* populations using PERMAP-Biplot and Scatter diagrams.

Keywords: *Plantago*; Populations; Asymmetry index; Karyotype and Egypt.

INTRODUCTION: The genus *Plantago* L. of family Plantaginaceae comprises about 483 species distributed throughout the world and the genus is the prevalent of the three genera on which family Plantaginaceae is based[1 & 2]. According to Pilger[3] the genus is divided into two subgenera: *Euplantago* Harms and *Psyllium* Harms. The genus subdivided into three subgenera including *Plantago*, *Coronopus* Rahn and *Psyllium* Rahn[4]. Recently, Rahn[5] proposed a new original taxonomic scheme of the genus according to which genus *Plantago* includes six subgenera: *Plantago*, *Coronopus* Rahn, *Albicans* Rahn, *Psyllium* Juss, *Littorella* Rahn and *Bougueria* Rahn. The *Plantago* genera are small, annual and perennial herbs of warm temperate, sandy provinces and are widely allocated this genus is with several critical groups, floral characters are sometimes subject to modification and the variations between populations of the same species are often considerable[2].

The seeds of several *Plantago* species have often been used medicinally due to their high mucilage content, they have proved effective against different diarrhea causing disease**.** The *Plantago lagopus* and *P.major* are very important plant with strong cytotoxic and radical scavenging activities that protect cells against free radical injuries and have been used for the treatment of ailments and diseases such as the common cold, wounds, viral infection, depression and in addition to inhibition of cancer cells proliferation due to presence of pro-oxidant effect of phenolics in higher concentrations[6 & 7].

Previous cytological knowledge of *Plantago* L. has revealed that this genus has three different basic chromosome numbers of x=4, 5 and 6. The basic number of x=6 is the original chromosome number from which x=5 & x=4 have been derived. This number of x=6 is present in the majority of species [8-10]. The first consistent chromosome counts in *Plantago* were reported by MucCullagh[11]. Other studies were made in connection with taxonomic studies to explain the relationships in particular groups of related species1[2-15]. Studies also covered the cytology of

Plantago species from different phytogeographical regions[16 & 17]. Badr and El-Kholy[18] studied *Plantago* chromosomes in the Egyptian flora and the basic chromosome number *x*=6 is the most common among the species *Plantago* which may be considered the ancestral number in the genus[19].

Cytological techniques determine the chromosome constitution of an organism to facilitate recognition of the individual chromosomes. In plant taxonomy, breeding and genetic studies; information about chromosome karyotype can be useful in species identification and analysis of hybrid populations[20-23].

Genetic variations defined as the variation of individual genotypes within and among species. It is important trait for long term survival of species and enables a population to adapt to new conditions caused by environmental change[24]. Cytotaxonomy is a branch of cytogenetics, devoted to the comparative study of karyological features for systematic and evolutionary purposes[25]. Today, a number of data can be obtained by chromosome studies: chromosome number, karyotype structure, karyotype formula and asymmetry, chromosome banding, FISH, GISH and chromosome painting[26-28].

Karyotype evolution is one of the most important aspects of the whole evolutionary processes[29] and considered as an isolating mechanism in speciation and has its own evolutionary trends independent of genetic evolution[30]. Stebbins[31] pointed out that the pattern of karyotype evolution is generally from symmetry to asymmetry in higher plants. Phenotypic traits are controlled by genes and affected by environment, but large numbers of accessions can adapt to environments[32]. Populations often go extinct following discrete changes in the environment. However, populations may avoid extinction by rapidly adapting to their altered environment[33].

The aims of this investigation are to: (1) report karyotype data on the ten populations of three species of genus *Plantago* (*P. lagopus, P. major, and P. squarrosa*), (2) contribute to the statistical correlations between the studied populations to elucidate the possible evolutionary trend, (3) examine the patterns of chromosome and genetic variability of *Plantago* L. populations growing naturally in Egypt which correlate with environmental adaptation and its impact on conservation strategies of gene banks.

MATERIALS AND METHODS:

Populations sampling: In this study plant materials were collected from ten populations of three *Plantago* L. species growing naturally in Egypt. The seeds of *Plantago lagopus* L. were collected from two localities namely, Abu AL-Matamir and Ganaklis Districts (El-Behiera Governorate), where *Plantago major* L. were collected from four localities namely, Mansoura and Aga Districts (El- Dakahlia Governorate), EL-Mahalla Al–Kubra District (El-Gharbia Governorate) and Motubas District (Kafr El-Sheikh Governorate), and *Plantago squarrosa* Murr. were collected also from four localities namely, Qalabshu-Bilqas District (El-Dakahlia Governorate), Baltim District and Al–Aqulah-El-Borullus District (Kafr El-Sheikh Governorate), and Idku District (El-Behiera Governorate) as shown in Table 1.

