ISSN : 0974 - 7427 Volume 8 Issue 4

BIOCHEMISTRY
An Indian Journal

Regular Paper

BCAIJ, 8(4), 2014 [129-137]

Genetic variability and evolutionary relationships between *Amaranthus spp*. as revealed by karyotype analysis

Reda Helmy Sammour*, Safaa Radwan, Mohammed Mira, Salwa Badar
Botany Department, Faculty of Science, Tanta University, Tanta, (EGYPT)
E-mail : redasammour54@gmail.com

ABSTRACT

This work aimed at identifing genetic variability and assesing the evolutionary relationships between 24 accessions of eight *Amaranthus* species, based on the morphological features of the basic chromosome numbers and numerical characterization of the karyotypes using total chromosome length (TCL), mean chromosome length (MCL) and mean centromeric index (MCI). The basic chromosome numbers were analyzed cytologically by Feulgen staining. They were 16 for all the studied accessions, except some accessions belong to *A. powellii* and *A. palmeria* which exhibited n=17. The karyotypes of the studied accessions had a predominance of metacentric chromosomes with some accessions characterized by subtelocentric chromosomes. The karyptype analysis showed a variation in the karyotype of the accessions of the same species. The variation may be considered of adaptive significance. Cluster analysis showed *A. hybridus* and *A. powelli* as a progenitor of Amaranthus species. The obtained data indicated that *A. powelli* could be considered the most advanced species, since it has the smallest chromosome length.

KEYWORDS

Karyotype;
Amaranthus;
Genetic diversity;
Cluster analysis.

INTRODUCTION

The genus *Amaranthus* includes about 60 species, some of them being cultivated for more 5000 years for their grains (*A. caudatus, A. cruentus* and *A. hypochondriacus*) or leaves (*A. blitum, A. dubius* and *A. tricolor*). Some others are useful as colorful ornamentals[1-6].

The genetic variability between *Amaranthus* species was confused by the extremely used range of phenotypic plasticity among species and the possible introgression and hybridization involving weedy and crop species[7-13]. This high genetic variability made the scholars to use other sources of taxonomic traits to collect information about the genetic or evolutionary origin of grain *Amaranthus*. Without such knowledge, scientific breeding, especially making use biotechnological methods, is not possible. One of thess sources is the karyotype analysis which was used effectively in the study of genetic variability between plant species[19-44].

Chromosome numbers vary only little in the genus *Amaranthus*. The two diploid numbers 2n = 32 and 2n = 34 were reported by[20-27]. In several cases both exist within single taxon (e.g. *A. albus* and *A.*

Regular Paper

graecizans). The role of aneuploidy in chromosome evolution of the genus *Amaranthus* is a well-known fact. It has been suggested that the gametic number n=17 has originated from n=16 through trisomy. Some species of the genus *Amaranthus* are polyploids (basic number x=8) and the chromosome number n=17 originated later by trisomy (2n+1)[28-33]. The evaluation of the chromosome analysis of *A. turicensis* hybrid (2n=34) showed that both parental species (*A. cruentus and A. retroflexus*) should hybridize relatively easily. However, most of the *Amaranthus* hybrids exhibit relatively high level of sterility which was already confirmed by Gupta and Gudu[34]. Most *Amaranthus* species are *n* = 16 or *n* = 17, but *A. dubius* is unusual for having *n* = 32[29-33]. The grain *Amaranthus* are paleo-allotetraploids, as indicated by observations of pairing in their hybrids[35-37]. However Lanta *et al.*[38-43] found that the species *Amaranthus retroflexus, Amaranthus cruentus and Amaranthus turicensis* have the same chromosome number 2n = 34. No higher ploidity level was detected. The chromosomes of all species studied uniform, short, and monotypic. No marked differences in chromosome counts and visual aspects (length, centromere position). The karyotypes of *Amaranthus* spp. are mainly comprised of many metacentric chromosomes and few submetacentric ones[44-50].

A. viridus could be the most advanced species amongst all the investigated taxa[44]. It exhibits the diploid number 2n = 34 and the shortest haploid genome length with more Karyotypic activity, concerning chromosome length and centromeric position, recorded among its different accessions[44]. This might have been produced as a result of differences in the degree of chromatin condensation and /or chromosomal changes such as translocations and pericentric inversions[44].

Sauer[50] has proposed the 3 weedy *Amaranthus*, namely, *Amaranthus powellii, Amaranthus hybridus and Amaranthus quitensis* as putative ancestors of the cultivated *Amaranthus*, namely, *Amaranthus hypochondriacus, Amaranthus cruentus* and *Amaranthus caudatus* respectively. This scheme has been refused by Pal and Khoshoo[51-56] on the basis of cytogenetic studies on *Amaranthus powellii and Amaranthus hypochondriacus* since the 2 species have different basic chromosome numbers (n = 17 and n = 16 respectively) and since the hybrid between the two was sterile. Further, they have also suggested that *Amaranthus hybridus* is the more likely ancestral species for *Amaranthus hypochondriacus*.

