Seed protein electrophoresis in six species and two F_1 s of *Cicer*

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Abstract. The water soluble fraction of the seed protein of 6 *Cicer* species and of the two F_1 hybrids of *Cicer arietinum* and *Cicer reticulatum* was analyzed by disc electrophoresis. Each species and F_1 of *Cicer* had its own pattern and also a different number of bands. In general banding pattern revealed variation in number and intensity of bands in all the species and F_1 s. A little difference was in the protein profile of the two strains, NEWC-21 and JM-2106 of *Cicer reticulatum*. Common and kabuli types of *Cicer arietinum* were found to possess 7 bands but one band in each type was at different Rf. The bands in *Cicer arietinum* had homologues in other wild species and none of the wild species possessed all 7 *Cicer arietinum* bands. All the species, except *Cicer arietinum* and *Cicer judaicum*, possessed some typical bands in their protein profiles in the sense that no one was found at that particular Rf in other species. As regards the similarity indices between hybrids and their respective parents it was found that each cross showed more than 50% homology with their parents. On the basis of seed protein profile, *Cicer reticulatum* seemed to be the most suitable for wild progenitor of *Cicer arietinum*. The difference between the profiles of *Cicer judaicum* and *Cicer pinnatifidum* supported the idea that they are indeed two separate species.

Keywords. Seed; protein; electrophoresis; *Cicer*.

1. Introduction

The seed protein profile is apparently a typical and diagnostic trait for plant species. The seed protein can not only serve as an additional tool in taxonomic studies but might also be helpful in determining and evaluating species relationship among the plant species. It's usefulness has been elaborated in biosystemic studies and the technique has been employed in more than 45 different genera (Ladizinsky and Hymowitz 1979). The disc electrophoresis technique offers the advantages of speed, simplicity, high resolution and great sensitivity. Several modifications have been introduced yielding a method which is simple and rapid, and which possesses excellent resolving power for all basic proteins (Shepherd and Gurley 1966). Larsen (1967) analyzed seed protein in 61 soybean varieties, *Glycine max* (L.) Merrill, by disc gel electrophoresis and was able to classify the varieties into two distinct groups based on two components identified by stained proteins in polyacrylamide gels. In other crops such as wheat (Johnson *et al* 1967), barley (McDaniel 1970) and cotton (Johnson and Thein 1970), species relationship revealed by conventional method and have been confirmed according to their seed protein profiles. Electrophoretic patterns of soluble seed protein have been used as genetic marker in many biosynthetic studies (Johnson 1969, 1972a, b; Yadav *et al* 1979; Jope and Jana 1980). In *Cicer*, where information on species relationship deduced from meiotic behaviour and fertility of the interspecific hybrid (Ladizinsky and Adler 1976a) is not available due to difficulty
in crossing the small cleistogamous flowers, the seed protein which reflect the genetic
constitution of various species might provide us with some clues (Ladizinsky and
Adler 1976b). The present study on different species and F₁s of Cicer was undertaken
to identify them on the basis of soluble seed protein banding pattern and also to find
out the genetic diversity and affinity among different species based on similarity
index.

2. Materials and methods

A total of 8 varieties or strains of different species of Cicer, viz., H-208 (common type)
and ICC-8923 (kabuli type) of C. arietinum, Jm-2103 of C. bijugum, SL-157 of
C. cuneatum, Jm-185 of C. judaicum, Jm-188 of C. pinnatifidum, NEWC-21 and Jm-
2106 of C. reticulatum were studied for their protein banding pattern. Along with these
species F₁ of C. arietinum (H-208) × C. reticulatum (NEWC-21) and F₁ of C. arietinum
(ICC-8923) × C. reticulatum (Jm-2106) were also studied electrophoretically.

Disc gel electrophoresis (Davis 1964) was adopted to separate the total protein
extracted in 0.1 M Tris HCl buffer, pH 8, and estimated by the method of Lowry
(1951) using bovine serum albumin as standard from the seed. The gels were stained
in imido black and destained in 7% acetic acid to remove the unbound dye in order to
visualize the protein bands. Sample was layered on 7.5% running gel and electrophoresis was conducted at 3 mA current per gel column. Rf value was calculated for
all visible bands and similarity index was calculated according to the method of
Sheen (1972).