Cytological studies: All seeds were germinated on moist filter paper in Petri dishes; 4–5 mm long root tips were excised and pre-treated with aqueous solution of 0.002 M 8-hydroxyquinoline for 4 h at 4–5°C[34]. The roots were fixed for 24 h in a mixture of ethanol: glacial acetic acid (3:1 v/v), and stored in the refrigerator. Roots were rinsed in distilled water, hydrolyzed with 1N HCl at 60°C for 8–9 minutes. Different staining techniques were used: 2% aceto-orcein after acid treatment[35] and modified carbol fuchsin [36 & 37]. Deeply stained root tips were squashed on clean slides with a drop of 45% acetic acid then specimens were squashed in a drop of 2% aceto-orcein. Cells with spread metaphase chromosomes were examined and photographed by olympus photomicroscope, using 100-phase oil immersion lens and Kodak film. At least 10 metaphases were drawn for each population (including 3–8 individuals), selecting the five best for measurements. The nomenclature used for the description of the chromosome morphology is that proposed by Abraham [38].

Karyotype analysis: Karyotype analysis was carried out using "Micro Measure 3.3" computer software[47] Karyotype asymmetry was estimated by many different methods, the karyotype asymmetry index (As K %), the total form percent (TF %), the Rec and Syi indices, the intrachromosomal asymmetry index (A_1) and interchromosomal asymmetry index (A_2), the dispersion index (DI), the degree of asymmetry of karyotype (A index) and the asymmetry index (AI). Equations and calculations of these parameters and their references were shown in Table 2.

RESULTS AND DISCUSSION: All ten populations are diploid and have 2n=2x=12 in somatic cells and represented in Figures 1 & 2. Karyotypic characters, mitotic metaphase chromosomes, karyograms and haploid ideograms are shown in Figures 1, 2 & 3. Analysis of somatic metaphases showed that the karyotype formula is nsm(-) & nm in all ten populations, while nsm(+) formula recorded in populations P. m3, *P.sq* 1, *P. sq*3 and *P.sq*4, nst(-) observed only in *P. sq*

1. The highest total chromosome length (TCL) found in *P. sq* 4 (32.676 µm), on the other hand the lowest TCL in *P. lag* 2 (9.985 µm), the TCL, arm ratio and centromeric index of all populations are observed in Tables 3 to12.

Table 1: Location and Habitat type of the ten collected populations from genus *Plantago* L. in Egypt.

Species	Code	Population No.	Life Form	Location (District)	Governorate	GPS Data		Habitat Type
						Latitude (N)	Longitude (E)	
Plantago lagopus	*P.lg* 1	1	Annual or short Perennial	Abu AL-Matamir	El -Beheira	30°54'15.30"	30°10'46.28"	Orchards
	p. lg 2	2	Annual or short Perennial	Ganaklis	El -Beheira	31° 7'14.66"	31° 7'50.15"	Orchards
Plantago major	*P.m* 1	3	Perennial	Mansoura	El-Dakahlia	31° 2'25.67"	31°21'30.43"	Canal banks
	P. m 2	4	Perennial	Aga	El-Dakahlia	30°56'30.29"	31°17'36.35"	Cultivated lands
	P.m 3	5	Perennial	EL- Mahalla Al- Kubra	El-Gharbia	30°58'42.35"	31° 9'50.18"	Garden
	P.m 4	6	Perennial	Motubas	Kafr El-Sheikh	31°17'36.07"	30°31'19.51"	Canal banks
Plantago squarrosa	*P. sq* 1	7	Annual	Qalabshu-Bilqas	El -Dakahlia	31°29'3.36"	31°19'53.14"	Sand flats
	P.sq 2	8	Annual	Baltim	Kafr El -Sheikh	31°33'17.48"	31° 5'28.09"	Sand dunes
	P. sq 3	9	Annual	Al –Aqulah-El-Borullus	Kafr El -Sheikh	31°13'15.89"	30°54'45.92"	Cultivated sandy soils
	P.sq 4	10	Annual	Idku	El - Beheira	31°19'55.93"	30°17'31.59"	Sand dunes

