



Species relationships in *Chenopodium quinoa* and *Chenopodium album* on the basis of morphology and SDS-PAGE profiles of soluble seed proteins

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ABSTRACT

Nineteen accessions of *Chenopodium*, comprising of 11 accessions of *C. quinoa* and 8 accessions of *C. album* were evaluated. Morphological variations were observed in leaf colour, shape and margins, type of pollen grains, seed colour, seed coat texture and morphology of seed edges amongst the accessions. SDS-PAGE profiling of soluble seed proteins revealed 22 bands ranging in molecular mass from 26-68 kDa. While 6 protein bands were monomorphic, 16 protein bands were polymorphic. One band representing protein with molecular mass of 35.76 kDa was detected only in the accession IC-341704 of *C. album*. Five exotic accessions viz. EC-507744, EC-507742, EC-507738, EC-5077391 and EC-507741, belonging to *C. quinoa*, showed the presence of a duplex of 33.55 and 29.59 kDa bands which was not detected in any other accession. The dendrogram generated from the scoring profiles of SDS-PAGE of soluble seed proteins segregated the accession as per their taxonomic classification into *C. quinoa* and *C. album*. While the accessions belonging to *C. album* showed a high degree of morphological heterogeneity, the SDS-PAGE profiles of seed proteins indicated low level of variations within the accessions of each species. Further, the accessions IC-411824 and IC-411825 showed more closeness with *C. quinoa* than *C. album*.

1. INTRODUCTION

The genus *Chenopodium* of Amaranthaceae family comprises about 150 species [1]. The domesticated species of *Chenopodium* viz. *C. quinoa*, *C. berlandieri* subsp. *nuttalliae*, *C. pallidicaule*, *C. album*, and *C. giganteum*, are important as grain crops as well as forage and leafy vegetable. While the leaves of *C. album* are known to be a good source of vitamins and micronutrients [2], *C. quinoa* is important for gluten free flour and high protein content of its grains [3,4]. While *C. quinoa* ($2n = 4x = 36$) is reported as tetraploid of putative allopolyploid origin, *C. album* is known as a complex of diploid ($2n=18$), tetraploid ($2n=36$) or hexaploid ($2n=54$) species with endopolyploidy and autopolyploidy as the origin of polyploidy [5,6,7]. *Chenopodium* has been one of the most taxonomically difficult representatives of the family Chenopodiaceae. While Fuentes-Bazan *et al.* [8] have clearly described the paraphyletic

nature of the genus on the basis of morphological and molecular data, a clear set of carpological characters, especially the fruit/seed anatomy, for resolving taxonomic issues in the genus are yet to be evolved. The domesticated species of *Chenopodium* are divided into two subsections on the basis of pericarp and perianth morphology and crossing relationships [9]. The first subsection Cellulata contains allotetraploids ($2n=4x = 36$) like *C. quinoa* and *C. berlandieri* subsp. *nuttalliae*. The second subsection Leiosperma includes domesticated and semi-domesticated forms like *C. pallidicaule* ($2n = 18$) and *C. album* ($2n = 18, 36, 54$) [10,11]. While most of the work on genetic diversity and phylogeny in *Chenopodium* has focused on domesticated species like *C. quinoa* and *C. berlandieri* subsp. *nuttalliae* [11,12,13,14,15], very few studies have been undertaken on important weed species like *C. album*, *C. berlandieri*, *C. ficifolium*, *C. glaucum*, *C. murale* and *C. strictum*.

Previous studies aimed at elucidating this taxonomic complex on the basis of cytology [16,17], karyotypic analysis [6,7], flavonoids [18], RAPD profiles [11,13,19], ISSR markers [20], directed amplification of minisatellite DNA (DAMDA) [13], microsatellite markers [14], ribosomal DNA [15] clearly indicate the existence of *C. album* as the most polymorphic plant species of

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genus *Chenopodium*. While some authors have recognized numerous segregate intergrading species in *C. album*, others have developed elaborate intra-specific hierarchies with numerous subspecies, varieties, forms, and even sub-forms [21]. Neither approach has, however, brought satisfactory and uncontroversial results. This lack of solid knowledge about the *Chenopodium* species has led to the need for a set of appropriate markers for proper identification of various species in this genus. Partap and Kapoor [22] have grouped the Himalayan *C. album* into four cultivars with black, brown, red and white seeds.