The objectives of this research are to identify genetic variability and asses the evolutionary relationships between 24 accessions of eight *Amaranthus* species.

MATERIAL AND METHODS

Plant material

Cytological study was carried out on twenty four accessions of *Amaranthus* species.The studied accessions were donated by the USAD (United State Department of Agriculture). The represented *Amaranthus* species are *A. hybridus, A. hypochondricus, A. palmeria, A. quitensis, A. retroflexus, A. spinosus, A. powellii and A. caudatus* belong to three sections. These species have shown worldwide distribution pattern. The sections, the origin and the accession number of these accessions are shown in TABLE 1.

Cytological preparations

For cytological preparations, one to two cm long roots of four days old seedlings of each of the twenty four accessions were detached and pretreated in super saturated solution by 1, 4 dichlorobenzine for 3-4 hours. Roots were then washed briefly in water and fixed in a mixture of 3:1 (v/v) ethyle alcohol: glacial acetic acid for 24 hrs and kept in 75% ethanol in a refrigerator until use[49].

Cytological preparations were carried out using the Feulgen squash technique. For Feulgen staining, root tips were hydrolyzed in 1 N HCl at 60°C for 8-9 min, washed in distilled water then well dried and stained in leuco-basic fuchsin for at least 2 hrs. at room temperature. The terminal 1-2 mm of the root tips were squashed in a drop of 45% acetic acid on a clean slide. Cover slips were separated by the freeze-drying method. Samples were then dehydrated in absolute ethanol for 2-3 min. For permanent preparations slides were mounted in D.P.X. and allowed to dry at room temperature. Cells with good spreading of chromosomes were photographed using a Zeiss Ultraphoto microscope equipped with automatic camera. The nomenclature used for the description of the chromosome morphology was that proposed by Levan *et al.*[57].

Regular Paper

TABLE 1 : The number and the origin of the studied accessions of *Amaranthus* species

	Section	Species	Accession No.	Origin	Plant name
1		*A. hybridus*	Ames 21188	South africa	Ames 21188
2		*A. hybridus*	PI 605351	Greece	RRC 847
3		*A. hybridus*	PI 636181	USA ,Delaware	RRC 1195
4		*A. hybridus*	Ames 23369	Brazil, Goias	GPAC 96-1
5		*A. hybridus*	Ames 26852	Portugal, Coimbra	Index seminum 110
6		*A. hypochondriacus*	PI 274279	India,Himachal pradesh	RRC 171
7		*A. hypochondriacus*	PI 337611	Uganda	P373
8		*A. hypochondriacus*	PI 477917	Mexico	RRC 1024
9		*A. hypochondriacus*	PI 540446	Pakistan	RRC 1004
10	*Amaranthus*	*A. quitensis*	PI 511744	Ecuador	HH 70
11		*A. retroflexus*	PI 572263	USA, lowa	DB, 8921
12		*A. retroflexus*	PI 607458	USA, kansas	Pop 56
13		*A.powellii ssp.bouchonii*	Ames 5304	USA, Washington	RRC 653
14		*A.powellii ssp.bouchonii*	Ames 15707	USA, California	AO- 30
15		*A.powellii ssp.bouchonii*	PI 572261	Germany	AMA 57/81
16		*A.powellii ssp.bouchonii*	PI 572262	France	AMA 31/80
17		*A. caudatus*	PI 166045	India	Chuu RRC 175
18		*A. caudatus*	PI 619264	Nepal	RRC 279
19		*A. caudatus*	PI 511679	Argentina	RRC 551
20		*A. palmeria*	PI 604557	Mexico,puebla	Mapes 820
21	*Centrusa Griseb*	*A. palmeria*	PI 607455	USA, kansas	Pop 53
22		*A. palmeria*	PI 607461	USA, kansas	Pop 59
23		*A. palmeria*	PI 632235	USA, Arizona	RRC 686
24	*saueranthus*	*A. spinosus*	PI619234	Indonesia, sumatra	RRC 114

Data analysis

For the numerical characterization of the karyotypes, the following parameters were calculated: (1) total chromosome length of the haploid complement (TCL); (2) mean chromosome length (MCL); (3) mean centromeric index (MCI). Comparisons of chromosome morphological features were made by arranging the chromosomes of each karyotype in pairs in order of their arm ratio and length as determined from the photographic prints. An idiogram for each sample was constructed using the total length of each pair of homologous chromosomes to represent the haploid chromosome number. The relative position of the centromere and their variation within the karyotype were expressed. A cluster analysis of the karyotype data was carried out to examine karyotype similarity among species and sections. A data matrix 24 OTUs (operational taxonomic units) × 6 variables was constructed. The TCL, CI, number of m, sm, and st chromosomes as well as the numbers of chromosomes were considered. The SYSTAT ver. 7 program was used to standardize the data matrix, calculate the average taxonomic distance, and generate a phenogram. Clustering was performed using the unweighted pair-group method (UPGMA).