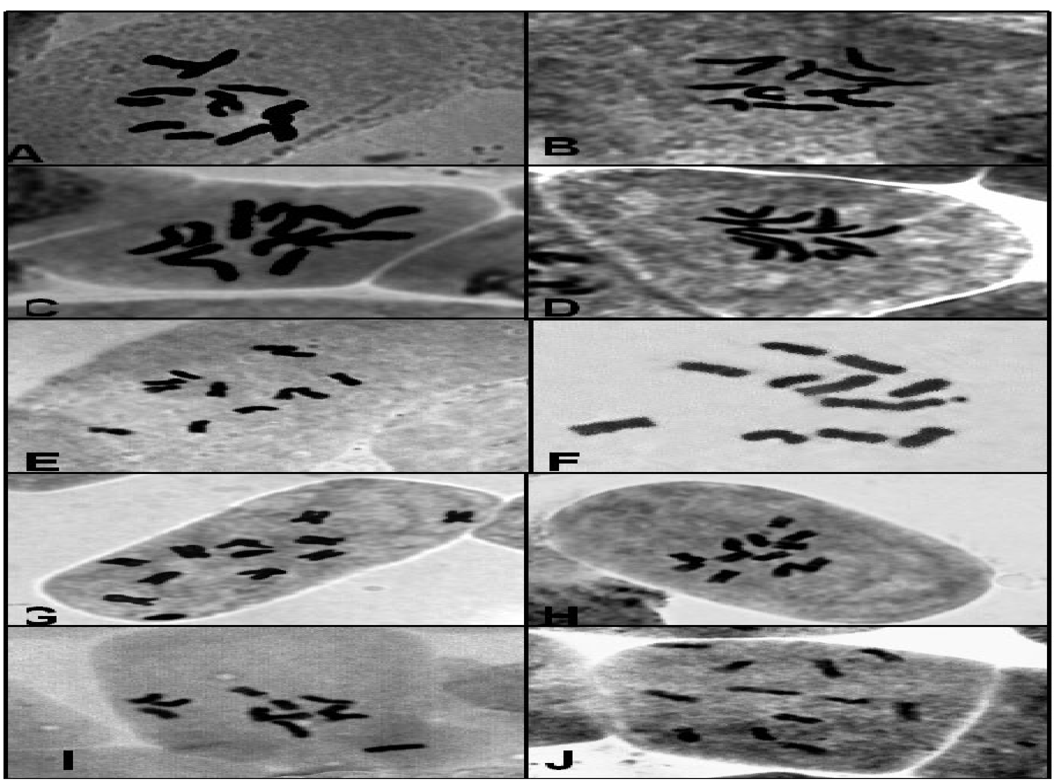

Figure 1: Somatic chromosomes of *Plantago* L., all populations with 2n =12. (A & B) *P. lag.*1; (C & D) *P. lag.* 2; (E & F) *P. m.* 1; (G & H) *P.m.* 2; (I & J) *P.m.* 3, (X=1000).

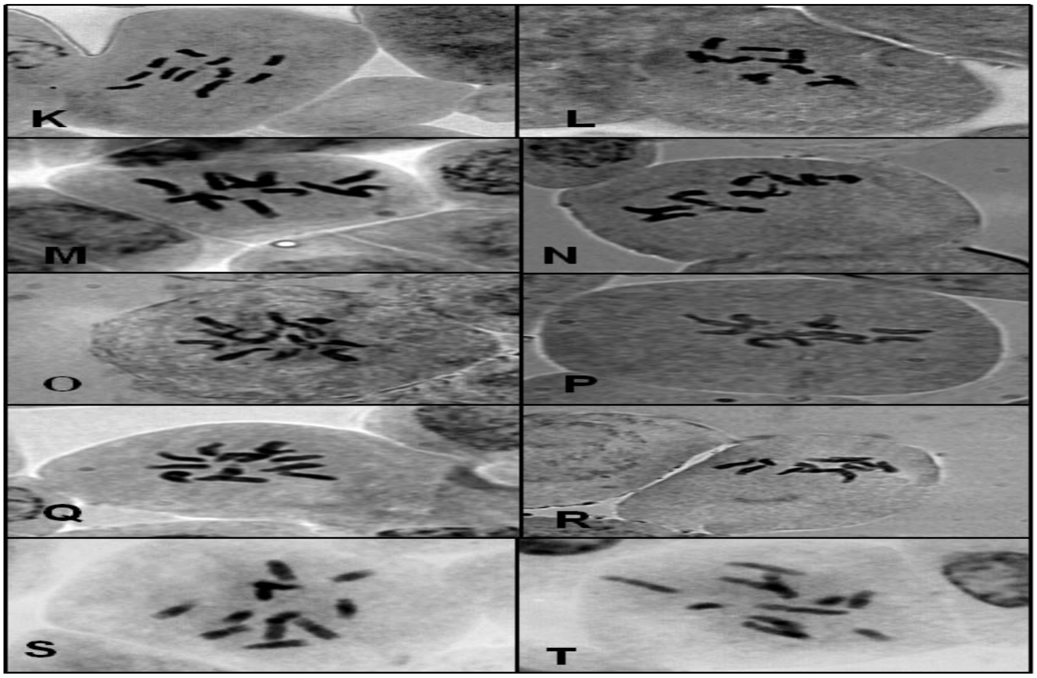

Figure 2: Somatic chromosomes of *Plantago* L., all populations with 2n =12. (K & L) *P.m.* 4; (M & N) *P. sq*. 1; (O & P) *P. sq*. 2; (Q &R) *P. sq.* 3; (S & T) *P.sq*. 4., (X = 1000).

Table 2: Calculations of karyotype asymmetry indices.

