Genetic diversity and relationships among some wild and cultivated species of *Chenopodium* L. (Amaranthaceae) using RAPD and DAMD methods

T. S. Rana[1], Diganta Narzary[1] and Deepak Ohri[2,*]

[1]Conservation Biology and Molecular Taxonomy and
[2]Genetics and Plant Breeding Laboratories, National Botanical Research Institute, Rana Pratap Marg, Lucknow 226 001, India

Genetic relationships in 55 accessions belonging to 14 species of *Chenopodium* have been studied by RAPD and DAMD markers. A UPGMA dendrogram based on 242 DNA markers divided the taxa into two main clusters. The first cluster joins all the accessions of *Chenopodium quinoa* with *Chenopodium berlandieri* subsp. *nuttalliae*, one *Chenopodium album* (4*x*) from Mexico and three north Indian 2*x* accessions of *C. album*. The other clusters comprises mainly 6*x* accessions of *C. album* and *Chenopodium giganteum* forming two subclusters. This clearly shows that *C. album* complex is a heterogenous assemblage and its taxonomic affinities need reassessment. Other wild species placed in the dendrogram are more or less according to their taxonomic position.

Keywords: *Chenopodium*, DAMD, genetic relationships, RAPD, molecular markers, wild and cultivated species.

CHENOPODIUM comprises about 250 species, which are herbaceous, suffrutescent and arborescent perennials[1] and belongs to the family Amaranthaceae (syn. Chenopodiaceae)[2]. The genus is economically important because many species e.g. *Chenopodium quinoa*, *Chenopodium berlandieri* subsp. *nuttalliae*, *Chenopodium pallidicaule*, *Chenopodium album* and *Chenopodium giganteum* have a long history of domestication as grain, vegetable or forage crops[3]. Among these, *C. quinoa* is a high protein pseudocereal and used as staple grain grown in South America, though recently its cultivation is spreading to many other parts of the world[4]. Both *C. quinoa* and *C. berlandieri* subsp. *nuttalliae* are allotetraploids ($2n = 36$)[5–8], though studies with regard to their exact genomic constitution, mode of origin and phylogenetic relationships with other related wild species are at a preliminary stage and many questions need to be explained[9]. Furthermore, *C. album* and *C. giganteum* show great morphological and cytological diversity as the former is represented by 2*x*, 4*x* and 6*x* and latter by only 6*x* types[8,10,11]. Previous attempts to study diversity and phylogenetic relationships between cultivated and wild taxa have been based on karyotypic analysis[6,8], allozymes[9,12–14], crossability relationships[13,15], flavonoids[16], random amplified polymorphic DNA (RAPD) studies[17,18], microsatellite markers[19] and ribosomal RNA genes[20]. However, these studies are based on very less number of species and their accessions. In this respect, the present study makes use of RAPD[21,22] and directed amplification of minisatellite DNA (DAMD)[23] markers to unravel the diversity and genetic relationships in 55 accessions of 14 species of *Chenopodium*.

The details of taxa studied are given in Tables 1 and 2. DNA was isolated from fresh young leaves collected from the germplasm plot maintained at Botanic Garden of National Botanical Research Institute, Lucknow using the cetyltrimethylammonium bromide (CTAB) method described by Doyle and Doyle[24]. DNA concentrations were estimated by gel electrophoresis on 0.8% agarose, staining with ethidium bromide and comparison with a set of concentration standards.

The RAPD primers were procured from Operon Tech. Inc., Alameda, CA, USA. Totally 60 RAPD primers were screened (B, N and U kits) and 12 primers (Table 3) that generated polymorphic profiles were selected for scoring data for all the accessions in the present study. All RAPD reactions were carried out in 25 µl volumes and contained 25 ng of template DNA, 10 pmoles of RAPD primer, 200 µM each dNTP, 2.5 mM Mg^{2+} ion concentration in suitable 1× assay buffer supplied along with the enzyme and 0.5 units of the thermostable *Taq* DNA polymerase (Bangalore Genei, Bangalore, India). The amplification of DNA was performed on a PTC-200™ (MJ Research, Inc. USA) thermocycler, which was programmed to include pre-denaturation at 94°C for 1 min, followed by 45 cycles of denaturation at 94°C for 1 min, annealing at 35°C for 1 min and extension at 72°C for 1 min. The final cycle allowed an additional 5 min period of extension at 72°C. The amplified products were separated on 1.5% agarose gel in 1× TBE buffer at constant voltage of 5 V/cm. After electrophoresis, the gel was stained in ethidium bromide and then visualized and photographed on a UV transilluminator using a Gel Documentation System (UV Tech, UK).