3. Results and discussion

A total of 30 bands were observed and these bands were numbered with increasing
magnitude of Rf values. The types of bands were decided on the basis of their colour
intensity, like thin and distinct, diffuse and dense. The protein banding pattern of all
the species and F₁s are presented in figure 1. Each species of Cicer and F₁s had its
own pattern and also a different number of bands. The number and kind of bands in
different species and F₁s are given in table 1. In general protein banding pattern
revealed variation in number and intensity of bands in all the 6 species and F₁s of
Cicer. However, little difference was in the protein profile of the two strains NEWC-
21 and Jm-2106 of C. reticulatum, where it was composed of 8 bands in both the
strains but one dense band in each case was at different Rf (0.63 and 0.69 respectively)
value. Similarly common and kabuli types of C. arietinum were found to possess 7
bands but one band in each type was found at different Rf (0.97 and 0.30 respectively)
value. They were not also homologue in the sense that the band in common
type was diffuse while the other band in kabuli type was thin and distinct. This was a
constant and conservative character in both the types. Ladizinsky and Adler (1976b)
found the same basic seed protein profile composed of 7 bands in 88 cultivars of C.
arietinum except two cultivars where the band at Rf 0.61 split into two close but
distinct bands. The per cent similarity index among the different species are given in
table 2. In the present study similarity of the protein profile between these two types
was 71.43%. Common and kabuli types of C. arietinum are treated as the same
species. However, Dixit (1932) suggested to place kabuli type in the species to be
named C. kabulium in the section Arietaria of the genus Cicer distinguishing it from
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common type by its large white flowers and large white seeds, and also by chromosome number (2n = 16) as he found 2n = 14 in common type. But it is several times established that both the types have same number of chromosomes (2n = 16).

The bands in C. arietinum had homologues in other wild species examined in the present study. However, none of these profiles possessed all 7 C. arietinum bands. The profile of C. bijugum was of 10 bands, 3 of which had homologues with those of common type C. arietinum and 4 of which had homologues with those of kabuli type C. arietinum, and their similarity indices were 35-29 and 44-45% respectively. Although C. bijugum is more close to the common type than the kabuli type from morphological point of view but that similarity did not reflect in the protein profile. C. bijugum showed more close association with kabuli type. Nine bands in C. bijugum were reported by Ladizinsky and Adler (1976b) of which 4 had homologues in C. arietinum profile. C. bijugum showed very poor relation in the protein profile of C. cuneatum, C. judaicum and C. pinnatifidum, where C. bijugum had only one homologue in the profile of these 3 species with 11-11, 9-52 and 10% similarity. However, Ladizinsky and Adler (1976b) found 4 and 2 bands of C. bijugum showing similarity in the protein profile of C. judaicum and C. pinnatifidum respectively. The morphological characters of these 4 species display tremendous variation particularly in the shape, size and colour of seeds, number of leaflets, photoperiodical response etc. Similarity indices in the protein profile between C. bijugum and C. reti-
Table 1. Number and kind of bands in some species of *Cicer* and *F₁*.

<table>
<thead>
<tr>
<th>Species/Species/</th>
<th>Total number</th>
<th>Kind of bands</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of bands</strong></td>
<td><strong>Thin and distinct band</strong></td>
<td><strong>Diffuse band</strong></td>
</tr>
<tr>
<td><em>C. arietinum</em></td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td><em>C. arietinum</em></td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td><em>C. arietinum</em></td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td><em>C. arietinum</em></td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td><em>C. arietinum</em></td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td><em>C. arietinum</em></td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td><em>C. arietinum</em></td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td><em>C. arietinum</em></td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td><em>C. cuneatum</em></td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td><em>C. cuneatum</em></td>
<td>11</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2. Estimation of per cent similarity indices for protein bands in some *Cicer* species.