The high degree of heterogeneity at morphological, cytological and molecular levels [2,6,13] clearly indicates that *C. album* in India is an aggregate taxon, thereby raising a question mark on the identity of accessions belonging to this species.

2. MATERIALS AND METHODS

2.1. Materials

Seeds of nineteen accessions of the genus *Chenopodium*, comprising of 11 accessions of *C. quinoa* and 8 accessions of *C. album* (Table 1), studied in the present investigation, were procured from National Bureau of Plant genetic resources, NBPGR, India. Plants of each accession were raised to maturity in the experimental fields of North Eastern Hill University, Shillong.

2.2. Morphology

Each accession was evaluated for various morphological parameters including colour, shape and arrangement of leaves, flower color, pollen morphology, colour, shape and surface features of seeds. Data was collected for two successive years with three replications for each accession. Seed coat morphology was determined by scanning electron microscopy after removal of the pericarp from the seeds and sputter-coating with Gold-platinum using FINE COAT 10N sputter JFC-1100. Scanning electron microscopy of pollen grains was carried out after fixing entire flowers with 3% glutaraldehyde for 4 hours followed by washing with phosphate buffer and dehydration by passing through a series of increasing concentrations (30 to 100%) of acetone at 4°C.

2.3. SDS PAGE

Soluble seed proteins were extracted from mature and healthy seeds of each accession using 20mM Tris-Cl extraction buffer (pH 8.0) containing 2mM EDTA and 1mM PMSF. Protein concentration in each sample was determined according to Bradford [23]. SDS-PAGE of the extracted seed protein was carried out on 15% polyacrylamide gel following the method of Laemmli [24]. The electrophoretic profile of seed proteins of each accession was recorded as presence (1) or absence (0) of a band of a particular molecular weight. The Jaccard's similarity coefficient between different accessions was derived from the binary data showing the pair wise similarity between the accessions.

3. RESULTS AND DISCUSSION

While the accessions of *Chenopodium* investigated in the study did not show any variations in flower colour, leaf arrangement and seed shape they showed variations in colour, shape and margins of leaves, colour and texture of seed coat, seed edge morphology and type of pollen grains (Table 1). Even though Bhargava et al. [25] have reported intra-specific variations in certain phenotypic traits including plant height, days to flowering, days to maturity, leaf area, seed size, inflorescence length, dry weight per plant amongst accessions of *C. quinoa*, our results do not reveal any variations in any of the qualitative morphological characters amongst different accessions of *C. quinoa* studied in the present investigation. On the other hand, *C. album* showed morphological heterogeneity with green/ reddish leaves, black/ brown/ white seeds, smooth/ reticulate seed coat with smooth as well as patterned edges.

Except for the accessions IC-341704 and IC-341700, which had reticulated seed surface, all other accessions of *Chenopodium* investigated in the present study had a smooth and finely lineated seed surface. This is in contrast with the observations of Karcz [26], who have observed reticulate and flatly tuberculate type seed surfaces in *C. berlandieri* and *C. quinoa* and smooth and finely lineated seed surface *C. album*. On the basis of surface features of their seeds, *C. quinoa* was suggested to be more closely related to *C. berlandieri* than to *C. album*. Besides the black and brown seeds observed in different accessions of *C. album*, we also observed white seeds in two accessions viz. IC-411824 and IC-411825, which have been identified by National Bureau of Plant Genetic Resources, India as *C. album* but have foveate type pollen grains, dull texture of seeds with a smooth surface which is typical of *C. quinoa*. Baar [27], who made the first attempt to describe seed heterogeneity in *C. album*, has reported the presence of both black and brown seeds within the same plant. Many others have, however, postulated the existence of cryptic heterospermy manifested by the presence of black seeds of various sizes and of their capability for rapid or delayed germination in *C. album* [28,29,30,31,32].