The minisatellite core sequence primers were custom synthesized from Bangalore Genei and DAMD reactions were carried out according to Zhou *et al.*[25]. The reaction mixture (25 µl) contained 10 mM Tris–HCl (pH8.3), 50 mM KCl, 2 mM Mg^{2+} ion concentration, 200 µM each dNTP, 50 pmoles primer, 1 unit *Taq* polymerase (Bangalore Genei) and approximately 60 ng genomic DNA. Optimal DNA amplification was obtained through 40 cycles (92°C for 1 min, 55°C for 2 min and 72°C for 2 min). The amplification of DNA was performed on a PTC-200™ thermocycler. The amplified products were separated on 1.5% agarose gel in 1× TBE buffer at constant voltage of 5 V/cm. After electrophoresis, the gel was

*For correspondence. (e-mail: ohri_deepak@rediffmail.com)

Table 1. *Chenopodium* taxa used in the present study

Sl no.	Taxon	2*n*	Accession code	Source
1	*Chenopodium album* L.	36	Chandigarh	Chandigarh, India
2	*Chenopodium album* L. 'Mexico'	36	Mexico	Mexico
3	*Chenopodium album* L.	54	CHEN 95/97	Gatersleben, Germany
4	*Chenopodium album* L.	18	Local	Lucknow, India
5	*Chenopodium album* L.	54	CHEN 85/82	Gatersleben, Germany
6	*Chenopodium album* L.	54	Czech	Czech Republic
7	*Chenopodium album* L.	54	CHEN 60/76	Gatersleben, Germany
8	*Chenopodium album* L. 'Michigan'	54	PI 605700	U.S.D.A.
9	*Chenopodium album* L. 'Chandanbathua'	18	Local	Lucknow, India
10	*Chenopodium album* L. 'Siliguri'	18	Siliguri	Siliguri, India
11	*Chenopodium album* L.	54	Local	Lucknow, India
12	*Chenopodium giganteum* D. Don	54	CHEN 46/85	Gatersleben, Germany
13	*Chenopodium giganteum* D. Don	54	PI 596371	U.S.D.A.
14	*Chenopodium giganteum* D. Don	54	PI 596372	U.S.D.A.
15	*Chenopodium giganteum* D. Don	54	Ames 86650	U.S.D.A.
16	*Chenopodium giganteum* D. Don	54	CHEN 86/85	Gatersleben, Germany
17	*Chenopodium quinoa* Willd.	36	Ames 21909	U.S.D.A.
18	*Chenopodium quinoa* Willd.	36	CHEN 33/84	Gatersleben, Germany
19	*Chenopodium quinoa* Willd.	36	PI 510536	U.S.D.A.
20	*Chenopodium quinoa* Willd.	36	PI 510532	U.S.D.A.
21	*Chenopodium quinoa* Willd.	36	PI 614883	U.S.D.A.
22	*Chenopodium quinoa* Willd.	36	CHEN 92/91	U.S.D.A.
23	*Chenopodium quinoa* Willd.	36	PI 510537	U.S.D.A.
24	*Chenopodium quinoa* Willd.	36	CHEN 58/77	Gatersleben, Germany
25	*Chenopodium quinoa* Willd.	36	PI 587173	U.S.D.A.
26	*Chenopodium quinoa* Willd.	36	Ames 13762	U.S.D.A.
27	*Chenopodium quinoa* Willd.	36	PI 596498	U.S.D.A.
28	*Chenopodium quinoa* Willd.	36	Ames 13719	U.S.D.A.
29	*Chenopodium quinoa* Willd.	36	Ames 13219	U.S.D.A.
30	*Chenopodium quinoa* Willd.	36	PI 478414	U.S.D.A.
31	*Chenopodium quinoa* Willd.	36	CHEN 84/79	Gatersleben, Germany
32	*Chenopodium quinoa* Willd.	36	PI 478414	U.S.D.A.
33	*Chenopodium quinoa* Willd.	36	PI 584524	U.S.D.A.
34	*Chenopodium quinoa* Willd.	36	Ames 22158	U.S.D.A.
35	*Chenopodium quinoa* Willd.	36	Ames 22156	U.S.D.A.
36	*Chenopodium quinoa* Willd.	36	PI 614881	U.S.D.A.
37	*Chenopodium quinoa* Willd.	36	PI 433232	U.S.D.A.
38	*Chenopodium quinoa* Willd.	36	CHEN 7/81	Gatersleben, Germany
39	*Chenopodium quinoa* Willd.	36	PI 510537	U.S.D.A.
40	*Chenopodium berlandieri* ssp. *nuttalliae* (Saff.) Wilson & Heiser	36	PI 568156	U.S.D.A.
41	*Chenopodium berlandieri* ssp. *nuttalliae* (Saff.) Wilson & Heiser	36	PI 568155	U.S.D.A.
42	*Chenopodium ugandae* (Aell.) Aell.	36	CHEN 77/78	Gatersleben, Germany
43	*Chenopodium giganteum* D. Don	54	H.P.	H.P., India
44	*Chenopodium giganteum* D. Don	54	IC 107297	N.B.P.G.R., India
45	*Chenopodium giganteum* D. Don	54	PRC 9862	N.B.P.G.R., India
46	*Chenopodium giganteum* D. Don	54	H.P.	H.P., India
47	*Chenopodium opulifolium* Schrad. ex DC	54	CHEN 43/96	Gatersleben, Germany
48	*Chenopodium ficifolium* Smith	18	CHEN 42/78	Gatersleben, Germany
49	*Chenopodium vulvaria* L.	18	CHEN 46/75	Gatersleben, Germany
50	*Chenopodium pallidicaule* Aellen	18	PI 510526	U.S.D.A.
51	*Chenopodium strictum* Roth.	54	CHEN 47/79	Gatersleben, Germany
52	*Chenopodium botrys* L.	18	CHEN 94/96	Gatersleben, Germany
53	*Chenopodium bushianum* Allen	54	Ames 22376	U.S.D.A.
54	*Chenopodium murale* L.	18	Local	Lucknow, India
55	*Chenopodium foetidum* Lam.	18	CHEN 19/75	Gatersleben, Germany
56	*Amaranthus viridis* L.		Out group	Lucknow, India

