Cytogenetic characterization of *Amaranthus caudatus* L. and *Amaranthus hybridus* subsp. *cruentus* (L.) Thell.

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Abstract The present study is aimed to identify genetic variability between two species of *Amaranthus* viz., *A. caudatus* and *A. hybridus* subsp. *cruentus*, two economically important species, cultivated mainly for grain production. Karyomorphological studies in *Amaranthus* are scarce, probably due to higher number of small sized chromosomes. Karyomorphological studies were conducted using mitotic squash preparation of young healthy root tips. Karyological parameters and karyotypic formula were established using various software programs and tabulated the karyomorphometric and asymmetry indices viz., Disparity index, Variation coefficient, Total formula percentage, Karyotype asymmetry index, Syi index, Rec index, Interchromosomal and Intrachromosomal asymmetry index and Degree of asymmetry of karyotypes. The mitotic chromosome number observed for *A. caudatus* was $2n = 32$ with a gametic number $n = 16$ and *A. hybridus* subsp. *cruentus* was $2n = 34$ with a gametic number $n = 17$. In *A. caudatus* the chromosome length during somatic metaphase ranged from 0.8698 to 1.7722 μm with a total length of 39.1412 μm . In *A. hybridus* subsp. *cruentus* the length of chromosome ranged from 0.7756 to 1.9421 μm with a total length of 44.9922 μm . Various karyomorphometry and

asymmetry indices analyzed revealed the extend of interspecific variation and their evolutionary status.

Keywords *Amaranthus caudatus* · *Amaranthus hybridus* subsp. *cruentus* · Cytogenetics · Karyomorphology

Introduction

The genus *Amaranthus* comprises about seventy herbaceous species grouped into three subgenera, they were used as vegetables, ornamentals and pseudo cereals. *Amaranthus* viz., *A. caudatus* and *A. hybridus* subsp. *cruentus* are two economically important amaranth species, cultivated mainly for grain production therefore named as “pseudo-cereals”. In terms of nutrition, the grain amaranths are characterized by rich fibre and mineral content and a balanced amino acid profile, which is better than most of the known and heavily utilized cereals and other food grains (Bressani 1989; Tucker 1986). Grain *Amaranthus* is produced commercially in hot and dry areas of the United States, Argentina and China (Sammour et al. 2012). Updated data have indicated that there are two basic chromosome numbers such as $x = 8$ and 9 in the family Amaranthaceae (Grant 1959a, b, c). The genus *Amaranthus* is tribasic, because three gametic numbers viz., $n = 14$, 16, and 17 were reported previously. The gametic number $n = 17$ has evolved from $n = 16$

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through primary trisomy (Pal et al. 1982). The new gametic number $n = 14$ was reported previously by Pal et al. (2000) in *A. tenuifolius* ($2n = 28$). This has added a new basic number ($x = 14$) in the list of genus *Amaranthus*. The reports of Pal et al. (2000) was confirmed by a recent cytogenetic study in *A. blitum* ($2n = 28$) by Srivastava and Roy (2014). The reduction in chromosome number from $n = 16$ to $n = 14$ was suggested to be due to aneuploid or dysploid reduction.

The taxonomy of the genus *Amaranthus* has been confused by the extremely used range of phenotypic plasticity among species and the possible introgression and hybridization involving weedy and crop species (Hauptli and Jain 1984). Much of the difficulty in taxonomic discrimination of species within the group may be attributed to attempts at recognizing taxa based on pigmentation or growth forms, which are extremely variable within *Amaranthus* (Sauer 1967). Taxonomic characterization is very difficult because of the occurrence of many hybrids and broad geographical distribution, which is the reason for many synonyms. Cytogenetic analysis is usually performed to clarify systematic and evolutionary aspects, which offer added information to disputed taxonomic families. It helps for the karyological characterization of the species, increase the knowledge of genetic resources and explore evolutionary trends. To explore the genetic system of any organism, a precise knowledge of its karyotype is essential. The genus *Amaranthus* is characterized by small sized chromosomes with indistinguishable secondary constriction, satellites etc. This fact has so far restricted the cytogenetical studies of this crop. Taxonomic delimitation and application of nomenclature in amaranth are still very tentative for its morphological variability and frequent hybridization. Cytogenetics employs number and morphology of chromosomes as the major distinctive characters. A comparative analysis helps in establishing both unique and common cytogenetic features of the species studied.

Apart from a cytogenetic study (Kolano et al. 2001), detailed karyomorphometric data of *Amaranthus* species was not available which is therefore attempted here. The present study is also aimed to reveal the genetic variability and evolutionary status of the species, which are indices of survival of a species to changing environmental conditions.