Even though seed coat morphology has been used as a phenotypic character in species identification in the genus *Chenopodium* [32,33], the existence of heteromorphs in different species of the genus, especially *C. album*, necessitates the use of other characters including molecular markers in taxonomic identification in this genus. SDS-PAGE profiles of seed proteins from the 19 accessions belonging to *C. quinoa* and *C. album* revealed a maximum of 22 bands in an accession with molecular weight ranging from 26-68 kDa out of which 6 were monomorphic and 16 were polymorphic in nature (Fig. 1). While seven bands representing proteins having molecular weights 68.7, 58.59, 55.85, 48.40, 46.14, 41.27 and 37.51 kDa were detected in all eleven exotic accessions belonging to *C. quinoa* viz. EC-507738, EC-507739, EC-507739-1, EC-507740, EC-5077401, EC-5077402, EC-507741, E-C507742, EC-507744, EC-507747, EC-507748 and

Table 1: Accessions of *Chenopodium quinoa* and *Chenopodium album* studied in the present investigation. Variations observed in the morphological parameters are listed. Parameters codes: A-Leaf color, B-Leaf shape, C-Leaf apex, D-Leaf margin, E-Seed color, F-Seed texture, G-Seed coat, H-Seed edge and I-pollen type.

Sl. no.	Accessions	Species	A	B	C	D	E	F	G	H	I
1	EC507738	<i>C. quinoa</i>	Green	Rhombic	Acute	Dentate	White	Dull	Smooth	Smooth	Foveate
2	EC507739	<i>C. quinoa</i>	Green	Rhombic	Acute	Dentate	White	Dull	Smooth	Smooth	Foveate
3	EC5077391	<i>C. quinoa</i>	Green	Rhombic	Acute	Dentate	White	Dull	Smooth	Smooth	Foveate
4	EC507740	<i>C. quinoa</i>	Green	Rhombic	Acute	Dentate	White	Dull	Smooth	Smooth	Foveate
5	EC5077401	<i>C. quinoa</i>	Green	Rhombic	Acute	Dentate	White	Dull	Smooth	Smooth	Foveate
6	EC5077402	<i>C. quinoa</i>	Green	Rhombic	Acute	Dentate	White	Dull	Smooth	Smooth	Foveate
7	EC507741	<i>C. quinoa</i>	Green	Rhombic	Acute	Dentate	White	Dull	Smooth	Smooth	Foveate
8	EC507742	<i>C. quinoa</i>	Green	Rhombic	Acute	Dentate	White	Dull	Smooth	Smooth	Foveate
9	EC507744	<i>C. quinoa</i>	Green	Rhombic	Acute	Dentate	White	Dull	Smooth	Smooth	Foveate
10	EC507747	<i>C. quinoa</i>	Green	Rhombic	Acute	Entire	White	Dull	Smooth	Smooth	Foveate
11	EC507748	<i>C. quinoa</i>	Green	Rhombic	Acute	Dentate	White	Dull	Smooth	Smooth	Foveate
12	IC411824	<i>C. album</i>	Green	Rhombic	Acute	Dentate	White	Dull	Smooth	Smooth	Foveate
13	IC411825	<i>C. album</i>	Green	Rhombic	Acute	Dentate	White	Dull	Smooth	Smooth	Foveate
14	IC341704	<i>C. album</i>	Green	Lanceolate	Obtuse	Entire	Black	Shiny	Reticulate	Pattern	Perforate
15	NIC22517	<i>C. album</i>	Red	Rhombic	Acute	Dentate	Black	Shiny	Smooth	Pattern	Perforate
16	IC341700	<i>C. album</i>	Red	Rhombic	Acute	Dentate	Black	Shiny	Reticulate	Pattern	Perforate
17	IC447575	<i>C. album</i>	Red	Rhombic	Acute	Dentate	Brown	Shiny	Smooth	Smooth	Perforate
18	EC359447	<i>C. album</i>	Red	Rhombic	Acute	Dentate	Black	Shiny	Smooth	Pattern	Perforate
19	EC359451	<i>C. album</i>	Red	Rhombic	Acute	Dentate	Black	Shiny	Smooth	Pattern	Perforate