Chromosome number 2*n* = 18 are diploid (2*x*), 2*n* = 36 are tetraploid (4*x*), and 2*n* = 56 are hexaploid (6*x*).

stained in ethidium bromide and then visualized and photographed as described here for RAPD analysis.

Data were scored as discrete variables, using '1' to indicate presence and '0' to indicate absence of a band.

A pairwise matrix of distances between genotypes was determined for the band data from each method using Jaccard's similarity coefficient in the FreeTree program[26]. From the pairwise distance data, the UPGMA trees were computed after allowing a 500 replicate bootstrap test using the same program. The trees were viewed, annotated and printed using Tree View (ver. 1.6.5)[27].

The suitability and reliability of two PCR methods were assessed for understanding the molecular diversity in 55 taxa of *Chenopodium* using *Amaranthus viridis* as an outgroup (Table 1). Sixty RAPD and four DAMD primers were screened to amplify genomic DNA out of which 12 RAPD and four DAMD primers generated polymorphic, reproducible and scorable bands after careful optimization of the PCR conditions.

A total of 242 polymorphic markers were generated from the 12 random primers yielding optimum RAPD profiles while four DAMD primers resulted in 107 polymorphic bands (Table 3, Figure 1). The number of bands per primer varied from 16 to 23 in case of RAPD and 25 to 31 in case of DAMD (Table 3). Cluster analysis generated a UPGMA tree for the combined data (Figure 2). The pairwise similarity as well as distances were calculated for the combined band data based on Jaccard's similarity coefficients by UPGMA method (data not shown).