Karyotype asymmetry type	Formula	Reference
AS K%	$\text{As K\%} = \frac{\text{Length of long armsin chromosome set}}{\text{Total chromosome length in set}} \times 100$	Arano[39]
TF %	$\text{TF \%} = \frac{\text{Length of short arms in chromosome set}}{\text{Total chromosome length in set}} \times 100$	Huziwara[40]
Rec	$\text{Rec} = \frac{\sum_{i=1}^{n} CLi/LC}{n} \times 100$	Venora *et al*[41]
Syi	$\text{Syi} = \frac{\text{Mean length of the short arms}}{\text{Mean length of the long arms}} \times 100$	Venora *et al*[41]
A_1	$A_1 = 1 - \frac{\sum_{i=1}^{n} \frac{qi}{pi}}{n}$	Romero Zarco[42]
A_2	$A_2 = \frac{SCL}{XCL}$	Romero Zarco[42]
DI	$CG = \frac{\text{Length of median short arm}}{\text{Length of median chromosome}} \times 100$ $CV = \frac{SCL}{XCL} \times 100$ $DI = \frac{CG \times CV}{100}$	Lavania and Srivastava[43]
A	$A = \frac{\sum_{i=1}^{n} \frac{pi-qi}{pi+qi}}{n}$	Watanabe *et al.*[44]
AI	$CV_{CI} = \frac{SCI}{xCI} \times 100$ $CV_{CL} = \frac{SCL}{XCL} \times 100$ $AI = \frac{CVCL \times CVCI}{100}$	Paszko[45]
TCV	$TCV = 2(\pi \times r^2 \times TCL)$	Levan *etal.* [46]

Figure 3: Karyogram of ten population of *Plantago* L. (A = *P. lag* 1. F = *P. m* 4; B = *P. lag* 2; C = *P. m* 1; D = *P.m* 2; E = *P. m*3; F = *P. m* 4; G = *P. sq* 1; H = *P. sq* 2; I = *P. sq* 3; J = *P. sq* 4.)

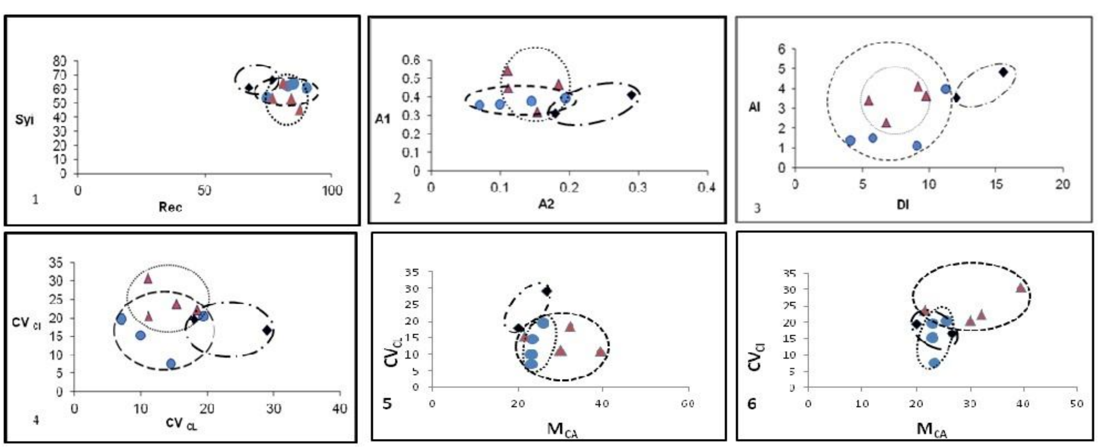

Figure 4: Scatter diagrams for Plantago populations : (1) The Rec index against the Syi index , (2) The A1 parameter against A2 parameter, (3) AI index against DI index , (4) The CV_{CL} parameter against the CV_{CI} parameter,(5) M_{CA} against the CV_{CL} parameter, (6) M_{CA} against the CV_{CI} parameter (▲) *Plantago lagopus* populations; (◆) *Plantago major* populations; (●) *Plantago squarrosa* populations.

