Differential Fluorescent Banding in Nine Varieties of *Cicer arietinum* L.

Kazi Nahida Begum¹ and Sheikh Shamimul Alam²*

¹Department of Botany, Jagannath University, Dhaka-1100, Bangladesh
²Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

Received January 7, 2016; accepted July 5, 2016

Summary  Nine varieties of *Cicer arietinum* L. (chickpea) released by Bangladesh Agriculture Research Institute (BARI), viz. BC-1 (BARI Chola-1), BC-2, BC-3, BC-4, BC-5, BC-6, BC-7, BC-8 and BC-9, represented a broad spectrum of variation for several phenotypic and agronomic traits. For authentic characterization, these varieties were investigated cytogenetically by differential fluorescent banding. The nine chickpea varieties were found to possess 2n=16 metacentric chromosomes. A wide range of CMA-positive bands (5–20) was found in the metaphase chromosomes of the nine varieties. Two satellites were found in the first pair of only BC-2, BC-3, BC-4, BC-6, BC-8 and BC-9 after DAPI-staining. No satellite was found after CMA-staining. The differential staining property of satellites revealed the stain specific nature of these satellites. Entirely DAPI-fluoresced chromosomes were frequent in these varieties. The number, location and distributions of GC- and AT-rich repeats are specific for each variety. Some CMA- and DAPI-bands were so unique that these chromosomes could easily be used as marker chromosomes for the respective varieties. Fluorescent banding revealed the occurrence of genomic alteration within these varieties. Therefore, each variety could be characterized authentically by fluorescent banding analysis.

Key words  CMA, DAPI, Karyotype, Chickpea.

*Cicer arietinum* L. (Chickpea) is an annual grain legume crop grown mainly for human consumption. It plays an important role in human nutrition as a source of protein, energy, fiber, vitamins and minerals for large population sectors in the developing world and is considered a healthy food in many developed countries.

Being an important pulse crop, breeders of Bangladesh have been trying to develop a better variety in respect of both yield and disease resistant capability. As a consequence, the Bangladesh Agriculture Research Institute (BARI) has released nine varieties of this species, namely BC-1, BC-2, BC-3, BC-4, BC-5, BC-6, BC-7, BC-8 and BC-9 (Mandal et al. 2011).

A successful breeding program depends on the complete knowledge and understanding of the genetic diversity within and among genetic resources of the available varieties. This will enable plant breeders to choose parental sources that generate diverse populations for selection (Esmail et al. 2008).

The earlier workers tried to characterize chickpea by cytotological research (Mercy et al. 1974, Lavania and Lavania 1982, Sharma and Gupta 1986, Venora et al. 1991, 1995, Galasso and Pignone 1992, Ocampo et al. 1992, Tayyar et al. 1994). Due to high condensation tendency and small size, the chromosomes of this species are difficult to stain and examine. As a result, comparative study of chromosome morphology and detail karyotypic features among genotypes in *Cicer arietinum* L. has not been well documented. Therefore, most of the earlier researches were confined to the counting of diploid chromosome number. As a consequence, information about detailed karyotypic features of chickpea is not available.

Karyotype analysis is one of the measures for proper characterization since it is a stable character and specific for each specimen. However, it is difficult in the varietal level as well as cultivars, as varieties usually possess the same chromosome numbers and even the same karyotypes (Sultana and Alam 2007, Sema et al. 2013, Afroz et al. 2013, Kondo and Hizume 1982, Hizume et al. 1988). In addition, conventional karyotype analysis is unable to express critically the differences among different germplasms of a species. Moreover, minute deletion, inversion, tandem duplication, etc. cannot be detected by conventional karyotype analysis.

In such a situation, modern cytogenetic approaches should be undertaken for comparative study among different varieties of a species. Using fluorescent banding with chromomycen A₃ (CMA) and 4’,6-diamidino-2-phenylindole (DAPI), it is now possible to determine the arrangement of GC-and AT-rich repeats in the genome, respectively (Akter and Alam 2005, Alam and Kondo 1995). With the help of fluorescent banding it was possible to identify even a particular chromosome (Schweizer 1976, Kondo and Hizume 1982, Hizume et al. 1988, Alam and Kondo 1995). It is well known that each chro-
mosome has one or more micro satellites composed of repetitive sequences. These are germplasm specific. If it is possible to mark those areas of chromosomes, karyotypes of a specimen could be authentically identified.

In the present study, the nine chickpea varieties were characterized on the basis of differential fluorescent karyotype analysis.