This dendrogram divides the taxa studied in two major clusters while some taxa appear as sister groups. The first cluster comprises mainly the various accessions of *C. quinoa* and its related species, i.e. *C. berlandieri* subsp. *nuttalliae*, a taxon belonging to *C. album* (4*x*) from Mexico and three 2*x* types of *C. album* from North Indian Plains (Figure 2). The second cluster includes various taxa considered under *C. album* and *C. giganteum* in addition to *C. strictum*, *C. bushianum*, *C. opulifolium* and *C. ficifolium*. Certain other wild species, i.e. *C. ugandae*, *C. botrys*, *C. foetidum*, *C. pallidicaule*, *C. murale* and *C. vulvaria* are present as separate branches in the tree (Figure 2).

All the accessions of *C. quinoa* studied form a major cluster which is divided into two subgroups. Twenty two accessions show a maximum homology of 85.1% between CHEN 92/91 and PI 587173 and a minimum of 51.1% between CHEN 33/84 and PI 22156 excluding PI510532, which did not show any affinity with the rest of the *C. quinoa* accessions. In the first group comprising 10 accessions, the minimum and maximum homology ranges between 58.0% (PI 510536 and PI 614883) and 85.1% (CHEN 92/91 and PI 587173) while in the second subgroup comprising 11 accessions, this ranges between 62.5% (PI 13219 and CHEN 7/81) and 81.1% (CHEN 84/79 and PI 478414). Such a structured pattern based on low level of genetic differentiation (where 178 out of a total of 350 RAPD and DAMD markers are shared by 21 accessions studied presently) is consistent with predominantly autogamous nature[28–32]. These *quinoa* accessions have also been shown to possess small but concrete dif-

Table 2. *Chenopodium* taxa with subgeneric classification used in the present study

Taxonomic hierarchy	Reference
subg. *Chenopodium*	39
sect. *Chenopodium*	
subsect. *Chenopodium*	
C. album L.	
C. giganteum D. Don	
C. opulifolium Schrad. ex DC.	
C. vulvaria L.	
C. strictum Roth.	
C. pallidicaule Aellen	
subg. *Chenopodium*	39
sect. *Chenopodium*	
subsect. *Favosa*	
C. quinoa Willd.	
C. berlandieri ssp. *nuttalliae* (Staff.) Wilson & Heiser	
C. ficifolium Smith	
C. bushianum Allen	
subg. *Chenopodium*	39
sect. *Chenopodium*	
subsect. *Undata*	
C. murale L.	
subg. *Ambrosia* Scott (1978)	
sect. *Botryoides*	
subsect. *Botrys*	
C. botrys L.	
C. foetidum Lam.	

Table 3. RAPD and DAMD primers used for PCR profiling of *Chenopodium* species DNAs and the extent of polymorphism detected with these primers

Primer	Sequence (5′–3′)	No. of amplified loci	Polymorphic loci (percentage)
RAPD			
OP-B08	GTCCACACAG	16	16 (100)
OP-B19	ACCCCCGAAG	23	23 (100)
OP-N01	CTCACGTTGG	23	23 (100)
OP-N02	ACCAGGGGCA	22	22 (100)
OP-N09	TGCCGGCTTG	23	23 (100)
OP-N10	ACAACTGGGG	18	18 (100)
OP-N16	AAGCGACCTG	21	21 (100)
OP-U08	GGCGAAGGTT	23	23 (100)
OP-U11	AGACCAGAG	19	19 (100)
OP-U12	TCACCAGCCA	16	16 (100)
OP-U13	GGCTGGTTCC	18	18 (100)
OP-U16	CTGGCGTGGA	20	20 (100)
	Total	242	242 (100)
DAMD			
M13	GAGGGTGGCGGTTCCT	26	26 (100)
33.6	GGAGGTTTTTCA	25	25 (100)
HBV	GGTGTAGAGAGAGGGGT	31	31 (100)
HVR	CCTCCTCCCTCCT	26	25 (96.15)
	Total	108	107 (99.07)

