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Short Communication

Molecular diversity in the Indian Chenopod (*Chenopodium album*) as revealed by DNA-based markers

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Abstract

Population structure of 41 accessions of *Chenopodium album* and its sub species *C. album* ssp. *amaranticolor* along with five accessions of *C. quinoa* from India, USA and Argentina was studied using 37 polymorphic microsatellite loci. *The C. album* from India was highly diverse and comprised of three distinct populations. The first population was genetically similar to *C. album*, the second was different from *C. album* as well as from *C. quinoa*, whereas the third population resembled *C. quinoa*. On the other hand, the ssp. *amranticolor* was genetically similar to *C. album*. The RAPD markers delineated an accession of *C. quinoa* into a separate group, whereas SSR markers showed this accession to be genetically similar to *C. album*. Genetic diversity patterns suggested the need for taxonomic review of the Indian accessions classified as *C. album* and *C. album* ssp. *amaranticolor*.

Key words: *Chenopodium album*, *C. album* ssp. *amaranticolor*, *C. quinoa*, genetic diversity, taxonomy

Chenopodium album L. (family Chenopodiaceae), a traditional crop of the Himalayan region, is rich for proteins, β–carotene, vitamin A, zinc, calcium and iron [1]. Its leaves contain high amount of good quality proteins and have been used to develop protein concentrates in Scandinavian countries [2]. Because of low fertilizer requirements, its ability to grow well in marginal soils and rich nutritional composition of its leaves and seed, the crop is considered ideal to alleviate nutritional deficiencies in poverty ridden regions of the world. Chenopod, however, remained neglected since the second half of 19th century because of shift to cultivation of limited number of crops during the green revolution era in India, and also due to changes in food habits and life styles. Presently, its cultivation is restricted to marginal lands by tribal farmers in the Himalayan region. Meager efforts have been made to estimate genetic diversity in the available genepool, conserve the diversity and initiate breeding programmes for its improvement.

C. album from India is an assemblage of heteromorphic [3-5] and heterocytotic forms (2n = 2x = 18, 2n = 4x = 36, 2n = 6x = 54) [5]. Because of morphological and cytological variability, the *hitherto* classified *C. album* is suspected to be an assemblage of more than one species [6-7]. In an effort to resolve this ambiguity, individuals having triangular, pinnately lobed leaves with mealy red on lower surface were classified as *C. album* ssp. *amaranticolor* Coste & Reynier [syn. *C. amaranticolor* [8], *C. giganteum* D. Don] [9, 10]. DNA-based markers, which have been used extensively to elucidate genetic structure of populations and resolve taxonomical ambiguities arising due to vague morphological descriptors can also be utilized to study population structure of the Indian chenopod. Additionally these markers might also be useful to resolve taxonomical discrepancies in Indian *C. album*. The present investigations were carried out with the objective to study genetic structure of the *hitherto* classified *C. album* complex from India using SSR and RAPD markers. The study also indicated that *C. album* from India was a group of three genetically diverse populations and a taxonomic review of this species is warranted.

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The experimental material comprised 46 accessions belonging to two *Chenopodium* species *viz*., *C. album* and *C. quinoa* and one sub-species, *C. album* ssp. *amaranticolor* (Table 1). Of these, *C. album* (31 accessions) and *C. album* ssp. *amaranticolor* (eight accessions) were from the Hiamalayan region (India), while *C. quinoa* (four accessions) was from the Andean region (USA and Argentina). An accession of the *C. quinoa* accessions was from the Himalayan region, whereas two of the *C. album* accession were from USA. The germplasm used in the present study was a subset of 166 accessions maintained in the medium term genebank at National Bureau of Plant Genetic Resources (NBPGR), Regional Station, Shimla, India.