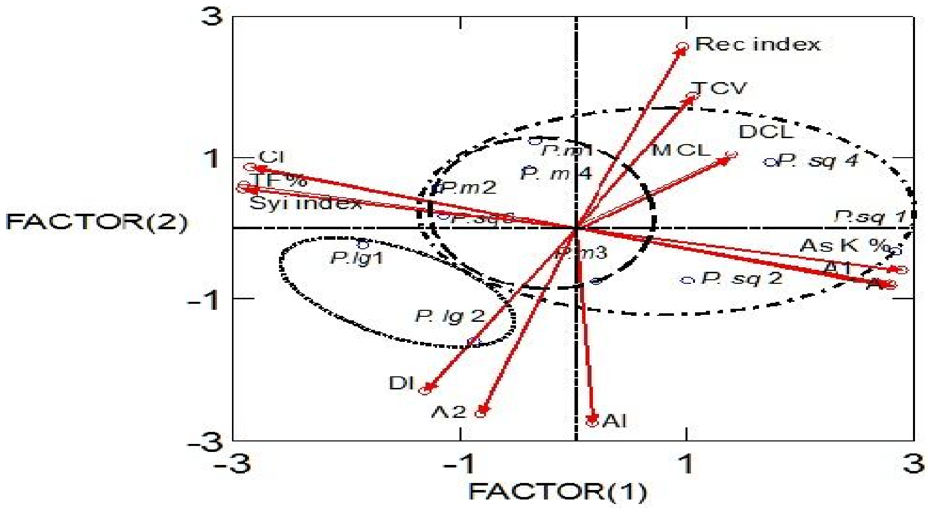

Figure 5: MDPREF (Biplot) analysis of the ten studied *Plantago* L. species populations in Egypt.

Table 3: The average measurements and arm ratios of somatic chromosomes of *P. lagopus* collected from Abu AL-Matamir District (El-Behiera Governorate).

Chromosome Pair	Total length (µm)	Long arm (µm)	Short arm (µm)	Arm ratio (long/short)	Centromeric index	Chromosome type
1	16.207	12.035	4.172	2.885	0.257	nsm(-)
2	13.320	8.499	4.820	1.768	0.362	nsm(-)
3	22.290	12.442	9.848	1.263	0.441	nm
4	19.516	11.989	7.526	1.593	0.385	nm
5	17.662	9.309	8.353	1.115	0.472	nm
6	14.771	7.977	6.793	1.174	0.459	nm

Table 4: The average measurements and arm ratios of somatic chromosomes of *P. lagopus* collected from Ganaklis District (El-Behiera Governorate).

Chromosome Pair	Total length (µm)	Long arm (µm)	Short arm (µm)	Arm ratio (long/short)	Centromeric index	Chromosome type
1	18.106	12.566	5.540	2.281	0.305	nsm(-)
2	14.735	9.423	5.311	1.773	0.360	nsm(-)
3	9.985	7.084	2.901	2.457	0.290	nsm(-)
4	25.093	13.582	11.511	1.182	0.458	nm
5	19.699	12.050	7.649	1.576	0.388	nm
6	14.541	8.731	5.809	1.504	0.399	nm

Table 5: The average measurements and arm ratios of somatic chromosomes of *P. major* collected from Mansoura District (El-Dakahlia Governorate).

Chromosome Pair	Total length (µm)	Long arm (µm)	Short arm (µm)	Arm ratio (long/short)	Centromeric index	Chromosome type
1	19.303	12.216	7.087	1.726	0.367	nsm(-)
2	18.491	13.093	5.398	2.426	0.291	nsm(-)
3	18.138	12.869	5.269	2.443	0.290	nsm(-)
4	20.792	11.869	8.923	1.334	0.429	nm
5	19.673	10.569	9.103	1.162	0.462	nm
6	16.754	9.418	7.336	1.305	0.437	nm

Table 6: The average measurements and arm ratios of somatic chromosomes of *P. major* collected from Aga District (El-Dakahlia Governorate).

Chromosome Pair	Total length (µm)	Long arm (µm)	Short arm (µm)	Arm ratio (long/short)	Centromeric index	Chromosome type
1	12.097	7.994	4.003	1.996	0.330	nsm(-)
2	11.783	8.189	3.788	2.167	0.321	nsm(-)
3	10.710	7.008	3.851	1.820	0.360	nsm(-)
4	14.439	7.576	6.863	1.104	0.475	nm
5	13.171	7.522	5.648	1.334	0.428	nm
6	12.155	7.211	4.944	1.461	0.406	nm

Table 7: The average measurements and arm ratios of somatic chromosomes of *P. major* collected from E-L Mahalla Al Kubra District (El-Gharbia Governorate).

Chromosome Pair	Total length (µm)	Long arm (µm)	Short arm (µm)	Arm ratio (long/short)	Centromeric index	Chromosome type
1	23.511	17.980	5.530	3.255	0.235	nsm(+)
2	19.921	13.886	6.035	2.325	0.302	nsm(-)
3	16.296	10.150	6.145	1.651	0.377	nsm(-)
4	18.498	11.061	7.437	1.488	0.401	nm
5	16.296	9.377	6.918	1.355	0.424	nm
6	12.926	7.506	5.419	1.394	0.419	nm

Table 8: The average measurements and arm ratios of somatic chromosomes of *P. major* collected from Motubas District (Kafr El-Sheikh Governorate).