Materials and methods

The nine varieties of *Cicer arietinum* L., viz. BC-1 to BC-9, were collected from the Pulse Research Center (PRC) of BARI. The accession number and pedigree of each variety are shown in Table 1. These nine varieties were maintained in the Botanic garden, Department of Botany, University of Dhaka.

Cytogenetical study

Healthy roots were collected from the mature plants and pretreated with 0.002 M 8-hydroxy quinoline for 1.5 h at 18°C followed by 15 min fixation in 45% acetic acid at 4°C. These were then hydrolyzed in a mixture of 1 N HCl and 45% acetic acid (2:1) at 60°C for 20 s. The root tips were stained and squashed in 45% acetic acid. For CMA- and DAPI banding, Alam and Kondo’s (1995) method was used with slight modification. The cover glasses were removed quickly from dry ice and allowed to air dry for at least 24 h before study. The air-dried slides were first pre-incubated in McIlvaine’s buffer (pH 7.0) for 30 min followed by Distamycin A (0.1 mg mL⁻¹) treatment for 10 min. The slides were rinsed mildly in McIlvaine’s buffer supplemented with MgSO₄ (5 mM) for 15 min. One drop of CMA (0.1 mg mL⁻¹) was added to the materials for 15 min in a hummid chamber and then rinsed with McIlvaine’s buffer with MgSO₄ for 10 min. Slides were mounted in 50% glycerol and kept at 4°C overnight before observation. These were observed under a Nikon (Eclipse 50i) fluorescent microscope with blue violet (BV) filter cassette. For DAPI-staining, after 24 h of air drying, the slides were first pre-incubated in McIlvaine’s buffer (pH 7.0) for 30 min and treated in Actinomycin D (0.25 mg mL⁻¹) for 10 min in a humid chamber. The slides were immersed in DAPI solution (0.01 mg mL⁻¹) for 20 min and mounted with 50% glycerol. These were observed under a Nikon (Eclipse 50i) fluorescent microscope with an ultra violet (UV) filter cassette.

Results and discussion

Nine varieties of *Cicer arietinum* L. were cytogenetically studied after CMA- and DAPI-staining for authentnic characterization.

Table 1. Accession number and pedigree of nine varieties of *Cicer arietinum* L.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Accession number</th>
<th>Pedigree</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC-1</td>
<td></td>
<td>Native</td>
</tr>
<tr>
<td>BC-2</td>
<td>ICCL-83228</td>
<td>P 1231×P 1265</td>
</tr>
<tr>
<td>BC-3</td>
<td>ICCL-83105</td>
<td>(K 850×T 3)×(JG 62×BEG 482)</td>
</tr>
<tr>
<td>BC-4</td>
<td>ICCL-85222</td>
<td>HMS 10×(P 436×H 223)</td>
</tr>
<tr>
<td>BC-5</td>
<td>RBH-228</td>
<td>ICC 14559</td>
</tr>
<tr>
<td>BC-6</td>
<td>ICCL-83149</td>
<td>(G 130×B 108)×(NP 34×GW 5/7)</td>
</tr>
<tr>
<td>BC-7</td>
<td>ICC-3274</td>
<td>P 3864-l</td>
</tr>
<tr>
<td>BC-8</td>
<td>ICCL-88005</td>
<td>(K 4×Chaffa)×ICCL 81001</td>
</tr>
<tr>
<td>BC-9</td>
<td>ICCV-95318</td>
<td>ICCV 2×ICCV 7344</td>
</tr>
</tbody>
</table>

The karyotypes of nine varieties were compared critically after CMA-staining and showed distinct CMA-banding pattern (Figs. 1–9, Table 2). Different numbers of CMA bands were found in the nine chickpea varieties, viz. 2 (BC-1, BC-4, BC-5, BC-7, BC-8), 3 (BC-3, BC-6), 4 (BC-9) and 6 (BC-2) with the percentage of GC-rich repeats ranging from 2.66 (BC-8) to 14.52 (BC-2) (Table 2). Moreover, the distribution and intensities of CMA bands varied in different varieties. Most of the CMA bands were present at the upper terminal regions of the respective chromosomes in the nine chickpea varieties (Figs. 19–27, Table 2). The presence of terminal CMA bands indicated a tendency of accumulating GC-rich repetitive sequences at the chromosomal ends (Zaman and Alam 2009). In addition to terminal bands, a few CMA bands were found at centromeric regions of the respective chromosomes in BC-2, BC-3, BC-4, BC-7, BC-8 and BC-9 varieties, revealing the presence of GC repeats in those regions (Figs. 20, 21, 22, 25, 26, 27).