RESULTS

A summary of the karyotype morphological characters, obtained from Feulgen stained preparations of the studied 24 accessions of *Amaranthus* was described in TABLE 2. The stained somatic chromosomes of the examined accessions and their idiograms were represented in Figures 1,2.

All the studied accessions had chromosome number $n^2 = 32$ except the two accessions of *Amaranthus palmeria* (accessions number PI 607455 from USA, kansas and PI 607461 also from USA, Kansas); two accessions of *A. powellii ssp.bouchonii* (accessions

Regular Paper

TABLE 2 : Accession number, karyotype formula (KF), total length of the haploid complement (TCL), mean chromosome length (MCL), mean centromeric index (MCI), metacentric (m), submetacetric (sm), subtelocentric (st)

No.	Section	Species	Accession No.	KF	TCL	MCL	MCI	Ch. no.
A		*Hybridus*	Ames 21188	26m + 6sm	12.65	.79	.897	32
B		*Hybridus*	PI 605351	28m + 2sm+ 2st	8.03	.50	.812	32
C		*Hybridus*	PI 636181	28m + 4sm	10.34	.65	.866	32
D		*Hybridus*	Ames 23369	24m + 6sm + 2st	7.45	.47	.885	32
E		*Hybridus*	Ames 26852	26m + 4sm + 2st	9.25	.58	.873	32
F		*Hypochondriacus*	PI 274279	24m + 8sm	8.74	.55	.893	32
G		*Hypochondriacus*	PI 337611	28m + 4sm	9	.56	.860	32
H		*Hypochondriacus*	PI 477917	26m + 6sm	8.55	.53	.890	32
I		*Hypochondriacus*	PI 540446	24m + 8sm	9.05	.57	.861	32
J	*Amaranthus*	*Quitensis*	PI 511744	16m+ 14sm+ 2st	10.12	.63	.794	32
K		*Retroflexus*	PI 572263	28m + 4sm	9.87	.62	.928	32
L		*Retroflexus*	PI 607458	16m+ 12sm+ 4st	9.56	.60	.780	32
M		*Powellii*	Ames 5304	22m+ 10sm+ 2st	8	.47	.825	34
N		*Powellii*	Ames 15707	30m + 4sm	10.61	.62	.866	34
O		*Powellii*	PI 572261	30m + 4sm	9.85	.58	.435	34
P		*Powellii*	PI 572262	28m + 4sm	7.57	.47	.425	32
Q		*Caudatus*	PI 166045	24m + 6sm + 2st	8.9	.56	.832	32
R		*Caudatus*	PI 619264	24m + 8sm	9.73	.61	.844	32
S		*Caudatus*	PI 511679	20m + 6sm + 6st	10.74	.67	.799	32
T	*Centrusa Griseb*	*Spinosus*	PI619234	26m + 8sm	9.35	.55	.833	32
U		*Palmeria*	PI 604557	24m + 6sm + 2st	7.625	.48	.893	32
V	*saueranthus*	*Palmeria*	PI 607455	30m + 4sm	9.93	.58	.868	34
W		*Palmeria*	PI 607461	30m + 4sm	9.93	.58	.866	34
X		*Palmeria*	PI 632235	24m + 8sm	8.98	.56	.873	32

number Ames 5304, Ames 15707 from USA, Washigton and California respectively) and accessions number PI 572261 from Germany that have $n^2 = 34$. The karyotypes of the studied accessions had a predominance of metacentric chromosomes with centromere in median and submedian region. Some samples were characterized by subtelocentric chromosomes such as the accessions of *Amaranthus hybridus* PI 605351, Ames 23369 and Ames 26852; accession of *Amaranthus palmeria* PI 604557, accession of *Amaranthus quitensis* PI 511744, accession of *Amaranthus retroflexus* PI 607458, accession of *A. powellii ssp.bouchonii* Ames 5304, and accessions of *Amaranthus caudatus* PI 511679, PI 166045. No satellite was observed on the karyotype of the examined accessions, all having normal structure.

All chromosomes were clearly discriminated and their relative size could be determined based on measurements of mitotic chromosome lengths. In the studied *Amaranthus* spp., a comparison of the total chromosome length (TCL) and the mean value of chromosome length (MCL) of the studied accessions revealed that they ranged from 7.45, 0.47 in *Amaranthus hybridus* Ames 23369 from Brazil to 12.65, 0.79 in *Amaranthus hybridus* Ames 21188 from South Africa. However, *Amaranthus retroflexus* PI 572263 from USA, lowa showed the highest mean centromeric index (MCI) 0.928 while *Amaranthus powellii ssp. Bouchonii* PI 572262 from France had the lowest (MCI) 0.425. A significant intraspecific variation was observed in the TLC, MCL and MCI in the accessions belong to the same species. This variation was highly detected in *A.hybridus, A.caudatus* and *A. powellii*. *A. powellii* PI572262 was recorded to have the shortest value of MCL and the smallest value of MCI among all the studied accessions.