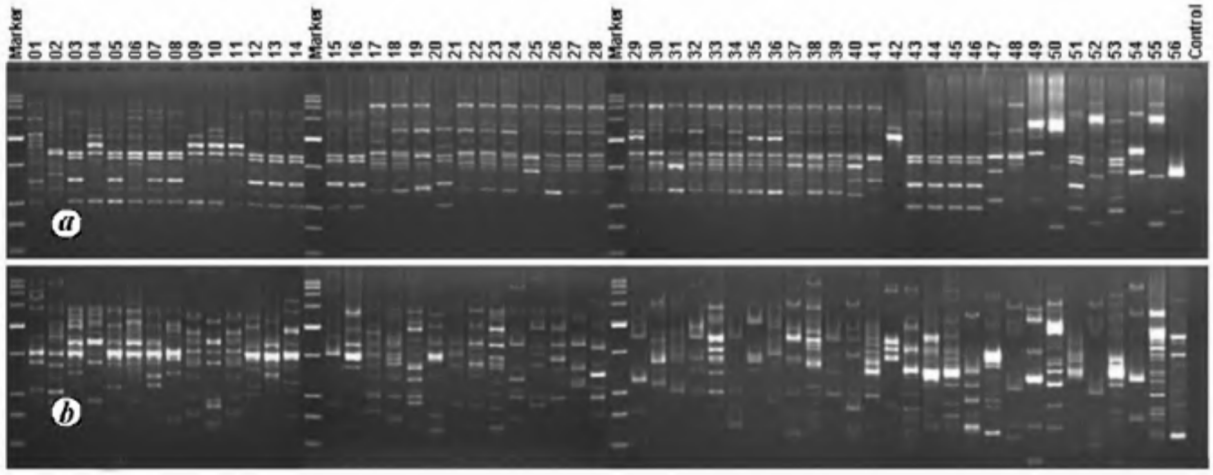

Figure 1. Gel profiles of the *Chenopodium* accessions amplified with RAPD primer OP-N09 (***a***) and DAMD primer HBV (***b***). The lanes indicated by 'Marker' contain low range DNA ruler as molecular weight marker and the lane 'Control' is negative control without adding template DNA in the PCR reaction. The other lanes are marked with the accession numbers as in Table 1. All profiles were resolved in 1.5% agarose gels in 0.5*x* TBE at constant voltage.

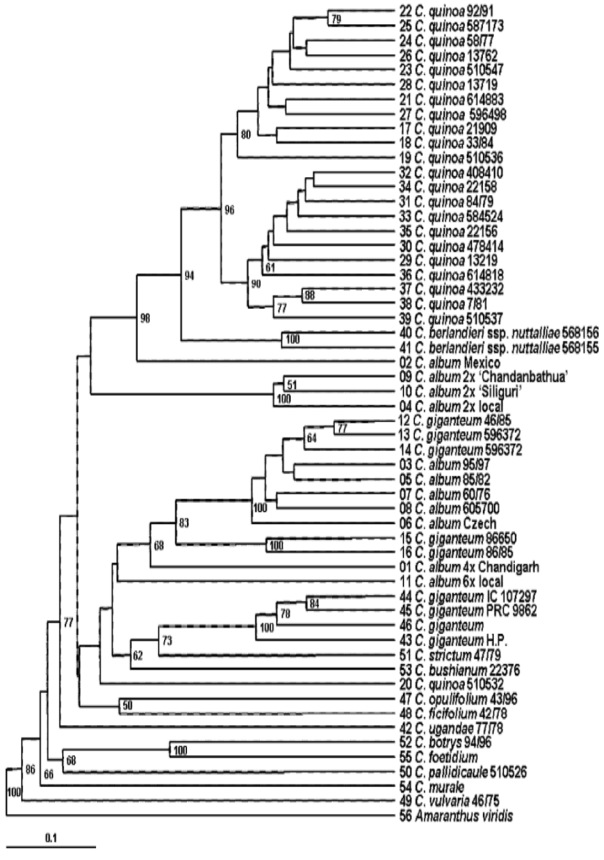

Figure 2. Cluster analysis of the combined RAPD and DAMD data. The UPGMA dendrogram was generated for the cumulative band after a 500 replicate bootstrap test. The accession names are abbreviated as in Table 1, and are indicated to the right of each branch. The numbers at the nodes are the bootstrap per cent values for the branches to the right of the node.