Chromosome Pair	Total length (µm)	Long arm (µm)	Short arm (µm)	Arm ratio (long/short)	Centromeric index	Chromosome type
1	27.708	18.074	9.633	1.876	0.347	nsm(-)
2	23.587	15.059	8.527	1.766	0.361	nsm(-)
3	21.450	13.631	7.818	1.743	0.364	nsm(-)
4	25.402	14.631	10.771	1.358	0.424	nm
5	22.336	13.297	9.038	1.472	0.404	nm
6	17.493	10.542	6.950	1.518	0.397	nm

Table 9: The average measurements and arm ratios of somatic chromosomes of *P. squarrosa* collected from Qalabshu- Bilqas District (El-Dakahlia Governorate).

Chromosome Pair	Total length (µm)	Long arm (µm)	Short arm (µm)	Arm ratio (long/short)	Centromeric index	Chromosome type
1	18.913	15.309	3.403	4.500	0.179	nst(-)
2	14.964	11.056	3.107	3.561	0.208	nsm(+)
3	21.132	13.830	7.102	1.949	0.336	nsm(-)
4	20.083	13.206	6.977	1.892	0.347	nsm(-)
5	17.992	12.178	5.463	2.229	0.303	nsm(-)
6	18.527	10.461	8.265	1.265	0.446	nm

Table 10: The average measurements and arm ratios of somatic chromosomes of *P. squarrosa* collected from Baltim District (Kafr El-Sheikh Governorate).

Chromosome Pair	Total length (µm)	Long arm (µm)	Short arm (µm)	Arm ratio (long/short)	Centromeric index	Chromosome type
1	22.680	15.102	7.578	1.994	0.334	nsm(-)
2	20.747	15.193	5.553	2.742	0.267	nsm(-)
3	16.450	11.065	5.385	2.055	0.3273	nsm(-)
4	15.842	11.804	4.037	2.923	0.254	nsm(-)
5	25.994	13.830	12.163	1.137	0.467	nm
6	18.593	11.445	7.148	1.601	0.384	nm

Table 11: The average measurements and arm ratios of somatic chromosomes of *P. squarrosa* collected from Al–Aqulah-El-Borullus District (Kafr El-Sheikh Governorate).

Chromosome Pair	Total length (μm)	Long arm (μm)	Short arm (μm)	Arm ratio (long/short)	Centromeric index	Chromosome type
1	19.778	15.577	4.200	3.753	0.212	nsm(+)
2	17.320	11.036	6.283	1.756	0.362	nsm(-)
3	24.642	12.598	12.044	1.045	0.488	nm
4	23.348	13.572	9.775	1.388	0.418	nm
5	19.272	11.238	8.034	1.407	0.416	nm
6	16.554	9.545	7.009	1.377	0.422	nm

Table 12: The average measurements and arm ratios of somatic chromosomes of *P. squarrosa* collected from Idku District (El-Behiera Governorate).

Chromosome Pair	Total length (μm)	Long arm (μm)	Short arm (μm)	Arm ratio (long/short)	Centromeric index	Chromosome type
1	29.199	22.049	7.150	3.084	0.244	nsm(+)
2	32.676	22.252	10.424	2.135	0.319	nsm(-)
3	28.178	17.647	10.531	1.676	0.373	nsm(-)
4	24.225	16.844	7.381	2.289	0.304	nsm(-)
5	27.323	16.518	10.805	1.528	0.395	nm
6	24.150	13.180	10.970	1.201	0.454	nm

Table 13: Karyotype parameters of ten *Plantago* L. populations in Egypt using different methods of evaluating karyotype asymmetry.

Populations	Chromosome No.	CI Mean±SE	DCL	MCL	TCV (μm^3)	As K%	TF%	Syi	Rec	A1	A2	DI	A	M_{CA}	AI	Karyotype formula
***P. lag*1**	2n=12	0.396±0.022	207.538	34.589	39.372	59.994	40.005	66.682	76.549	0.312	0.18	12.025	0.200	20	3.534	4nsm(-) +8 nm
***P. lag* 2**	2n=12	0.367±0.017	204.323	34.053	40.284	62.096	37.903	61.040	67.239	0.410	0.29	15.583	0.268	26.8	4.827	6 nsm(-) +6 nm
***P. m* 1**	2n=12	0.379±0.021	226.309	37.718	122.616	61.893	38.106	60.770	90.044	0.355	0.070	4.1129	0.231	23.1	1.395	6 nsm(-) +6 nm
***P. m* 2**	2n=12	0.387±0.017	148.714	24.785	49.375	61.193	39.135	63.954	84.958	0.359	0.099	5.790	0.231	23.1	1.514	6 nsm(-) +6 nm
***P.m* 3**	2n=12	0.359±0.021	214.900	35.816	79.449	65.111	34.888	53.5833	74.558	0.392	0.194	11.210	0.258	25.8	3.975	2 nsm(+) + 4 nsm (-) +6 nm
***P.m* 4**	2n=12	0.383±0.008	275.958	45.993	159.207	61.775	38.224	61.875	82.869	0.377	0.145	9.071	0.234	23.4	1.105	6 nsm(-) +6 nm
***P. sq* 1**	2n=12	0.303±0.026	223.227	37.204	53.024	68.130	30.749	45.133	87.412	0.543	0.11	5.433	0.394	39.4	3.404	2nst(-) + 2 nsm(+) + 6 nsm (-) +2nm
***P. sq*2**	2n=12	0.339±0.021	240.616	40.102	52.513	65.200	34.799	53.372	76.421	0.466	0.185	9.185	0.321	32.1	4.110	8 nsm(-) + 4nm
***P. sq* 3**	2n=12	0.386±0.026	241.833	40.305	81.124	60.841	39.158	64.360	80.993	0.319	0.153	9.783	0.216	21.6	3.653	2 nsm(+) +2 nsm (-) + 8nm
***P. sq* 4**	2n=12	0.348±0.020	331.508	55.251	301.192	65.452	34.547	52.781	84.074	0.447	0.111	6.817	0.300	30	2.282	2nsm(+) +6nsm (-) +4nm