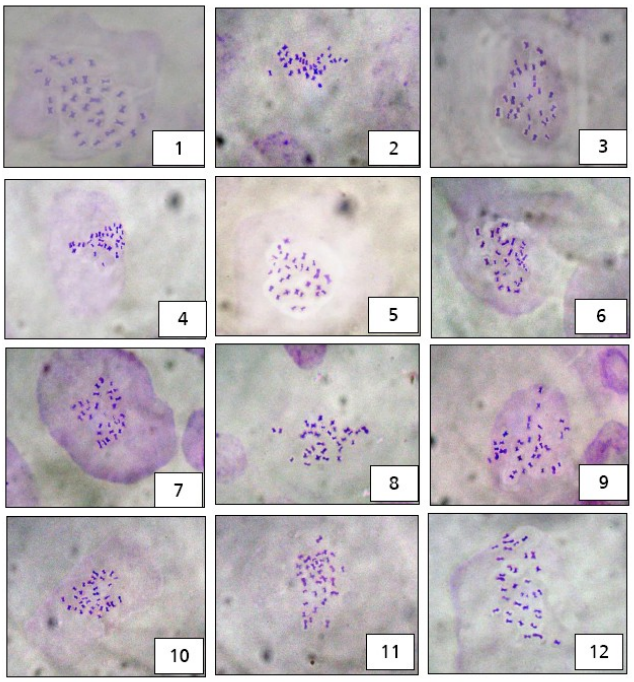

Figure 1A : The somatic chromosomes of the studied accessions in *Amaranthus* species. 1. *A. hybridus* Ames 21188, 2. *A. hybridus* PI 605351, 3. *A. hybridus* PI 636181, 4. *A. hybridus* Ames 23369, 5. *A. hybridus* Ames 26852 , 6. *A. hypochondriacus* PI 274279, 7. *A. hypochondriacus* PI 337611, 8. *A. hypochondriacus* PI 477917, 9. *A. hypochondriacus* PI 540446, 10. *A. palmeria* PI 604557, 11. *A. palmeria* PI 607455, 12. *A. palmeria* PI 607461

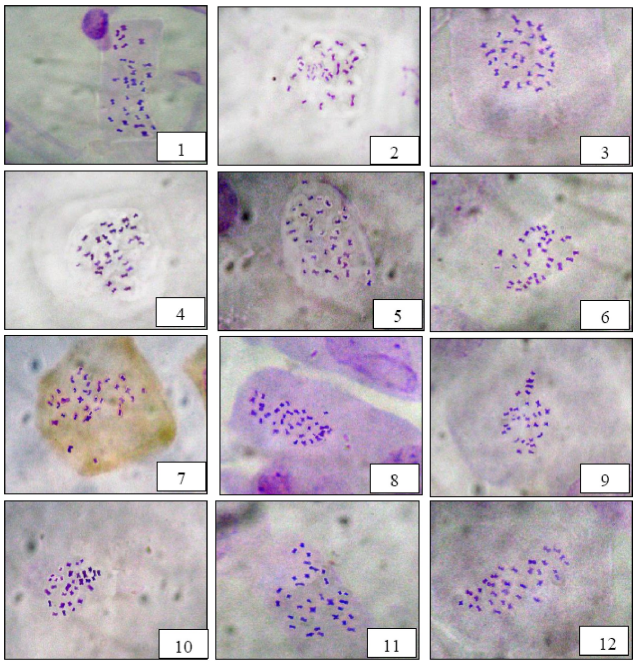

Figure 1B : The Somatic chromosomes of the studied accessions in *Amaranthus* species. 1. *A. palmeria* PI 632235, 2. *A. quitensis* PI 511744, 3. *A. retroflexus* PI 572263, 4. *A. retroflexus* PI 607458, 5. *A. spinosus* PI619234, 6. *A.powellii ssp.bouchonii* Ames 5304, 7. *A.powellii* ssp.bouchonii Ames 15707 , 8. *A.powellii* ssp.bouchonii PI 572261, 9. *A.powellii* ssp.bouchonii PI 572262, 10. *A .caudatus* PI 166045, 11. *A .caudatus* PI 619264, 12. *A .caudatus* PI 511679

The dendrogram constructed on the basis of karyotype characters show two major clusters (Figure 3). The first cluster comprised all the accessions carry $n^2 = 34$. It included three accessions of *Amaranthus powellii* Ames 15707, PI 572261 and Ames 5304 and two accessions of *Amaranthus palmeria* PI 607455 and PI 607461. These two accessions originated in USA, Kansas. They seem to be genetic identical duplicates. The second cluster included all the rest of the studied accessions which carry $n^2 = 32$. It separated one accession of *Amaranthus hybridus*, PI Ames 21188 from the other accessions. This accession was characterized by the largest TCL and MCL. The rest of the accessions were divided into two groups. The first one included two accessions of *Amaranthus hybridus*, PI Ames 23369 and PI 605351, one accession of *Amaranthus powellii* PI 572262 and one accession of *Amaranthus palmeria* PI 604557. The second group included the rest of accessions. Other accessions of *A. hybridus* were scattered among the second cluster. In this cluster all the studied accessions of *Amaranthus hypochondricus* PI 540446, PI 337611, PI 274279 and PI 477917 were clustered together. Moreover, all the studied accessions of *Amaranthus retroflexus* PI 572263 and PI 607458 were clustered together.

