Development of Marker Chromosomes in Three Varieties of
Vigna radiata L. (Fabaceae)

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Summary Karyotype analysis of 3 released varieties in Vigna radiata L. viz. Barimung-2, Barimung-3, Barimung-5 has been carried out after staining with orcein, CMA and DAPI. These varieties were found to possess 2n=22 chromosomes. Four terminal CMA-positive bands were found in Barimung-2, 3 such bands in Barimung-3 and 4 in Barimung-5. The terminal CMA positive bands showed deep DAPI-negative reversible bands in the 3 varieties of V. radiata. One chromosome of Barimung-5 was fluoresced entirely with CMA and DAPI. DAPI-positive and DAPI-negative band occurred in another chromosome of Barimung-5. A pair of interstitial CMA-negative band was observed in chromosome pair V of only Barimung-2. These portions were stained brightly with DAPI. These kinds of reversible banded chromosomes could be easily identified. Polymorphism regarding the number and location of DAPI-positive bands were observed in these varieties. That may due to either minute deletion or high condensation of heterochromatic region at the respective loci. Few chromosomes of each variety showed characteristic CMA and DAPI bands. Therefore, with the help of CMA and DAPI it was possible to develop marker chromosomes for authentic identification of three varieties in V. radiata.

Key words Fluorescent banding, Marker chromosome, Vigna.

Vigna L. is a member of grain legumes belongs to Fabaceae. It has above 200 species distributed throughout the world (Fery 2002). Vigna radiata L. (locally known as ‘mung’, ‘sonamung’ etc.) is an important grain legume. Mung has originated in south and south-east Asia (India, Myanmar, Thailand region) and widely grown in India, Pakistan and Bangladesh. It is also distributed in different areas of East and central Africa, West Indies, USA and Australia. In Bangladesh, about 70% of mungbeans are grown in the southern districts viz. Barisal, Jhalakati, Patuakhali, Barguna, Bhola and Pirojpur.

Being legume, mungbean has some important agronomic characters. These are: (i) can grow in flood free soil nearly throughout the year, ii) able to fix atmospheric nitrogens, thereby reducing the cost of production, iii) adaptable to a wide range of environment, iv) rich in protein and minerals v) tolerant to water logging condition, vi) green parts used as animal fodder etc. (Gowda and Kaul 1982).

The above mentioned features of V. radiata attracted the plant scientists and agriculturists in Bangladesh. Bangladesh Agricultural Research Institute (BARI) and Bangladesh Institute of Nuclear Agriculture (BINNA) have been collected different germplasms of these species from different parts of the country. At present they have released 14 improved mungbean varieties in Bangladesh (Afzal et al. 2004).

The germplasms and released varieties of Vigna radiata are characterized solely on the basis...
of their morphological features. This kind of characterization is not reliable, because due to phenotypic plasticity a released variety or any germplasm may show morphological change in different environment. It creates problems like overlapping, misidentification etc. Therefore, an authentic characterization and streamlining of these germplasms becomes essential otherwise the conservation would be meaningless.

It is well known that karyotype analysis is one of the dependable ways by which genomic characterization of any germplasm would be possible authentically. Classical method of karyotypic characterization in varietal level is not possible since varieties usually possess same chromosome numbers and even same karyotypes. However, there are some modern cytogenetic methods by which karyotypes of varieties could be characterized. Fluorescent chromosome banding is one of such techniques. Two common and effective fluorochromes viz. Chromomycin A3 (CMA) and 4–6′ Diamidino-2-phenyl Indole (DAPI) have been widely using in cytogenetics (Schweizer 1976, Kondo and Hizume 1982, Hizume et al. 1988, Alam and Kondo 1995, Alam et al. 1995). CMA and DAPI bind with GC- and AT-rich repeats in the genome, respectively. With this technique it is possible to mark individual chromosome and to know the amount/distribution of GC- and AT-rich repeats in a particular karyotype (Alam and Kondo 1995, Alam et al. 1995).

Recently Akter and Alam (2005) were able to characterize the karyotypes of 3 different varieties in Cicer arietinum with the help of differential fluorescent banding. Now it is important to know whether the same techniques are applicable for characterizing the karyotypes of other legumes. With this view the present study was undertaken to develop marker chromosomes for authentic characterization of 3 varieties in V. radiata.

Materials and methods

The following 3 varieties of V. radiata viz Barimung-2, Barimung-3 and Barimung-5 were studied in this investigation. These varieties were collected from the gene bank of BARI. The plants were grown and maintained in the Botanic garden, Department of Botany, University of Dhaka, Bangladesh.

Healthy roots were collected and pretreated with 2 mM 8-hydroxyquinoline for 2.20 h at room temperature followed by 15 min fixation in 45% acetic acid at 4°C. These were then hydrolysed in a mixture of 1 N HCl and 45% acetic acid (2 : 1) at 60°C for 5 s. The root tips were stained and squashed in 1% aceto orcein.