DCL:Diploid complement length; MCL: Mean chromosome Length; TCV: Total chromosome volume; TF(%):Total forms; S(%): Symmetry percent; Rec: Resemblance between chromosome; A1: Intrachromosomal asymmetry index; A2: Interchromosomal asymmetry index

Table 14: Pearson correlations for asymmetry indices between the karyotype formulae of ten populations.

	AS K%	TF %	Syi index	Rec index	A1	A2	DI	A	AI
As K%	1								
TF%	-.996	1							
Syi index	-.998	.997	1						
Rec index	.149	-.170	-.172	1					
A1	.930	-.936	-.931	.101	1				
A2	-.117	.125	.132	-.980	-.030	1			
DI	-.292	.295	.309	-.959	-.239	.963	1		
A	.933	-.946	-.935	.131	.989	-.059	-.262	1	
AI	.226	-.233	-.215	-.780	.220	.788	.711	.269	1

Karyotype analysis: Karyotype analysis includes chromosomes number, arm ratio, centromeirc index, diploid complement length (DCL), mean chromosome length (MCL), total chromosome volume (TCV), total form percentage (TF%), intrachromosomal asymmetry index(A_1), interchromosomal asymmetry index(A_2),the symmetric indices (Syi), resemblance between chromosomes (Rec), the karyotype asymmetry index (As K%), the dispersion index (DI), the degree of asymmetry of karyotype (A), the asymmetry index (AI), as well as karyotype formulae of all populations of *Plantago* L. are recorded Table 13. Karyotypic variations exist not only between different species but also within the same species. The mean chromosome length (MCL) was varied from 24.785 µ in population of *P. major* (*P.m*2) collected from Aga District to 55.251µ in population of *P. squarrosa* (*P.sq*4) collected from Idku District. However, the highest total chromosome length (DCL) was found in population of *P. squarrosa* (*P.sq*4) collected from Idku District (331.508 µ) and the least DCL in population of *P. major* (*P.m*2) collected from Aga District (148.714µ). Also the largest total chromosome volume (TCV) was recorded in population of *P. squarrosa* (*P.sq*4) collected from Idku District (301.192µm^3), while the smallest was observed in population of *Plantago lagopus* (*P.lag* 1) collected from Abu Al-Matamir District (39.372µm^3). The evolution of karyotype is estimated by the indices of symmetry. According to these parameters, TF% values ranged between 30.749% in population of *P. squarrosa* (*P. sq* 1)collected from Qalabshu-Bilqas District to 40.005 % in population of *P. lagopus* (*P. lag*1) collected from Abu AL-Matamir. The highest value of Rec index was observed in population of *P. major* (*P. m* 1) collected from Mansoura District (90.044) and also the highest value of Syi index was found in population of *P. lagopus* (*P. lag*1) collected from Abu AL-Matamir District (66.682). Population of *P. squarrosa* (*P.sq*1) collected from Qalabshu-bilqas District had the highest values of A_1 (0.543), where the highest value of A_2 (0.29) was recorded in population of *P. lagopus* (*P. lag* 2) collected from Ganaklis District. The analysis of index formulae and the association between quantitative indices and karyotype characteristics showed in Table (13).The As K % index had a perfect negative correlation with two indices: the TF% and the Syi. Pearson Correlations between ten populations based on karyotypic parameters were shown in Table (14), the variation in TF%, Syi index, As K %, Rec index, A_1, A_2, DI, AI and A had a perfect or almost positive or negative correlation with each other, while the highest positive correlation was observed between TF% and Syi index (0.997) and the lowest positive correlation was recorded between Rec index and A_1 (0.101). The AI value and scatter diagram based on CV_{CI} and CV_{CL} seem best suited to assess overall classification strength and display relationships among ten populations with respect to the heterogeneity of the centromeirc index. Scatter diagrams between AI and DI and between M_{CA} and CV_{CL} gave populations of *P. lagopus* (*P. lag* 1 &*P.lag* 2) collected from Abu AL-Matamir and Ganaklis Districts in a separate group, while Populations of *P. major* and *P. squarrosa* were super imposed and the center of the rest populations pool was occupied by *P.squarrosa* populations. Despite the scatter diagrams between Syi and Rec, A_1 and A_2, CV_{CI} and CV_{CL}, and between M_{CA} and CV_{CI} showed that the three groups of all ten populations were intermingled (Figure 4).