DISCUSSION

The genetic variability and evolutionary relationships between eight species of *Amaranthus* species were studied based on chromosome features. The studied species were *A. hybridus, A. hypochondricus, A. palmeria, A. quitensis, A. retroflexus, A. spinosus, A. powellii and A. caudatus*. These species belongs to three sections; *Amaranthus, Centrusa Griseb, Saueranthus*.

The karyotpe analysis of *Amaranthus caudatus, A. quitensis* and *A. hypochondricus* exhibited somatic chromosome number 32 (2n =32). This agrees with all of previous karyological studies on this species[58-60]. The accessions of *Amaranthus spinosus* and *A.*

Regular Paper

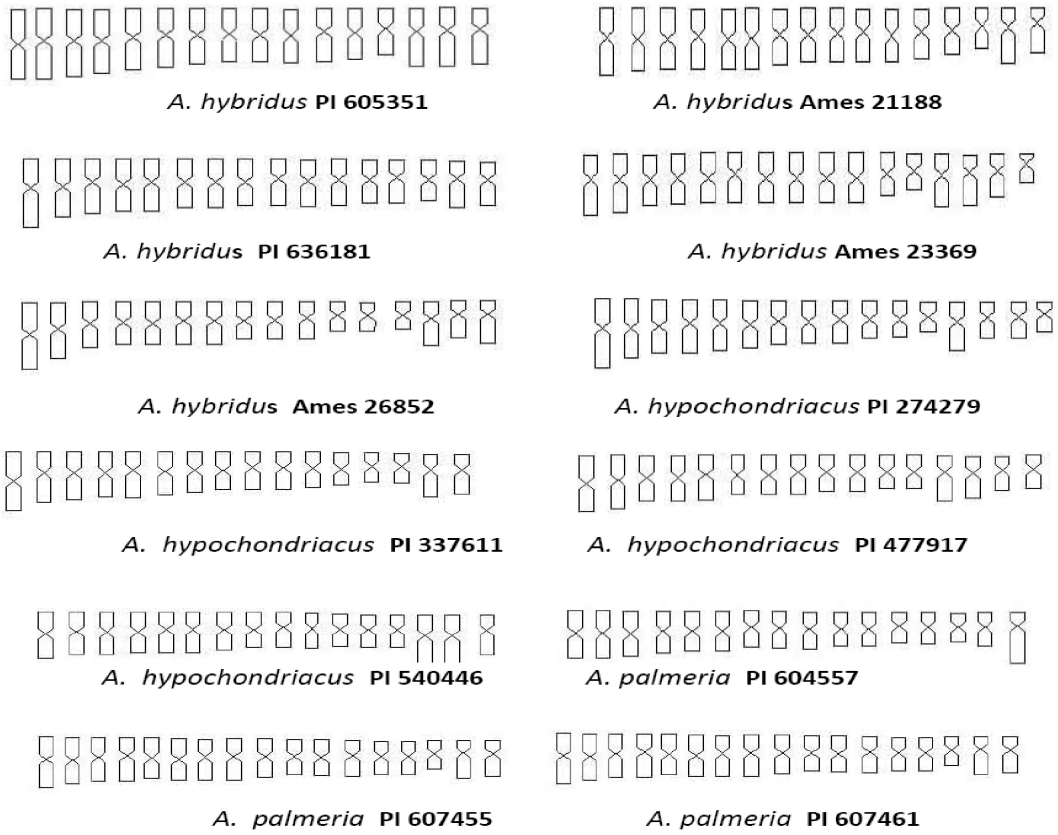

Figure 2A : The idiograms of the specifiesd accessions in *Amaranthus* species

Figure 2B : The idiograms of the specifiesd accessions in *Amaranthus* species

Regular Paper

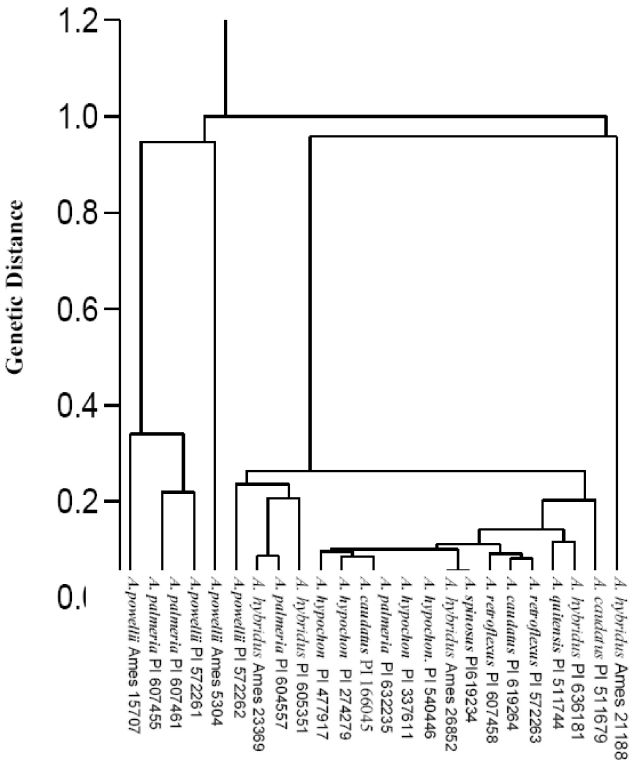

Figure 3 : Dendrogram showed the phylogenetic relationships among the studied species of *Amaranthus* using average linkage method

retroflexus had 2n=32. This data supported the opinion of Pandy[61] who reported that 2n of *Amaranthus spinosus* and *A. retroflexus* was 32 but does not agree with the results of Ge *et al.*[62-67] and Song *et al.*[44] who showed that these species may carry 2n=30 or 32 or 34. The contradication in the data between Pandy[61], and Ge *et al.*[62] and Song *et al.*[44] may be due to the variation in the origin of these accessions. On the other hand, our analysis showed that some accessions of *Amaranthus powellii* and *A. palmeria* had 2n=32 agreeing with Pandy[61] and some other had 2n=34 agreeing with Sauer[50]. This data indicated that the studied accessions cover a wide range of habitates.

Our results indicated that chromosome morphology (most chromosomes were either metacentric or submetacentric) of the studied accessions were uniform. This finding supported the conclusion of Hamoud *et al.*[43] and Song *et al.*[44], that most of species displayed uniformity in chromosome morphology. Furthermore, the karyotype analysis showed a variation in the karyotypes of different accessions of the same species. This variation may be considered of adaptive significance. It implicates that these species have some kinds of karyotypic activity, concerning chromosome length and centromeric position, recorded among its different accessions. This might have been produced as a result of differences in the degree of chromatin condensation and /or chromosomal changes such as translocations and pericentric inversions[68-71].