**CMA-karyotype**

The karyotypes of nine varieties were compared critically after CMA-staining and showed distinct CMA-banding pattern (Figs. 1–9, Table 2). Different numbers of CMA bands were found in the nine chickpea varieties, viz. 2 (BC-1, BC-4, BC-5, BC-7, BC-8), 3 (BC-3, BC-6), 4 (BC-9) and 6 (BC-2) with the percentage of GC-rich repeats ranging from 2.66 (BC-8) to 14.52 (BC-2) (Table 2). Moreover, the distribution and intensities of CMA bands varied in different varieties. Most of the CMA bands were present at the upper terminal regions of the respective chromosomes in the nine chickpea varieties (Figs. 19–27, Table 2). The presence of terminal CMA bands indicated a tendency of accumulating GC-rich repetitive sequences at the chromosomal ends (Zaman and Alam 2009). In addition to terminal bands, a few CMA bands were found at centromeric regions of the respective chromosomes in BC-2, BC-3, BC-4, BC-7, BC-8 and BC-9 varieties, revealing the presence of GC repeats in those regions (Figs. 20, 21, 22, 25, 26, 27).

**Heteromorphism in respect of CMA-staining** was found in chromosome pair II of BC-2. In this pair, one chromosome had upper terminal band and an interstitial band was present in its homologue. This banding pattern indicated the probable occurrence of a paracentric inversion either from terminal to interstitial or vice versa (Fig. 20).

Pair II of BC-3, pair I and V of BC-4, pair VIII of BC-6, pair VI and VII of BC-7, pair I and VI of BC-8 and pair I, III, IV and VIII of BC-9 showed heteromorphism in respect of CMA banding pattern (Figs. 21, 22, 24, 25, 26, 27). In these pairs, a member had a CMA

### Table 2. Comparative CMA- and DAPI-karyotype analysis in nine varieties of *Cicer arietinum* L.

<table>
<thead>
<tr>
<th>Variety</th>
<th>No. of CMA bands</th>
<th>% of GC-rich banded region</th>
<th>CMA-banded karyotype formula</th>
<th>No. of DAPI bands</th>
<th>DAPI-satellites</th>
<th>% of AT-rich banded region</th>
<th>DAPI-banded karyotype formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC-1</td>
<td>2</td>
<td>4.69</td>
<td>(2\alpha + 1\delta)</td>
<td>4</td>
<td>—</td>
<td>7.04</td>
<td>(2\alpha + 1\delta)</td>
</tr>
<tr>
<td>BC-2</td>
<td>6</td>
<td>14.52</td>
<td>(5\alpha + 1\Omega + 10\delta)</td>
<td>2</td>
<td>2</td>
<td>2.53</td>
<td>(2\alpha + 1\delta)</td>
</tr>
<tr>
<td>BC-3</td>
<td>3</td>
<td>8.98</td>
<td>(2\alpha + 1\Omega + 13\delta)</td>
<td>—</td>
<td>2</td>
<td>4</td>
<td>1(\alpha + 1\delta)</td>
</tr>
<tr>
<td>BC-4</td>
<td>2</td>
<td>4.94</td>
<td>(1\alpha + 1\Omega + 14\delta)</td>
<td>1</td>
<td>2</td>
<td>1.32</td>
<td>1(\Omega + 1\delta)</td>
</tr>
<tr>
<td>BC-5</td>
<td>2</td>
<td>6.36</td>
<td>(2\alpha + 14\delta)</td>
<td>4</td>
<td>—</td>
<td>11.13</td>
<td>(2\alpha + 2\beta\delta)</td>
</tr>
<tr>
<td>BC-6</td>
<td>3</td>
<td>7.09</td>
<td>(2\alpha + 1\Psi + 13\delta)</td>
<td>4</td>
<td>2</td>
<td>5.91</td>
<td>3(\alpha + 1\Omega + 1\delta)</td>
</tr>
<tr>
<td>BC-7</td>
<td>2</td>
<td>2.96</td>
<td>(1\alpha + 1\Omega + 14\delta)</td>
<td>4</td>
<td>—</td>
<td>11.83</td>
<td>4(\alpha + 1\Omega + 1\delta)</td>
</tr>
<tr>
<td>BC-8</td>
<td>2</td>
<td>2.66</td>
<td>(1\alpha + 1\Psi + 14\delta)</td>
<td>2</td>
<td>—</td>
<td>2.66</td>
<td>2(\alpha + 1\Omega + 1\delta)</td>
</tr>
<tr>
<td>BC-9</td>
<td>4</td>
<td>6.29</td>
<td>(2\alpha + 2\Omega + 12\delta)</td>
<td>4</td>
<td>2</td>
<td>15.12</td>
<td>2(\beta + 2\beta\delta)</td>
</tr>
</tbody>
</table>

\(\alpha\)=Band in terminal region of short arm, \(\beta\)=Band in terminal region of long arm, \(\lambda\)=Band in terminal region of short arm and long arm, \(\Omega\)=Band in centromeric region, \(\Psi\)=Dot-like band, \(\theta\)=Fluoresced almost throughout the length and \(\delta\)=No band.

band while no such band was observed in its homologue suggesting deletion of the banded region from the respective chromosomes.