The data matrix of karyotypic parameters were standardized and compute coordinates for plotting Biplot mapping by using perceptual mapping (PERMP) (Figure 5), populations of *P. lagopus* delimitated in a separate group from the rest of studied populations. The center of rest populations was occupied by *P. major* populations. PERMAP-Biplot showed that the importance of A_2, DI, CI, TF % and Syi index to distinguish the *P. lagopus* populations from the other studied populations.

Genetic diversity plays a very important role in survival and adaptability of a species because when species environment changes, slight gene variations are necessary to produce changes in the organisms. Anatomy enables it to adapt and survive. A species that has a large degree of genetic diversity among its population will have more variations from which to choose the fit alleles[48].

The diploid chromosome number 2*n*=12 has been observed in all populations these counts agree with the previous counts for these species[7, 10, 11, 18, 9 & 50]. The importance and reliability of cytological methods for studying evolutionary problems have been recognized for a long time. Cytological studies used for determining similarities and differences between various taxa include chromosome number, their formula and behavior.

The information on chromosome number is the role of numerical variations, karyotypic studies used in understanding the role of structural changes and variations in the evolutionary process. Important karyotypic aspects used to evaluate relationships between different populations include absolute and relative size of chromosomes, position of primary constrictions and number of chromosomes.[51]

Karyotypes are one of the most important parameters by which authentic identification of a specimen is possible.[52] Despite this stability in chromosome number, the large variations in chromosome size have played an important role in the chromosome evolution of the *Plantago* L. populations. Total chromosome length (TCL) was recorded higher in *P. sq* 4 population (331.508µ), while lower TCL was observed in *P. m* 2 population (148.714 µ). The differences in TCL also indicate that during the diversification of the genus, cyclic variations in genome size may have occurred.[53]

Karyotype analysis was characterized by nearly metacentric and nearly sub-metacentric chromosomes in all populations, where only nearly sub-telocentric chromosomes were observed in population of *P. sq* 1. Changes to an asymmetric karyotype can arise by shifts in position of centromere towards the telomere (intrachromosomal) and/or by the addition or deletion of chromatin from some but not all chromosomes,which leading to differences in size between the largest and smallest chromosome (interchromosomal).[54]

The evolution of karyotype parametersare estimated by the indices of symmetry. These values range theoretically from 0 to 100 for Rec and Syi indices[40]; from 0 t0 50 for TF%.[56] According to As K% , TF % and Syi indices, *P. lagopus* population collected from Abu AL-Matamir District (*P. lag*1), and Al–Aqulah District (*P. sq* 3) may be more symmetrical, (*P. lag* 1) was the most symmetrical karyotype, while *P. squarrosa* population collected from Qalbshu- Bilqas District (*P. sq* 1) may be the most asymmetrical, also population of *P. squarrosa* collected from Al –Aqulah District (*P. sq* 3) was more symmetrical.

According to A_1 and A index *P. lagopus* population collected from Abu AL-Matamir District (*P. lag* 1) was the most symmetrical karyotype, while *P. squarrosa* population collected from Qlbsh- Bilqas District (*P. sq*1) was the most asymmetrical karyotype. Also, population of *P. squarrosa* collected from Al –Aqulah District (*P. sq* 3) was more symmetrical. The TF % and Syi-Rec values decrease with increasing asymmetry, while the As K%, the A_1-A_2 and the A values increase with increasing asymmetry.[56 & 57]

The DI index was a new asymmetry index, AIwas proposed to measure karyotype asymmetry and had the potential to display even minor karyotypic variations**.** The highest value of AI index was recorded in *P. lagopus* population collected from Ganaklis District (*P. Lag* 2), while the higher values of the AI index are considered to indicate higher levels of karyotypic heterogeneity. Scatter diagrams between DI and AI & M_{CA} and CV_{CL} was similar to the result of PERMAP-Biplot were showed that populations of *P. lagopus* were in a separate group, where populations of *P. major* and *P. squarrosa* were super imposed.

CONCLUSION: The results of this study determine the chromosome numbers, karyotypes, idiograms and karyotype asymmetry degrees of ten populations of *Plantago* L. The high genetic variation among populations collected from different geographic regions, high similarity indices suggest that the populations of the species have close genetic relationship as well as populations were closely related to each other in the same geographical regions. This study suggests that the utilization of asymmetry indices for the establishment of the evolutionary relationships in *Plantago* L. in Egypt.

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