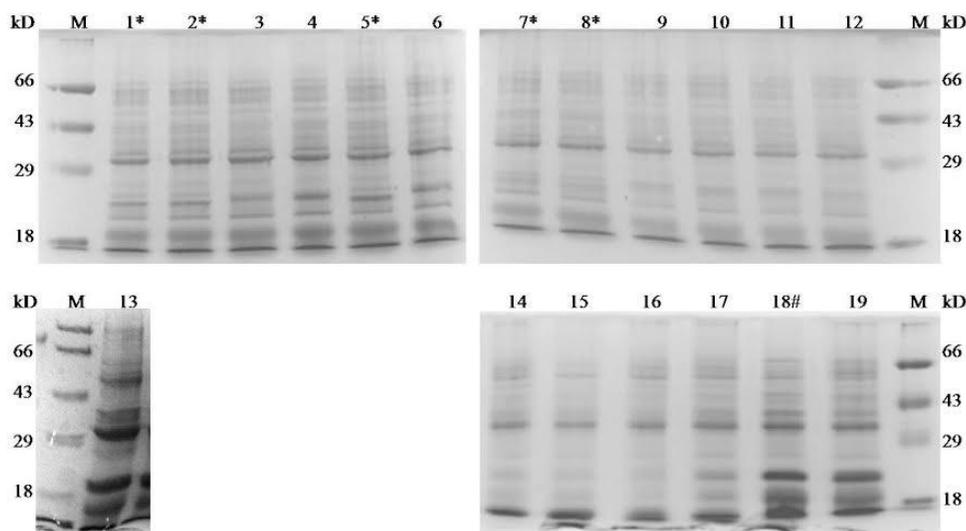


Fig. 1: SDS-PAGE profile of soluble seed proteins extracted from different accessions of *Chenopodium quinoa* and *C. album* investigated in the present study. (1) EC-507744, (2) EC-507742, (3) IC-411824, (4) IC-411825, (5) EC-507738, (6) EC-507739, (7) EC-5077391, (8) EC-507741, (9) EC-5077401, (10) EC-5077402, (11) EC-507740, (12) EC-507748, (13) EC-507747, (14) EC-359447, (15) IC-447575, (16) IC-341700, (17) NIC-22517, (18) IC341704, (19) EC-359451. While '*' indicates five accessions of *C. quinoa* detected with the duplex of 33.55 and 29.59 kDa protein bands, '#' indicates the accession IC-341704 of *C. album* with one protein band of molecular mass 35.76 kDa. M: standard molecular weight markers.

two accessions viz. IC-411824 and IC-411825 identified as *C. album*, five bands representing proteins having molecular mass of 67.62, 60.48, 54.97, 50.76 and 36.91 kDa were detected only in six accessions viz. IC-341704, NIC-22517, IC-341700, IC-447575, EC-359447, EC-359451 which belong to *C. album*.

An important feature of the profile was the presence of one band representing protein with molecular mass of 35.76 kDa in IC-341704 of *C. album*. This protein band was not detected in any other accession investigated in the present study. The accession IC-341704 has lanceolate shaped leaves with an obtuse apex and entire margin whereas all the other accessions have rhombic shaped leaves with an acute apex and dentate margins.

Another important feature of the profiles was the detection of a duplex of 33.55 and 29.59 kDa protein bands in five accessions of *C. quinoa* viz. EC-507744, EC-507742, EC-507738, EC-5077391 and EC-507741. This duplex could not be detected in any other accession of *Chenopodium* studied in the present investigation. Fairbanks et al. [34] have reported the presence of three polymorphic polypeptides having molecular masses of 34.3, 35.6 and 36.2 kDa from the globulin fraction of seed proteins of *C. quinoa*. On the other hand Bhargava et al. [35] have reported 72 bands from 40 species of *Chenopodium* and Drzewiecki et al. [36] have reported 41 protein bands in SDS-PAGE profiles of seed proteins of *C. quinoa*.