ferences with respect to morphological and quality traits, seed protein profiles, karyotypic features and inflorescence types[8,10,32,33]. Furthermore, no genetic differentiation has been observed with regard to the light and black seeded accessions as black seeded accessions (PI 510547, PI 510536, PI 478414 and PI 510537) are included in both the subgroups with no clear separation between these two types (Figure 2). Other studies based on RAPDs[17], field populations[34], and allozyme and morphometric analysis of pure populations of cultivated and weedy forms[9,12,35–37] of *quinoa* have also shown very low levels of differentiation. In fact *C. quinoa* has been mainly divided into the highland Andean and coastal Chilean ecotypes which show closer affinity to the free living wild types of their respective geographical regions than with each other thereby suggesting that Andean *quinoa* crop/weed complex is a monophyletic coevolving unit[9]. This major separation into two ecotypes, i.e. Andean and coastal Chilean, has been confirmed from microsatellite markers[19], IGS sequence polymorphism[20], AFLP markers[7] and SNPs[38].

Two accessions of *C. berlandieri* subsp. *nuttalliae* show 74.6% similarity with each other and form a sister group to *C. quinoa* accessions (Figure 2), which is in accordance with taxonomic grouping of these two species (Table 2)[39]. These two accessions of *C. berlandieri* subsp. *nuttalliae*, however, show genetic similarity with *C. quinoa* accessions ranging from 46.4% (between *C. berlandieri* subsp. *nuttalliae* PI 568156 and *C. quinoa* PI 21909) and 62.8% (between *C. berlandieri* subsp. *nuttalliae* PI 568155 and *C. quinoa* PI 433232). A close genetic similarity between the two cultivated species has been noted on the basis of very high PCR conservation of microsatellite markers (99.5%)[19] though RAPD profiles

show separation of *C. quinoa* and *C. berlandieri* subsp. *nuttalliae* in separate groups at the level of 80% similarity[17]. These species also differ markedly in DNA amounts as both the accessions of *C. berlandieri* subsp. *nuttalliae* show 8.31% less DNA value than the average of 21 accessions of *C. quinoa*[40] and three 5S loci and one or two 45S loci in the former instead of two and one of each respectively in the latter species[20]. *C. berlandieri* subsp. *nuttalliae* has been considered to be conspecific with *C. quinoa*[41] and on the basis of grain characters it was suggested to represent an 'early migrant quinoa population'[42]. However, studies showing genetic complementation for light fruited condition[43], morphological and electrophoretic differences and crossability data strongly indicate independent origin of both the cultigens[13]. Moreover, the hybrids between *C. berlandieri* subsp. *nuttalliae* and *C. quinoa*/*C. hircinum* (Andean complex) show very low pollen stainability of 3.4% and complete seed sterility after selfing, though pollen stainability increases after backcrossing the hybrid with *C. berlandieri* subsp. *nuttalliae*[13,15]. Nevertheless, *C. berlandieri* subsp. *zschackei* produces fertile hybrids in crosses with *C. quinoa*/*C. hircinum* (Andean complex) indicating a close affinity to *C. quinoa*[13]. The morphological and isozyme data also show *C. berlandieri* subsp. *zschackei* as a basal element intermediate between North American and Andean cultigen/weed complexes therefore suggesting a southward migration of a North American tetraploid which most likely acted as a progenitor of Andean crop/weed (*C. quinoa*/*C. hircinum*) complex[13]. This relationship is also supported by the similarity of subrepeat sequence of IGS regions and in case of one of the two repeat classes of 5S NTS sequence of *C. quinoa* with that of *C. berlandieri* subsp. *zschackei*, suggesting that the two cultivated allotetraploid species have originated from at least one common ancestor[20]. The present results also support the possibility of some phyletic relationship between *C. quinoa* and *C. berlandieri* subsp. *nuttalliae* though both have evolved independently and accumulated genetic differences as shown by high sterility of F1 hybrids[9] and significant differences in karyotypes and genome sizes[8,40].