A. powellii could be considered the most advanced species among all the investigated taxa. It exhibited the smallest mean chromosome length with more karyotypic activity, concerning chromosome number, chromosome length and centromeric position, recorded among its different accessions. This data contradct the data of Hamoud *et al.*[43] who reported that *A. viridus* could be the most advanced species amongst *Amaranthus* species. It exhibits the diploid number 2n = 34 and the shortest haploid genome length with more Karyotypic activity, concerning chromosome length and centromeric position, recorded among its different accessions.The Mean chromosome length of *A. powellii* (0.47mm) is smaller than that of *A. viridus* (0.69)[44]. The contradiction between the data may be due to that Hammoud *et al*[44] did not include *A. powellii* in his study.

In cluster analysis of karyotypic characters of the accessions of *Amaranthus* species, two major clusters were observed. The left cluster showed that *A. powellii Ames is the* core to most of species and the secone one exhibited that *A. hybridus* Ames is the core to most of species. This finding supported partly the hypothesis of Sauer[50] of that A. hypridus and A. powelli and *Amaranthus quitensis are the progenitor of Amaranthus species*. However the *Amaranthus quitensis* was grouped in the second cluster where *A. hypridus* is the progenitor of these species.This may be due to its being an ancestor to most of other species as *A. cruentus* according to Michael[72], ancestor to *A. hypochondriacus* according to Sauer[50], Pal and Khoshoo[51], also ancestor to *A. caudatus* according to Chan and Sun[73-75].

It was noticed that accessions of some species, such as *A. hypochondriacus* and *A. retroflexus* were grouped in the same cluster. This finding was attributed to that these accessions are geographically located on the same latitude. This interpretation indicated that the variation analyzed by cluster analysis is determined not only by genetic factors but rather by environmental dif-

Regular Paper

ferences.

The dendrogram also showed that both accessions number PI607455 and PI 607461 belong to *A. palmeri* had 100% genetic similarity. This result may declare them as genetic identical duplicates. Thus, one of them could be selected for the core collection used in the breeding programs. However, this finding should be undergo further analysis for a wider range of the genome using the vast polymorphic screening molecular markers.

REFERENCES

[1] D.Brenner, D.Baltensperger, P.Kulakow, J.Lehmann, R.Myers, M.Slabbert, B.Sleugh; Plant.Breeding.Reviews, **19**, 227-285 **(2000)**.

[2] R.H.Sammour, M.A.Hamoud, A.S.Haidar; Cytologia, **56**, 289-291 **(1991)**.