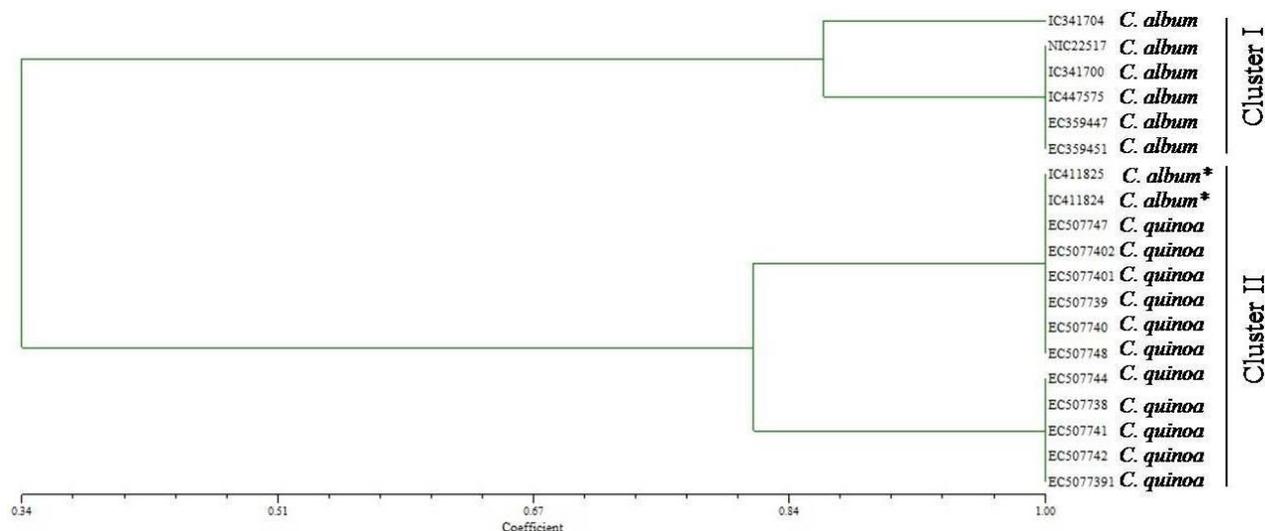


Fig. 2: Dendrogram generated on the basis of UPGMA (Unweighted Pair Group Method with Arithmetic Mean) analysis of the similarity matrix of SDS-PAGE profiles of soluble seed proteins of different accessions of *Chenopodium* studied in the present investigation. *Two accessions identified as *C. album* by NBPGR grouped together with *C. quinoa*. Numbers prefixed with IC/ EC/NIC indicates the accession numbers of the plants.

The difference in the number of proteins detected could be ascribed to the differences in the composition of the extraction buffers. While Drzewiecki et al. [36] focused on profiling of total seed proteome, our investigation focused only on the soluble seed proteins.

Statistical analysis of the SDS-PAGE profile revealed an average polymorphic Information Content (PIC) value of 0.42 (Fig. 2). The UPGMA dendrogram generated from the similarity matrix of binary data of SDS-PAGE profiles resolved the accessions of chenopods into two broad clusters wherein *C. album* grouped as Cluster I and *C. quinoa* grouped as Cluster II with inter cluster Jaccard's similarity co-efficient value of 0.34. These results are in conformity with the observations of Bhargava et al. [35] who have also reported the segregation of *C. quinoa* and *C. album* into two clusters on the basis of their seed protein electrophoretic profiles. The accession IC-341704 of *C. album* emerged as a separate clade in cluster I with a similarity co-efficient value 0.84 with other five accessions of *C. album* that clustered together as another clade with a similarity co-efficient value of 1.0 in cluster I. Two accessions viz. IC-411824 and IC-411825 of *C. album*, which have white coloured seeds, clustered together with other accessions in one of the clades of Cluster II. Cluster II segregated into two sub-clusters wherein each sub-cluster showed a similarity co-efficient value of 1.0 and the two sub-clusters showed an inter cluster similarity coefficient of 0.78. Wilson [37], Raus et al. [19] and Bhargava et al. [35] have also reported a low level of variation in allozyme, RAPD and seed protein profiles amongst the accessions of *C. quinoa*. While Bhargava et al. [35] have suggested *C. album* to be a heterogeneous assemblage of species, our results on SDS-PAGE profiles of soluble seed proteins indicate a high level of genetic similarity amongst the accessions of *C. album* except IC-341704. It is possible that the "heterogeneous assemblage of species" as observed by Bhargava et al. [35] may be actually an assemblage of "cytotypes" in

C. album. While Bhargava et al. [35] had investigated the SDS-PAGE profiles of seed proteins in three cytotypes (2n=18, 4n=36, 6n=54), all the accessions of *C. album*, except IC-411824 and IC-411825, studied in the present investigation were hexaploid (6n=54). The accessions IC-411824 and IC-411825 were tetraploid (2n=36).

These observations clearly indicate a relationship between heteromorphy and ploidy level in accessions of *C. album*. Our observations also suggest the accessions IC-411824 and IC-411825 to be *C. quinoa* rather than *C. album*.

4. CONCLUSIONS

The work establishes the identity of accessions IC-411824 and IC-411825 as *C. quinoa* rather than *C. album*. Our observations also indicate a relationship between ploidy level and heteromorphy in *C. album*.

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