The taxa under *C. album* are distributed into two main groups of the dendrogram (Figure 2) and represent a very heterogenous assemblage which comprises three ploidy levels, diploid ($2n = 18$), tetraploid ($2n = 36$) and hexaploid ($2n = 54$)[8,15,43–47]. A Mexican tetraploid ($2n = 4x = 36$) taxon included in *C. album* shows close similarity to *C. berlandieri* subsp. *nuttalliae* (Figure 2). Both hexaploid and tetraploid taxa referred to *C. album* are known to occur in South America[1,48] along with other wild species related to *C. quinoa*. The close relationship of such *C. album* taxa with *C. berlandieri* is also supported by RAPD studies[17]. Three diploid *C. album* taxa occurring in North India join all the above species forming a sister group (Figure 2). The close similarity among these taxa is corroborated by their crossability behaviour resulting in fertile hybrids (unpublished data). Such minor but concrete differences among these 2*x* taxa are also apparent with respect to morphological differences, seed protein profiles and karyotypes[8,10,49]. Similar differences in some narrow- and broad-leaved types of 2*x* types have been observed with regard to seed protein, isozyme and RAPD profiles[18]. In the dendrogram, they show closer affinity with *C. quinoa* and *C. berlandieri* subsp. *nuttalliae* which is corroborated by the fact that these three 2*x* *C. album* taxa are intercrossable with *C. quinoa* and the hexaploid obtained after treating the resultant triploid with colchicine is fully fertile (unpublished data). The other two North Indian taxa of *C. album* comprising 4*x* and 6*x* cytotypes are included in the second group of the dendrogram showing 42.8% similarity (Figure 2). They form sister groups with *C. giganteum* (6*x*) and *C. album* (6*x*) accessions of European and American origin (Figure 2) with which they show 29.2–49.0% similarity. The 4*x* and 6*x* taxa of North Indian *C. album* show greater similarity with each other than with three 2*x* *C. album* taxa from the same region which shows that 2*x* is more dissimilar among these cytotypes, this agrees with the results obtained from seed protein profiles[49]. Furthermore, these three cytotypes are cross incompatible (unpublished results) as reflected by the differences in their karyotypes, genome size and seed protein profiles[8,40,49] and different genomic constitutions[18]. The 6*x* populations of North India have been shown to be allopolyploids involving two 2*x* types of *C. album* and *C. murale* (2*x*) as ascertained from seed protein, isozyme and RAPD profiles[18]. The genomic constitution of 4*x* type has not been studied so far. In this regard, it may be mentioned that all the material of *C. album* of British, European, American and Australian origin is uniformly hexaploid[50,51]. Cole[50] has considered 4*x* types of *C. album* from USA as *C. berlandieri* Moquin subsp. *zschackei* (Murr.) Zobel to which perhaps 4*x* form of North India is also referable even though the latter shows greater resemblance to *C. album* (*sensu stricto*) in the presence of anthocyanin pigment, nature of inflorescence and seed coat markings (smooth testa with radial markings instead of deep honey comb-like pittings as in *C. berlandieri* subsp. *zschackei*) (S. C. Verma, pers. commun.). The diploid types of Europe have been assigned to *C. suecicum* J. Murr[51] with which the North Indian 2*x* types do share some morphological features though being very different from 4*x* and 6*x* types (S. C. Verma, pers. commun.). Therefore, North Indian *C. album* is an aggregate species and it is still a dilemma as to which of its components conforms to Linnean *C. album*. In this respect, further studies with more populations from diverse areas are required to know the extent of variability and relationships between various cytotypes. It is worth mentioning here that 6*x* *C. album* from Europe and some related taxa show flavonoid profiles which are the exact summation of those of diploids *C. suecicum* and *C. ficifolium* Sm[16].