<table>
<thead>
<tr>
<th>Species/Species/</th>
<th>C. arietinum</th>
<th>C. bijugum</th>
<th>C. cuneatum</th>
<th>C. judaicum</th>
<th>C. pinnatifidum</th>
<th>C. reticulatum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ICC-8923</strong></td>
<td>71.43</td>
<td>35.29</td>
<td>26.67</td>
<td>22.22</td>
<td>35.29</td>
<td>66.67</td>
</tr>
<tr>
<td><strong>Jm-2103</strong></td>
<td>44.45</td>
<td>26.67</td>
<td>11.11</td>
<td>5.22</td>
<td>35.29</td>
<td>53.33</td>
</tr>
<tr>
<td><strong>ISC-8923</strong></td>
<td>11.11</td>
<td>11.11</td>
<td>9.52</td>
<td>10.00</td>
<td>22.22</td>
<td>22.22</td>
</tr>
<tr>
<td><strong>NEWC-30</strong></td>
<td>21.05</td>
<td>22.22</td>
<td>12.50</td>
<td>12.50</td>
<td>28.57</td>
<td>52.63</td>
</tr>
<tr>
<td><strong>Jm-188</strong></td>
<td>22.22</td>
<td>22.22</td>
<td>22.22</td>
<td>22.22</td>
<td>22.22</td>
<td>22.22</td>
</tr>
<tr>
<td><strong>NEWC-21</strong></td>
<td>87.50</td>
<td>87.50</td>
<td>87.50</td>
<td>87.50</td>
<td>87.50</td>
<td>87.50</td>
</tr>
</tbody>
</table>