Materials and methods

The studies were conducted using two species of *Amaranthus* viz., *A. caudatus* L. and *A. hybridus* subsp. *cruentus* (L.) Thell. collected from Malappuram district of Kerala state.

Karyomorphological studies were conducted using mitotic squash preparation of young healthy root tips. The basic chromosome numbers were analysed cytologically by acetoorcein staining. Young healthy twig of the plant was allowed to germinate in autoclaved sand. The germinated roots were collected at peak mitotic period and were subjected to pretreatment in cold paradicholoro benzene (PDB) for $1\frac{1}{2}$ h. The pretreated root tips were washed thoroughly in distilled water and fixed in modified Carnoy's fluid for 1 h. Fixed root tips were subjected to hydrolysis in 1 N HCl for 1–10 min. After thorough washing in distilled water, the roots were stained with acetoorcein for 3 h. Micro slides were prepared, screened and photomicrographs were taken with the help of AmScope MU Series digital camera attached to the Olympus CX21 microscope. Computer based programs such as Adobe photoshop, AutoCAD and Microsoft Excel were used to determine various parameters as well as for karyogram and idiogram preparation.

The well spread mitotic metaphase chromosomes were subjected to analyze various parameters. On the basis of length, arm ratio and centromeric indices, homologous chromosome pairs were identified and were classified according to Abraham and Prasad (1983). Karyotypic formula was determined based on the number of chromosome and position of centromere. The karyograms and idiograms were drawn and arranged based on chromosome size in decreasing order. Various karyomorphometric and asymmetry indices calculated includes Disparity index (DI) (Mohanty et al. 1991), Variation coefficient (VC) (Verma 1980), Total forma percentage (TF%) (Huziwar 1962), Karyotype asymmetry index (As K%) (Arno 1963), Syi index (Greilhuber and Speta 1976), Rec index (Venora et al. 2002), Intrachromosomal asymmetry index (A1) and Interchromosomal asymmetry index (A2) (Zarco 1986) and Degree of asymmetry of karyotypes (A) (Watanabe et al. 1999).

Results and discussion

The mitotic squash preparation of both the species led to the disclosure of their karyological identity (Fig. 1). The mitotic metaphase chromosome number observed in the present study for *A. caudatus* was $2n = 32$ with a gametic chromosome number of $n = 16$ and for *A. hybridus* subsp. *cruentus* was $2n = 34$ ($n = 17$). The karyotype formula of *A. caudatus* and *A. hybridus* subsp. *cruentus* drawn from the karyomorphometric data was 32 nm and $30 \text{ nm} + 4 \text{ nsm} (-)$ respectively. The karyotypes of the studied species had a predominance of nearly median chromosomes but 4 chromosomes of *A. hybridus* subsp. *cruentus* shown nearly sub median centromere. The karyotype analysis showed slight variations in karyotype formulae and asymmetry indices. Detailed data pertaining to the variations in karyotype and asymmetry indices between the species studied are mentioned in Table 1.

Fig. 1 Normal chromosome complement images of *A. caudatus* and *A. hybridus* subsp. *cruentus*. **A** *A. caudatus* cell at mitotic metaphase with $2n = 32$ chromosomes; **B** *A. hybridus* subsp. *cruentus* cell at mitotic metaphase with $2n = 34$ chromosomes. Bar 5 μm

The karyogram and idiogram drawn based on chromosome size and position of centromere for both the species are represented in Figs. 2 and 3, respectively.

Both species possess chromosomes with small size, of which *A. hybridus* subsp. *cruentus* shows slightly higher value (1.9421) for the largest chromosome. The range of chromosome length observed for *A. caudatus* was 0.8698–1.7722 μm , summing up to a total of 39.1412 μm . The range of chromosome length for *A. hybridus* subsp. *cruentus* was 0.7756–1.9421 μm , summing up to a total of 44.9922 μm . The total length of each chromosome, length of long arm, short arm, arm ratio, centromere index and position of centromere observed for both the species are mentioned in Tables 2 and 3, respectively. The number of secondary constriction was in equity for both species. Secondary constriction was observed in the first two pairs of chromosomes in both species. The study carried out on the chromosome morphology of the species studied have indicated similarity in number of chromosome pairs with secondary constriction and variations in the chromosome pairs with primary constriction. According to the present observation *A. caudatus* with $2n = 32$, *A. hybridus* subsp. *cruentus* with $2n = 34$ chromosome showed slight variations in karyotype formula and asymmetry index. The various parameters of DI, VC, TF%, As K%, Syi and Rec calculated for *A. caudatus* were found to be 34.15, 21.52, 44.14, 56.02, 78.78 and 69.01. In the case of *A. hybridus* subsp. *cruentus*, they were 42.92, 22.81, 42.00, 57.99, 72.44 and 68.14, respectively. The variation in karyotype of different species could be brought about by dysploidy, pericentric inversion, deletion, unequal translocation, spontaneous mutation, interspecific hybridization etc. An important and well recognized component in the evolutionary history of *Amaranthus* species is interspecific hybridization. Interspecific gene flow may lead to chromosomal evolution by chromosomal rearrangement. This hybridization has been proposed as a critical stimulus for invasiveness and it perhaps aids in the evolution of adaptations critical for the success of *Amaranthus* as weeds. Cytogenetic studies already revealed the paleo allotetraploid nature of several *Amaranthus* species. There are several groups of angiosperms having variable chromosome numbers as a result of dysploidy/aneuploidy. *Amaranthus* is one such group of plant. Dysploidy is a process by which euchromatin on