Genomic DNA was isolated from young leaves (0.5 g) using CTAB method. Ten SSR primers [QAAAAT2, QATG019, QATG020, QATG026, QATG028, QATG035, QCA005, QCA012, QCA026, QAAT006; 11] and 33 decamer primers (OPA-01, OPA-02, OPA-04, OPA-06, OPA-07, OPA-10, OPA-18, OPA-19, OPA-20, OPD-17, OPD-08, OPD-19, OPD-03, OPD-09, OPD-04, OPF-01, OPF-02, OPF-03, OPF-04, OPF-05, OPF-06, OPF-07, OPF-09, OPF-12, OPF-15, OPJ-05, OPJ-06, OPJ-10, OPJ-12, OPJ-13, OPJ-14, OPJ-18,OPJ-20; Operon Technologies Inc., USA) were used for amplification of genomic DNA. DNA amplifications were carried out in 25 µl volumes in a thermal cycler (Gene Amp PCR System 9700, Applied Biosystems, USA). The PCR conditions for SSR primers were as follows: 94^{o}C for 1 min; followed by 5 cycles of 94^{o}C for 30 s, 55^{o}C for 30 s (decreasing 1^{o}C every cycle), 72^{o}C for 1 min; 10 cycles of 94^{o}C for 30 s, 50^{o}C for 30 s, 72^{o}C for 1 min; 5 cycles of 94^{o}C for 30 s, 50^{o}C for 30 s (decreasing 1^{o}C every cycle), 72^{o}C for 1 min; 10 cycles of 94^{o}C for 30 s, 45^{o}C for 30 s, 72^{o}C for 1 min, followed by 72^{o}C for 5 min. The PCR conditions for RAPD were: initial cycle of 94^{o}C for 5 minute; 40 cycles of 94^{o}C for 1 minute, 37^{o}C for 1 min and 72^{o}C for 2 min; and final extension at 72^{o}C for 5 min. SSR products were resolved in 3% agarose SFR (Amresco, Solon, USA) gels in 1X Tris acetate-EDTA buffer whereas RAPD products were resolved in 1.4% agarose gel. The PCR products were visualized and photographed using the Gel-Documentation Unit (Bio-Rad, USA). The presence (1) and absence (0) of each band was scored manually and data analyzed using the Jaccard's Coefficient of Similarity in SIMQUAL programme of NTSYSpc package [12]. Cluster analysis of different genotypes was based on Unweighted Pair Group Method with Arithmetic mean (UPGMA) option in the SAHN programme of NTSYSpc package.

Table 1. List of *Chenopodium* accessions and their place of origin

Name of the species	Accession no.	Place of origin
Chenopodium album	EC507733, EC359451 IC341696, IC341698, IC341699, IC341700 & IC341703	U.S.A U.P.
	IC258254, IC258332, IC381078 & IC381106	Lahaul, H.P.
	NIC22505, NIC22516, NIC22531, NIC22532 & IC343192	Kinnaur, H.P.
	IC109480, IC109734, IC328854 &	Chamba, H.P.
	IC258253	Pangi, H.P.
	IC107515, IC108088, IC329470, IC329494, IC329521 & NIC15022	Kullu, H.P.
	IC107263, IC341705, IC341708, IC363733 & IC415405	Shimla, H.P.
	IC108082	Mandi, H.P.
	IC108816	Kangra, H.P.
Chenopodium album ssp. *amaranticolor*	NC50229	Delhi
	IC469275	Jammu, J & K
	IC341701	U.P
	NIC22519 & IC341710	Kinnaur, H.P.
	IC109731, IC328877	Chamba, H.P.
	IC108086	Kullu
Chenopodium quinoa	EC507740 & EC507743	U.S.A
	EC507748 & EC507749	Argentina
	IC258331	Lahaul, H.P.

U.P.= Uttar Pradesh, H.P.= Himachal Pradesh, J & K.= Jammu and Kashmir

The amplification of genomic DNA of 46 accessions using 10 *C. quinoa* SSR primer pairs generated 39 bands (3.9 bands per primer), out of which 37 (94.9%) were polymorphic. All the 10 *C. quinoa* SSR primer pairs used in the study generated amplified products in *C. album* as well as in *C*. *album* ssp. *amranticolor* indicating high transferability rates of SSRs between *C. quinoa* and *C*. *album*. The dendrogram obtained after analysis of SSR data is presented in Fig. 1. The diversity analysis showed high inter- and intra-species diversity and the 46 accessions were delineated into three distinct groups. Majority of the *C. album* accessions from India (27 out of the 31), *C. album* from USA and *C. album* ssp. *amaranticolor* from India were in Group I. This group also included an accession of *C. quinoa* (EC507749) from Argentina. Overall genetic