C. cuneatum was 22.22%. Two bands of *C. bijugum* had homologues in *C. reticulatum* profile. Morphologically these two species are somewhat closer than *C. cuneatum*, *C. judaicum* and *C. pinnatifidum*, and this similarity, however, reflected a little in the protein profile study. *C. cuneatum* is the only species which shows climbing habit and more number of leaflets per leaf and differs apparently from other annual species of
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The seed protein profile of *C. cuneatum* was composed of 8 bands, two of which had homologues in *C. arietinum* (both common and kabuli type) profile. Ladizinsky and Adler (1976b) reported 7 bands in *C. cuneatum* two of which had homologues in *C. arietinum* profile. With *C. judaicum*, *C. pinnatifidum* and *C. reticulatum*, *C. cuneatum* showed 21-05, 22-23 and 12-50% similarity in the protein profile. Out of 8 bands, two had homologues in *C. judaicum* and *C. pinnatifidum*, but only one had homologue in *C. reticulatum*. *C. judaicum* possessed 11 bands, 3 of which had homologues in the protein profile of *C. pinnatifidum*. The protein profile of *C. pinnatifidum* was composed of 10 bands. However, Ladizinsky and Adler (1976b) found 9 and 7 bands for these two species respectively. In the present study these two species showed 28-57% similarity indices in their protein profile. Morphologically they differ from each other in leaflet shape, number of leaflets and seed size. Maesen (1972) following more detailed morphological comparisons decided to keep them as two separate species, since various botanists considered them as variants of the same species. Ladizinsky and Adler (1976b) strongly supported this conclusion by the difference in their seed protein profile. They further mentioned that of the total 16 bands in the profile of these two species, only 3 pairs could be matched. The present investigation has confirmed the homology of 3 pairs of bands out of 18. Thus the conclusion made by Maesen (1972) is strongly supported by the difference in their seed protein profile. On the other hand *C. judaicum* showed more similarity in the protein profile of *C. reticulatum* than that of *C. bijugum*, *C. cuneatum* and *C. pinnatifidum*. However, *C. judaicum* showed different similarity indices with two strains of *C. reticulatum*, i.e., with NEWC-21 similarity index was 52-63% and with Jm-2106 it was 42-10%. Out of 11 bands in *C. judaicum*, 5 bands had homologues in the protein profile of NEWC-21 and 4 had homologues in that of Jm-2106. Morphologically *C. judaicum* and *C. reticulatum* display tremendous variation. *C. pinnatifidum* had two homologues in the protein profile of both the strains of *C. reticulatum* and similarity index was only 22-22%. This species also differs tremendously in morphological characteristics from *C. reticulatum*. The similarity index in protein profile of NEWC-21 and Jm-2106 was 87-50%. NEWC-21 had 6 homologues in the protein profile of Jm-2106. On the other hand both the strains of *C. reticulatum* had 5 homologues in the protein profile of common type *C. arietinum* with 66-67% similarity. Out of 8 bands in both the strains of *C. reticulatum* 4 bands had homologues in the protein profile of kabuli type *C. arietinum* and similarity index between them was 53-33%. Morphologically, *C. arietinum* (common type) and *C. reticulatum* are very close than any other species examined in the present study. They differ from each other mainly in the seed coat structure. *C. arietinum* (kabuli type) differs from *C. reticulatum* mainly by flower colour and the shape, size and colour of seeds. Ladizinsky and Adler (1976b) reported 6 bands in the protein profile of *C. reticulatum* and all of which had homologues in *C. arietinum* and it lacked only one band. Except *C. arietinum* and *C. judaicum*, all the species possessed some typical bands in their protein profile in the sense that no one was found at that particular *R*<sub>f</sub> in other species. *C. bijugum* had two thin and distinct bands at *R*<sub>f</sub> = 0.40 and 0.82. *C. cuneatum* had 3 thin and distinct bands at *R*<sub>f</sub> = 0.32, 0.56 and 0.95. *C. pinnatifidum* had a single thin and distinct band at *R*<sub>f</sub> = 0.25. Ladizinsky and Adler (1976b) found two such typical bands at *R*<sub>f</sub> = 0.64 and 0.69 in the protein profile of *C. pinnatifidum*. *C. reticulatum* (Jm-2106) had also one dense band at *R*<sub>f</sub> = 0.69 in the present investigation. It was constant in species specific character and therefore it may be considered as conservative characteristic in their protein profile.
As regards the similarity indices between hybrids and their respective parents (table 3), it was found that each cross showed more than 50% homology with their respective parents. This proves the greater diversity of the hybrids over pure lines (Singh et al. 1983). Protein profile in the hybrid of *C. arietinum* (H-208) × *C. reticulatum* (NEWC-21) was composed of 9 bands, 5 of which had homologues in the protein profile of both the parents. The similarity indices of the hybrid with its maternal and paternal parent were 62.50 and 58.50% respectively. Thus it appeared that loci controlling the bands in maternal parent were dominant. The hybrid of *C. arietinum* (ICC-8923) × *C. reticulatum* (Jm-2106) had 11 bands, 5 of which had homologues in the protein profile of maternal parent and 6 had homologues in that of paternal parent with 55.56 and 63.16% similarity indices respectively. Thus it appeared that loci controlling the bands in paternal parent were dominant. Three bands (R of 0.36, 0.86 and 0.93) present in the hybrid of *C. arietinum* (H-208) × *C. reticulatum* (NEWC-21) were absent in both the parents, showing complementary nature of their inheritance. Similar bands (R of 0.21, 0.36, 0.63 and 0.80) of the hybrids of *C. arietinum* (ICC-8923) × *C. reticulatum* (Jm-2106) were absent in both the parents. This discrepancy in effect at band level suggested that reliable inferences regarding the inheritance of individual bands can not be drawn unless individual F₂ seeds in all the possible crosses are analyzed electrophoretically (Singh et al. 1983).

On the basis of seed protein profile of 6 species of *Cicer* and F₁s, it appeared that *C. reticulatum* was close to the cultivated species *C. arietinum* than any other wild species examined in the present study. The next most interesting point in the evolution of annual species was the origin of cultivated species. The seed protein profile of this species was found as constant conservative character which indicated monophyletic origin. Ladizinsky and Adler (1976) stated that if the seed protein profile of the present day *C. arietinum* cultivars was virtually with that of the type domesticated thousands of years ago, it was probably also similar to that of its wild progenitor and hence the latter should be detectable among the wild species. In the present study, none of the wild species had identical profile to that of the cultivated species and from this point of view none could be regarded as the immediate progenitor of cultivated species. On the basis of similarity indices in the protein profile of *C. arietinum* and *C. reticulatum* and also of their F₁s, *C. reticulatum* might be the wild progenitor of *C. arietinum* or its close relative.

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