Table 1 A comparison of karyotype and asymmetry indices of the species studied

Sl. No.	Karyotype and asymmetry indices	<i>A. caudatus</i>	<i>A. hybridus</i> subsp. <i>cruentus</i>
1	Chromosome number	2n = 32 (n = 16)	2n = 34 (n = 17)
2	Karyotype formula	32 nm	30 nm + 4 nsm (–)
3	Chromosome with secondary constriction	4	4
4	Range of chromosome length (RCL)	0.8698–1.7722 μm	0.7756–1.9421 μm
5	Total chromosome length (TCL)	39.1412 μm	44.9922 μm
6	Average chromosome length (ACL)	1.2231 μm	1.3233 μm
7	Disparity index (DI)	34.15	42.92
8	Variation coefficient (VC)	21.52	22.81
9	TF value (%)	44.14	42.00
10	Karyotype asymmetry index (As K%)	56.02	57.99
11	Syi index	78.78	72.44
12	Rec index	69.01	68.14
13	Intrachromosomal asymmetry index (A1)	0.95	0.97
14	Interchromosomal asymmetry index (A2)	0.21	0.22
15	Degree of asymmetry of karyotypes (A)	0.11	0.15

Fig. 2 Karyogram and idiogram of *A. caudatus* with 2n = 32 chromosomes

Fig. 3 Karyogram and idiogram of *A. hybridus* subsp. *cruentus* with $2n = 34$ chromosomes

a genome is rearranged by translocation on to a greater or lesser number of centromeres, which is evidenced in morphology of chromosomes, whole chromosome loss at diploid level in high number. It is assumed that dysploidy reduction from relatively high chromosome number has taken place in *Amaranthus* due to repeated chromosome manipulation during hybridization. Extensive dysploidy reduction from high primitive chromosome number to lower chromosome number species associated with derived morphological and ecological variations may represent most common cytogenetic evolutionary pattern in angiosperms. *Amaranthus* represent a genus that shows great variability in chromosome number and ploidy level.

As a whole, symmetric karyotypes are usually considered as primitive and asymmetrical as advanced. The presence of nearly median centromere in all the chromosomes of *A. caudatus* is indicative of its primitiveness. The substantial information about a species is more precisely obtained from chromosome number which makes it an important cytotaxonomic

parameter (Anil et al. 2013). Karyosystematics is regarded as one of the most relevant parameter in the evaluation of the genetic relationship and divergence among species or populations (Guerra 2008). Karyotype asymmetry can be considered to be the driving force behind speciation since symmetrical karyotypes represents ancestral condition in evolution (Stebbins 1971). Various parameters determining asymmetry of karyotype were used in the present study. The divergence and evolution in higher plants have been related to the variations in morphological characters of the genome (Zarco 1986). DI determines the asymmetry of karyotype based on the variations in the size of chromosome. The homogeneity of chromosome is depicted by low DI value, while high DI value indicates the heterogenous assemblage. Based on the DI value, the heterogenous assemblage of chromosomes were more prominent in *A. hybridus* subsp. *cruentus*. VC is a similar parameter as DI, which well determines the variations in chromosome size and distribution. Among the two species studied

Table 2 Detailed karyomorphometric data of *A. caudatus* with $2n = 32$ chromosomes