diversity in this group was 55%. Group I could further be subdivided into six sub groups (IA-IF). The accessions of ssp. *amaranticolor* were not even delineated into a separate sub group within Group I, instead these shared different sub groups with different *C. album* accessions (see Group I, Fig. 1). Some of these accessions had very high similarity to those of *C. album* accessions e.g. ssp. *amaranticolor* accession, IC341701 from UP had 92.5% similarity to *C. album* accession (IC341696) from the same region (subgroup IB), IC341710 from Kinnaur was similar (89.0% similarity) to *C. album* accession (IC343192) from Kinnaur and IC107263 from Shimla (subgroup IC), NIC22519 from Kinnaur was genetically closer (88.6% similarity) to *C. album* accession (NIC22516) from the same region (subgroup ID). The ssp. *amaranticolor* differs with respect to triangular, pinnately lobed leaves, and mealy red on lower surface [9, 10] from *C. album* which has linear, lanceolate leaves when young and broad lobed later, mealy white on lower surface. Despite morphological differences, the close genetic relatedness of *amranticolor* to *C. album* (present study) and almost similar seed protein profiles of *C. album* (6x) and ssp. *amranticolor* from India [13] question the existence of a subspecies within *C. album*. It might be possible that the leaf morphology and colour, the two characters used to differentiate *C. album* and ssp. *amaranticolor* are governed by a few genes whereas rest of the genetic make up of these taxa is almost similar.

The Group II had three accessions of *C. album* (IC381078 and IC381106 from Lahaul valley, Himachal Pradesh and IC258253 from Pangi subdivision of Chamba, Himachal Pradesh) from India with low similarity (41.4%) to *C. album* accessions in Group I. These three accessions were not only genetically distinct but also had morphological differences to *C. album* accessions in Group I. While the accessions in