Therefore, the pairwise CMA banding comparison revealed the occurrence of structural aberration such as deletion, tandem duplication and inversion. Generally GC-rich repeats are heterochromatic in nature (Schweizer 1976). As a result, the changes in heterochromatic region did not have an acute impact on the morphology of a variety.

**DAPI-karyotypes**

The karyotype of nine varieties showed distinct banding pattern after DAPI-staining (Figs. 1–18, Table 2). Different numbers of DAPI bands were found in the nine chickpea varieties, viz. 1 (BC-4), 2 (BC-2, BC-8) and 4 (BC-1, BC-5, BC-6, BC-7, BC-9) with the percentage of AT-rich repeats ranging from 0.00 to 15.12 (Table 2).

Some chromosomes fluoresced entirely with DAPI fluorochrome (Figs. 32, 36, Table 2). The occurrence of entirely fluoresced chromosome indicated that AT-rich repeats were less confined and rather distributed throughout the length of banded chromosomes. The probable reason for entire fluorescence was i) either these chromosomes were completely AT-rich by nature or ii) due to successive duplication of AT-rich repeats (Hiron et al. 2006, Mahbub et al. 2007). In addition, some terminal and few centromeric bands were also observed in the nine chickpea varieties. A pair of centromeric DAPI bands was found, one in each member of pair I in BC-6, BC-7 and BC-8 (Figs. 33, 34, 35, arrow). However, no such band was found in this pair in the other varieties. One pair upper terminal DAPI band was observed in BC-2 and BC-6 (Figs. 29, 33). This data indicated the occurrence of deletion of the banded region from this pair in the other varieties.

Heteromorphism in respect of DAPI band was found in three varieties, viz. BC-1 (pair III, IV, V, VII), BC-4 (pair II) and BC-5 (pair II, V). In these varieties, DAPI bands were found only in a member of respective pairs whereas no such band was present in their homologue member (Figs. 28, 31, 32). Heteromorphism in DAPI banding pattern was reported earlier by several workers in different specimens (Afroz et al. 2013, Zaman and Alam 2009, Khatun and Alam 2010). In the earlier reports the authors mentioned that deletion was the result of heteromorphism. Therefore, heteromorphism found in the present study was due to deletion of the banded portion from the respective chromosome.

A pair of dot-like DAPI bands was found on the upper terminal region in two chromosomes of BC-6 (Fig. 33, arrow head). These types of banding chromosome were not found in the other varieties. These chromosomes could easily be isolated from the rest due to their unique DAPI-banding pattern; thus these chromosomes could be used as marker chromosomes for this variety.

**Satellite**

A pair of satellites was found, one in each member of pair I, in all varieties except BC-1, BC-5 and BC-7 after DAPI staining (Figs. 29, 30, 31, 33, 35, 36, arrow). On the other hand, no satellite was found in any variety after CMA staining. This preferential staining revealed that these satellites were staining specific. Alam and Kondo (1995) also reported on the nature of a satellite and a small chromosome in *Drosera ericksonea*. They carried out sequential staining of the same metaphase plate with Giemsa, CMA and DAPI. They found a satellite and a small chromosome in Giemsa and DAPI, whereas it was not observed in CMA. From these observations, they suggested that there were stain specific satellites and chromosomes. In the present study, the pair of satellites that were not observed after staining with CMA might possess some kind of DNA sequence which made it stain-specific. However, no report about stain specific satellite was available for *Cicer arietinum*.

The above results regarding differential fluorescent karyotype analysis revealed a number of reshuffling of GC- and AT-rich segments among the genome of the nine varieties. This redistribution made diversification in the karyotypes of the nine chickpea varieties. Therefore, the nine varieties of *Cicer arietinum* L. could be characterized authentically by differential fluorescent banding.

**Acknowledgements**

Kazi Nahida Begum is grateful to Jagannath University, Dhaka for approving study leave during the research period. This work was supported by the Ministry of Science and Technology, People’s Republic of Bangladesh for the “Bangabandhu Scholarship” (P012/2010).

**References**