No. of Chr.	c (μm)	s (μm)	l (μm)	R_1 (s/l)	R_2 (l/s)	I_1 (s/c %)	I_2 (l/c %)	Nature of PC
2 ^a	1.7722	0.7337	1.0385	0.7064	1.4154	41.4005	58.5994	nm
2 ^a	1.6967	0.7227	0.974	0.7419	1.3477	42.5944	57.4055	nm
2	1.4545	0.6806	0.7739	0.8794	1.137	46.7927	53.2072	nm
2	1.3602	0.5804	0.7798	0.7442	1.3435	42.6701	57.3298	nm
2	1.3561	0.61	0.7461	0.8175	1.2231	44.9819	55.018	nm
2	1.3428	0.6234	0.7194	0.8665	1.1539	46.4253	53.5746	nm
2	1.2677	0.5392	0.7285	0.7401	1.351	42.5337	57.4662	nm
2	1.1951	0.5016	0.6935	0.7232	1.3825	41.9713	58.0286	nm
2	1.1731	0.4546	0.7185	0.6327	1.5805	38.752	61.2479	nm
2	1.0698	0.4597	0.6101	0.7534	1.3271	42.9706	57.0293	nm
2	1.0679	0.4893	0.5786	0.8456	1.1825	45.8188	54.1811	nm
2	1.0463	0.4894	0.5569	0.8787	1.1379	46.7743	53.2256	nm
2	0.993	0.4683	0.5247	0.8925	1.1204	47.1601	52.8398	nm
2	0.9774	0.4111	0.5663	0.7259	1.3775	42.0605	57.9394	nm
2	0.928	0.4626	0.4993	0.9264	1.0793	49.8491	53.8038	nm
2	0.8698	0.4126	0.4572	0.9024	1.108	47.4361	52.5638	nm

Chr. chromosome, *c* total length of chromosome, *l* long arm length, *s* short arm length, R_1 arm ratio 1, R_2 arm ratio 2, I_1 centromeric index 1, I_2 centromeric index 2, *PC* primary constriction, *nm* nearly median

^a Chromosome with secondary constriction

Table 3 Detailed karyomorphometric data of *A. hybridus* subsp. *cruentus* with $2n = 34$ chromosomes

No. of Chr.	c (μm)	s (μm)	l (μm)	R_1 (s/l)	R_2 (l/s)	I_1 (s/c %)	I_2 (l/c %)	Nature of PC
2 ^a	1.9421	0.7742	1.1679	0.6628	1.5085	39.864	60.1359	nm
2 ^a	1.861	0.7284	1.1326	0.6431	1.5549	39.1402	60.8597	nm
2	1.6637	0.7252	0.9382	0.7729	1.2937	43.5895	56.3923	nm
2	1.5104	0.5496	0.9607	0.572	1.7479	36.3877	63.6056	nsm (–)
2	1.4553	0.6063	0.849	0.7141	1.4	41.6615	58.3384	nm
2	1.4083	0.615	0.7932	0.7753	1.289	43.6696	56.3232	nm
2	1.3747	0.6092	0.7655	0.7958	1.256	44.3151	55.6848	nm
2	1.332	0.6416	0.6904	0.9293	1.076	48.1681	51.8318	nm
2	1.2706	0.513	0.7576	0.677	1.4768	40.3746	59.6253	nm
2	1.2185	0.5211	0.6974	0.7472	1.3383	42.7656	57.2343	nm
2	1.2033	0.5233	0.6799	0.7696	1.2992	43.4887	56.5029	nm
2	1.1575	0.4934	0.6641	0.7429	1.3459	42.6263	57.3736	nm
2	1.1401	0.3808	0.7593	0.5015	1.9939	33.4005	66.5994	nsm (–)
2	1.1318	0.4566	0.6752	0.6762	1.4787	40.3428	59.6571	nm
2	1.0757	0.4934	0.5822	0.8474	1.1799	45.8678	54.1228	nm
2	0.9755	0.4578	0.5177	0.8842	1.1308	46.9297	53.0702	nm
2	0.7756	0.3608	0.4147	0.87	1.1493	46.5188	53.4682	nm

Chr. chromosome, *c* total length of chromosome, *l* long arm length, *s* short arm length, R_1 arm ratio 1, R_2 arm ratio 2, I_1 centromeric index 1, I_2 centromeric index 2, *PC* primary constriction, *nm* nearly median, *nsm* (–) nearly submedian

^a Chromosome with secondary constriction

A. caudatus showed low VC value (21.52), which reflects the homogeneity and primitiveness of the karyotype. Lower TF% value of *A. hybridus* subsp. *cruentus* (42.00) indicates its asymmetric karyotype. As K% value was almost similar in both species (56.02 and 57.99). Higher values of As K% is a sign of increasing asymmetry of karyotype. The A1–A2 and A values also indicated the asymmetric nature of *A. hybridus* subsp. *cruentus*.

A comparison between the normal chromosome complement of two species of *Amaranthus* based on karyomorphometric and asymmetry indices revealed that *A. hybridus* subsp. *cruentus* possess asymmetric karyotype. According to the asymmetry indices and centromeric position, *A. hybridus* subsp. *cruentus* was found to be the evolved among the two species of *Amaranthus* studied.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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