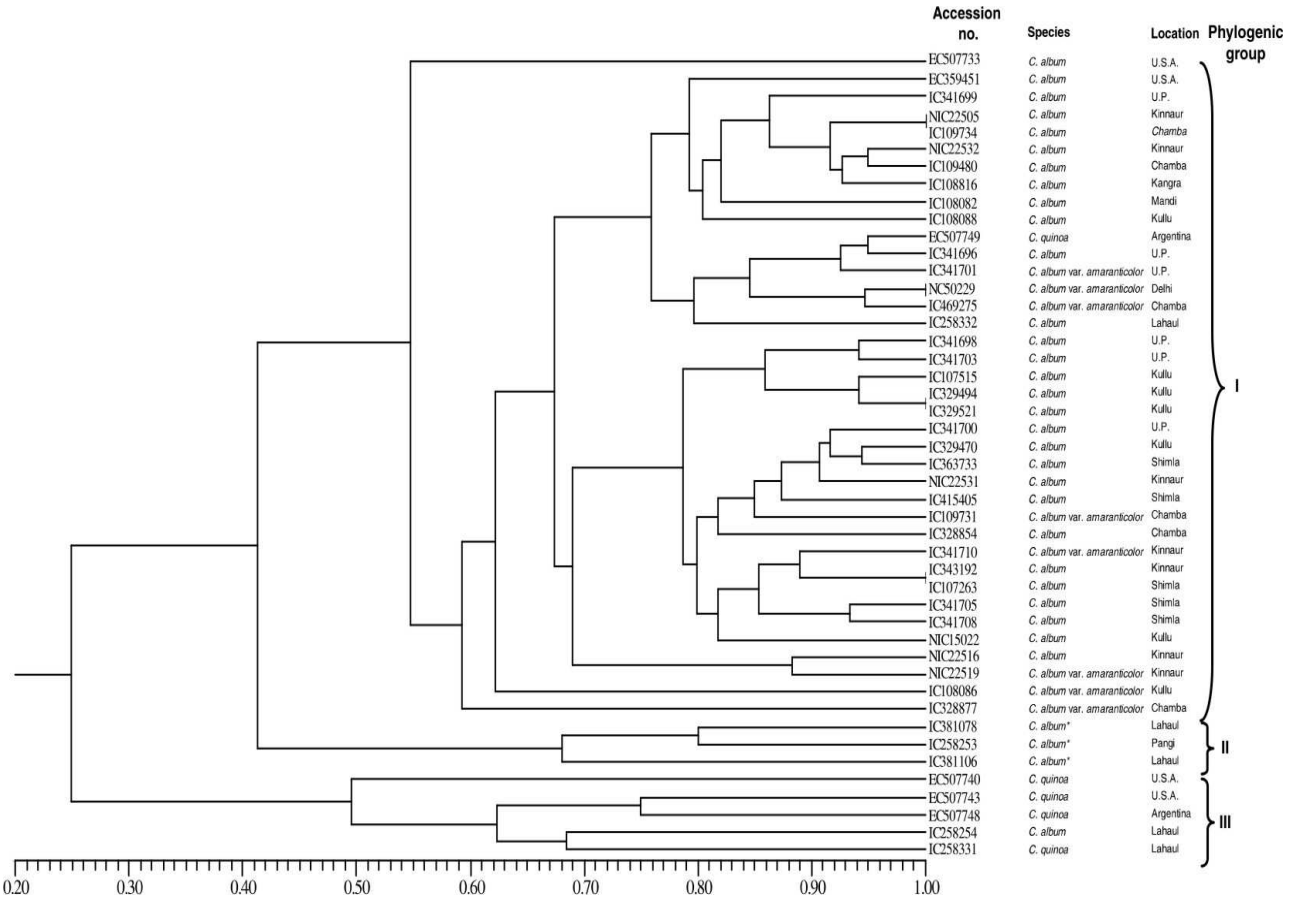

Fig. 1. Gentic relationships among 46 accessions of *Chenopodium* species (*C. album*, *C. album* ssp. *amaranticolor* and *C. quinoa*) based on SSR marker data. The marker data were analyzed using NTSYS software (Rohlf, 1993) with Jaccard's Coefficient and UPGMA option to obtain the dendrogram. The accession numbers, species names, locations and phylogenic group numbers are given on the termini of branches whereas subgroups are shown above the clusters

Group I had green stems, linear leaves, leaves lanceolate when young and broadly lobed later, generally compact inflorescence and black coloured seed, those in Group II had red stems, lanceolate leaves, loose and red inflorescence and brownish white seed (personal observation). Low genetic similarity to *C. album* in Group I and morphological distinctness suggested that accessions in Group II might not be *C. album*. Group III was comprised of four accessions of *C. quinoa*, two from the USA, one each from the Argentina and India and an accession of *C. album* (IC258254 from Lahaul, India). It appeared that this accession has been misclassified as *C. album*. Apart from genetic similarity to *C. quinoa*, this accession was also morphologically akin to *C. quinoa* except brown black seed colour.

The RAPD analysis carried out using 33 RAPD primers generated 247 loci [240 (97.17%) polymorphic, seven (2.83%) monomorphic] with a frequency of 5.37 bands per primer. Compared to SSR, RAPD data generated almost similar results (data not shown). The only major difference was the delineation of an accession (EC507749) of *C. quinoa* from Argentina into a separate group. SSR markers on the other hand showed this accession to be genetically similar to *C. album* (see Group I, Fig 1).

The controversy over classification of domesticated Indian *C. album* is not new. Earlier, plants classified as *C. album* have been considered to be the domestic forms of either *C. album* [14] or *C. giganteum* D. Don [15], *C. quinoa* [16], *C. berlandieri* and *C. suecicum* [7]. It was also suggested that the 2x accessions of *C. album* were closer to *C. berlandieri* whereas 4x and 6x accessions were similar to *C. giganteum* [8]. It is worth mentioning here that *C. giganteum* is synonym to *C. album* ssp. *amranticolor* [8]. Our findings suggest that the *hitherto* classified *C. album* from India was a group of at least three species, *C. album* (see Fig. 1. Group I), *C. quinoa* (Group III) and a species other than *C. album* or *C. quinoa* (Group II), whereas classification as ssp. *amranticolor* with in *C. album* is not relevant. Further taxonomic studies are needed to establish the species of individuals in Group II and Group III which are presently classified as *C. album*. The present study suggested that ca 10%-12% accessions in the Indian genebanks were genetically dissimilar to *C. album* and might belong to some other species. The decline in cultivation of *C. album*, misclassification of other species as *C. album* (present study), and low number of *C. album* accessions (166) in Indian genebanks have implications for conservation of Himalayan chenopod. To conserve whole of the diversity within *C. album*, the core set of germplasm should represent accessions from all the genetic groups and sub groups.

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