[3] S.Badr, A.A.Mustafa, W.Tahr, R.H.Sammour; Cytologia, **74**, 101-111 **(2009)**.

[4] R.H.Sammour; Folia Geobotanica et Phytotaxonomica, **26**, 95-100 **(1991)**.

[5] R.H.Sammour; Feddes Repertorium, **105**, 191-196 **(1994)**.

[6] R.H.Sammour; Bot.Bull.Acad.Sci., **40**, 121-126 **(1999)**.

[7] H.Hauptli, S.Jain; Theoretical and Applied Genetics, **52**, 177–185 **(1978)**.

[8] H.Hauptli, S.Jain; Theoretical and Applied Genetics, **69**, 155-165 **(1984)**.

[9] R.H.Sammour; FABIS Newsletter, **18**, 30-32 **(1987)**.

[10] R.H.Sammour; Journal of Agronomy and Crop Science, **160**, 271-276 **(1988)**.

[11] R.H.Sammour; Plant Breeding, **104**, 196-201 **(1989)**.

[12] R.H.Sammour; Egypt J.Bot., **33**, 169-174 **(1990)**.

[13] R.H.Sammour; Bot.Bull.Acad.Sin., **38**, 171-177 **(1994)**.

[14] P.Goldblatt; Monogr.Syst.Bot.Missouri Bot.Gard., 13 **(1985)**.

[15] R.H.Sammour, A.E.Z.Mustafa, S.Badr, W.Tahr; Acta.Agric.Slovenica, **88**, 33-43 **(2007)**.

[16] R.H.Sammour, A.E.Z.Mustafa, S.Badr, W.Tahr; Acta.Bot.Croat., **66**, 1–13 **(2007)**.

[17] R.S.Sammour, S.A.Radwan, M.Mira; Research and Review of Bioscience, **6**, 351-360 **(2012)**.

[18] R.H.Sammour; Acta.Agronomica Hungarica, **55**, 131-147 **(2007)**.

[19] R.H.Sammour; Genetic Diversity and Allele Mining in Soybean Germplasm, In: Soybean, In: Dora Krezhova, (Ed); Soybean -Genetics and Novel Techniques for Yield Enhancement, In Tech, **(2011)**.

[20] P.Goldblatt; Monogr.Syst.Bot.Missouri Bot.Gard., 23 **(1988)**.

[21] P.Goldblatt, D.Johnson; Monogr. Syst.Bot.Missouri Bot.Gard., 69 **(1998)**.

[22] R.H.Sammour; Journal of Agronomy and Crop Science, **159**, 282-286 **(1987)**.

[23] R.H.Sammour; Feddes Repertorium, **105**, 283-286 **(1990)**.

[24] R.H.Sammour, M.A.Hamoud, A.S.Haidar, A.Badr; Feddes Repertorium, **104**, 251-257 **(1993)**.

[25] M.A.Karam, R.H.Sammour, M.F.Ahmed, F.M.Ashour, L.M.El-Sadek; J.Union Arab.Biol., **9**, 269-279 **(1999)**.

[26] R.H.Sammour; Thesis (Ph.D.), Ph D thesis, Tanta University, Tanta, Egypt, **(1985)**.

[27] E.Greizerstein, L.Poggio; Darwiniana, **31**, 159-165 **(1992)**.

[28] R.H.Sammour; Plant Varieties and Seeds, **12**, 11-210 **(1999)**.

[29] M.F.Ahmed, M.A.Karam, L.M.El-Sadek, R.H.Sammour; J.Fac.Sci., U.A.E.Univ., **8**, 127-144 **(1994)**.

[30] R.H.Sammour, M.A.Hamoud, S.A.A.Alla; Bot.Bull.Acad.Sin., **34**, 37-42 **(1993)**.

[31] R.H.Sammour; Turk.J.Biol., **30**, 207-215 **(2006)**.

[32] S.A.Radwan, S.Bader, M.Mira, R.H.Sammour; Acta.Botanica Hungarica, **54**, 391–408 **(2012)**.

[33] V.Gupta, S.Gudu; Euphytica, **52**, 33-38 **(1991)**.

[34] M.Pal, R.Pandey, T.Khoshoo; J.Heredity, **73**, 353-356 **(1982)**.

[35] E.Greizerstein, L.Poggio; Plant Breeding, **114**, 448-450 **(1995)**.

[36] E.Griezerstein, L.Poggio; Cytologia, **59**, 25-30 **(1994)**.

[37] V.Lanta, P.Havranek, V.Ondrej; Plant Soil.Environ., **49**, 364-369 **(2003)**.

[38] R.H.Sammour, S.A.Radwans, A.El-Koly; Seed Technology, **29**, 50-59 **(2007)**.

[39] R.H.Sammour, A.R.El-Shanoshoury; Bot.Bull. Academica Sinica, **23**, 185-190 **(1992)**.

[40] R.H.Sammour; Turk.J.Bot., **29**, 177-184 **(2005)**.