The second main group of the dendrogram makes clear distinction between 6*x* *C. album* and *C. giganteum* accessions of American and European origin on the one hand and of Indian origin on the other as they are divided between two subgroups (Figure 2). In the first subgroup five accessions each of *C. giganteum* (6*x*) and *C. album* (6*x*) of European and American origin are closely joined (Figure 2). Similarly, the second subgroup joins *C. giganteum* of Indian origin (Figure 2). *C. strictum* joins this group which is congruent with its taxonomic position (Table 2)[39]. This subgroup is then joined by *C. bushianum* which is quite an unexpected result as *C. bushianum* along with *C. quinoa* and *C. berlandieri* subsp. *nuttalliae* belongs to subg. *Chenopodium* sect. *Chenopodium* subsect. *Favosa* (Table 2)[39]. The accession of *C. bushianum* studied presently shows 23.9–31% similarity with various accessions of *C. quinoa* and *C. berlandieri* subsp. *nuttalliae*. *C. bushianum* has been reported to be a tetraploid ($2n = 36$)[15,52] which is cross-compatible with *C. quinoa* and *C. berlandieri* subsp. *nuttalliae* producing sterile and semi-fertile hybrids with these species respectively[15]. The material presently studied is hexaploid ($2n = 54$)[8] therefore aberrant behaviour of this accession of *C. bushianum* may be because of difference in genomic constitution as reflected in distinct karyotypic differences as compared with those of *C. quinoa* and *C. berlandieri* subsp. *nuttalliae*[8]. *C. opulifolium* and *C. ficifolium* join all the taxa in this subgroup (Figure 2). *C. opulifolium* along with *C. album* (*s.l.*) and *C. strictum* belongs to subg. *Chenopodium* sect. *Chenopodium* subsect. *Chenopodium* and *C. ficifolium* belongs to subg. *Chenopodium* sect. *Chenopodium* subsect. *Favosa*[39].

C. ugandae is the first species to join two main clusters of the dendrogram as an outgroup (Figure 2). *C. botrys*, *C foetidum* and *C. pallidicaule* are the next three species to join (Figure 2). *C. botrys and C. foetidum* belong to subg. *Ambrosia* sect. *Botryoides* subsect. *Botrys*[53], which is also supported by karyotypic and genome size studies[8,40]. *C. pallidicaule*, however is quite aberrant taking into account its taxonomic relationships (Table 2). Next to join is *C. murale* which also shows very low similarity with the rest of species in the dendrogram. This is in accordance with its taxonomic position as it belongs to subg. *Chenopodium* sect. *Chenopodium* subsect. *Undata*[39]. *C. vulvaria* is clearly separated out from all other species in the dendrogram. It belongs to subg. *Chenopodium* sect. *Chenopodium* subsect. *Chenopodium* along with *C. album*, *C. giganteum* and *C. opulifoilium*. However, Mosyakin and Clemants[39] have recommended separation of *C. vulvaria* into an independent subsection which is corroborated by the present study. This is also supported by significant differences in genome size of *C. vulvaria* as compared to the species of subsect. *Chenopodium*[40].

The present study based on RAPD and DAMD markers shows that these DNA markers are useful tools not only to assess intraspecific variation within cultivated species like *C. quinoa*, *C. album* or *C. giganteum* but also to reveal interrelationships among various species in this large genus and to solve taxonomic problems both at or below the species level.

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Spatio-temporal analysis of the Indus urbanization

Kavita Gangal[1,3], M. N. Vahia[1,2] and R. Adhikari[3,*]

[1]Tata Institute of Fundamental Research, Homi Bhabha Road, Mumbai 400 005, India
[2]Centre for Excellence in Basic Sciences, Kalina, Mumbai 400 098, India
[3]The Institute of Mathematical Sciences, CIT Campus, Chennai 600 113, India

The greater Indus valley was home to Neolithic cultures starting from 7000 BCE. They formed the antecedents of the urban Harappan civilization, whose rise and decline are dated to 2600 BCE and 1900 BCE respectively. At its peak, the Harappan civilization covered an area of more than a million square kilometres, making it the largest urbanized civilization of the Bronze Age. In this communication, we integrate GIS information on topography and hydrology with radiocarbon and archaeological dates of 1874 sites, to

*For correspondence. (e-mail: rjoy@imsc.res.in)