[41] A.R.El-Shanshoury, M.El-Sayed, R.H.Sammour, W.El-Shouny; Can.J.Microbiol., **41**, 99-104 **(1995)**.

[42] R.H.Sammour, A.E.Z.Mustafa; Research and Review of Bioscience, **7**, 19-26 **(2013)**.

[43] M.Hamoud, R.H.Sammour, S.Abdalla; Sci.Int.

(Lahore), **6**, 255-260 **(1994)**.
[44] Ba-Hua Song, Xue-Jie Zhang, Fa-Zeng Li, Peng Wan; Acta Phytotaxonomica Sinica, **40**, 428–432 **(2002)**.
[45] R.H.Sammour, S.Radwan, A.El-Koly; BioTechnology- An Indian Journal, **9**, 319-326 **(2014)**.
[46] R.H.Sammour; Research & Reviews in Biosciences, **8(2)**, 78-84 **(2014)**.
[47] R.H.Sammour, S.M.Abdel-Momen, E.A.Elagamey; Research & Reviews in BioSciences, **8(6)**, 228-236 **(2014)**.
[48] R.H.Sammour; Research & Reviews in BioSciences, **8(9)**, 325-336 **(2014)**.
[49] R.H.Sammour; Research & Reviews in BioSciences, **8(7)**, 277-284 **(2014)**.
[50] J.Sauer; In: N.W. Simmonds, (Ed); Evolution of crop plants. Longman Group Ltd., London, 4–7 **(1976)**.
[51] M.Pal, T.Khoshoo; In: J.B.Hutchinson, (Ed); Evolutionary studies in world crops: diversity and change in the Indian subcontinent. Cambridge University Press, UK, 129-137 **(1974)**.
[52] R.H.Sammour; Research & Reviews in BioSciences, **8(9)**, 347-358 **(2014)**.
[53] R.H.Sammour, S.M.Abdel-Momen, E.A.Elagamey; Applied Cell Biology, **2(4)**, 156-165 **(2013)**.
[54] R.H.Sammour, S.Badr, A.A.Mustafa, M.El-Esawi; Applied Cell Biology, **2(4)**, 136-143 **(2013)**.
[55] R.H.Sammour, S.A.Radwan, M.Mira; Research & Reviews in BioSciences, **6(11)**, 351-360 **(2012)**.
[56] A.Diab, S.Amin, S.Badr, J.A.da Silva Teixeira, P.Van Thanh, B.Abdelgawad, R.H.Sammour; International Journal of Plant Breeding, **6**, 14-20 **(2012)**.
[57] A.Levan, K.Fredga, A.Sandberg; Hereditas, **52**, 201-220 **(1965)**.
[58] P.Goldblatt; Monogr.Syst.Bot.Missouri Bot.Gard., 5 **(1981)**.
[59] G.Palomino, R.Ruby; In Resúmenes, Primer Congreso Internacional del Amaranto 22-27 setiembre, 24 **(1991)**.
[60] R.Sammour, M.Hamud, S.Abd Alla; Bot.Bull.Acad.Sin., **34**, 37-42 **(1993)**.
[61] R.Pandy; Genetic Resources and Crop Evolution, **46**, 219-224 **(1999)**.
[62] J.Ge, K.Li, Y.Zhou; Acta.Bot.Yunnan., **9**, 333-338 **(1987)**.
[63] R.H.Sammour A.A.Mustafa, S.Bader, W.Tahr; Acta.Agriculturae Slovennica, **88**, 33-43, 77 **(2007)**.
[64] R.H.Sammour; Russian Journal of Plant Physiology, **52**, 365–373 **(2005)**.
[65] M.A.Karam, R.H.Sammour, M.F.Ahmed, F.M.Ashour, L.M.El-Sadek; J.Union Arab.Biol., **9**, 269-279 **(1999)**
[66] R.H.Sammour, M.N.El-Shourbagy, A.M.Abo-Shady, A.M.Abasery; Arab Gulf Journal of Scientific Research, **13**, 591-604 **(1995)**.
[67] M.A.El-Haak, A.Sharaf El-Din, R.H.Sammour; Pak.J.Bot., **25**, 41-46 **(1993)**.
[68] G.Stebbins; Edward Arnold, London, **(1971)**.
[69] R.H.Sammour; DRASAT, **18B**, 54-65 **(1990)**.
[70] R.H.Sammour; PhD diss., Tanta University, Tanta, Eygpt, **(1985)**.
[71] S.Badr, W.Taher, R.H.A.Sammour; Journal of Biological **(2007)**.
[72] R.H.Sammour; African Crop Science Journal, **(2005)**
[73] K.Chan, M.Sun; Theoretical and Applied Genetics, **95**, 865-873 **(1997)**.
[74] S.Badr, W.Taher, R.H.A.Sammour; Pakistan Journal of Biological Sciences, **10**, 49-56 **(2007)**.
[75] R.H.Sammour; African Crop Science Journal, **13**, 27-39 **(2005)**.