For fluorescent banding, Alam and Kondo’s (1995) method was used with slight modification. After hydrolysing and dissecting, the materials were squashed with 45% acetic acid. The cover glasses were removed quickly on dry ice and allowed to air dry for at least 48 h before study. The air-dried slides were first preincubated in McIlvaine’s buffer (pH 7.0) for 30 min followed by Distamycin A (0.1 mg/ml) 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) was added to the materials for 15 min and rinsed with McIlvaine’s buffer with Mg2+ for 10 min. Slides were mounted in 50% glycerol and kept at 4°C for overnight before observation. These were observed under Nikon (UFX-IIA) fluorescent microscope with Blue Violet (BV) filter cassette. After CMA-staining, same slides were used for DAPI-staining. Slides were rinsed with distilled water followed by destaining in 45% acetic acid for 15 min, washed in distilled water and air dried overnight. The destained preparations were immersed in McIlvaine’s buffer (pH 7.0) for 20 min and treated in Actinomycin D (0.25 mg/ml) for 10 min. The slides were then immersed in DAPI solution (0.01 mg/ml) for 15 min and mounted with 50% glycerol. These were observed under a Nikon (UFX-IIA) fluorescent microscope with Ultra Violet (UV) filter cassette.
Results and discussion

CMA-banding

Three varieties of *Vigna radiata* were found to possess \(2n=22\) chromosomes. These varieties showed two common characteristics in CMA-staining such as (i) CMA-positive bands appeared at the terminal regions of respective chromosomes and (ii) bands were thick, bright and prominent (Figs. 1, 2, 3, 7, 9 and 11 arrows).

On the other hand the 3 varieties of *V. radiata* showed mark differences in CMA-banding pattern. Barimung-2 possessed four terminal CMA-positive bands. These were located on both the member of pair III and VIII (Figs. 1, 7 arrows). In Barimung-3, there were only 3 terminal CMA-positive bands of which two on both the members of pair II and one in a member of pair III (Figs. 2, 9 arrows). Four CMA-positive bands were found in Barimung-5. Both the members of pair II in this variety had a terminal CMA-positive band. One member of pair V had a terminal CMA-positive band while the other fluoresced entirely (Figs. 3, 11 thin arrow).

The noticed polymorphism of CMA-positive banding pattern among the 3 varieties of *V. radiata* are: (i) pair II of Barimung-3 and Barimung-5 had CMA-positive bands but there was no band in pair II of Barimung-2 (Figs. 7, 9, 11 arrows), (ii) pair III of Barimung-2 had two terminal CMA-positive bands but one band found in pair III of Barimung-3 (Figs. 1, 2, 7, 9 arrows), (iii) pair VIII of Barimung-2 had 2 bands whereas no band was found in pair VIII in Barimung-3 and Barimung-5 (Figs. 7, 9, 11 arrows). The reason of Polymorphism between homologous chromosomes is unclear. However, it indicates either the probable occurrence of minute structural aberration or due to highly condensation of heterochromatic region at metaphase that prevented CMA to bind.

A pair of interstitial CMA-negative band was found in chromosome pair V of Barimung-2 (Figs. 1, 7 arrow head). This kind of band is totally absent in other varieties. The CMA-negative banded chromosomes of this variety have become a unique and could easily be identified. This kind of banding pattern was absent in the other two varieties. This chromosome was also unique and could easily be identified.

DAPI-banding

The three varieties of *V. radiata* showed two common features in counter-staining with DAPI such as: (i) DAPI-negative bands occurred exactly at the same location where CMA-positive bands were present, (ii) since the DAPI-negative bands appeared at the terminal region, the respective chromosomes looked shorter when compared to that of CMA-positive one (Figs. 4, 5, 6, 8, 10, 12 arrowhead). Reversible banding (CMA-positive and DAPI-negative) indicated that those portions were completely rich in GC-repeats.

Polymorphism regarding the number and distribution of DAPI-positive bands was observed in these varieties. DAPI-positive bands indicated the presence of AT-repeats. Eight DAPI-positive bands were found in Barimung-2 (Figs. 4, 8 arrows). Members of pair V in this variety, showed interstitial bright DAPI-positive bands. A pair of CMA-negative bands was found at the same locations (Figs. 7, 8, arrowhead). The reversible banding (DAPI-positive and CMA-negative) indicates the presence of complete AT-rich areas at those portions. Two and 4 DAPI-positive bands were found in Barimung-3 and Barimung-5, respectively (Figs. 5, 6, 10, 12 arrows).

Marker chromosomes

Few chromosomes in 3 varieties of *V. radiata* showed unique characters after staining with CMA and DAPI. The reversible banded chromosomes of 3 varieties (CMA-positive and DAPI-negative) could easily be identified (Figs. 1–12 arrows, arrowheads). Pair V of Barimung-2, showed a CMA-negative band at the interstitial area of short arm (Figs. 1, 7 arrowhead). CMA-negative band was not observed in other chromosomes of not only this variety but other varieties also. This CMA-
Figs. 1–12. CMA- and DAPI-stained mitotic metaphase chromosomes and karyotypes of three varieties in *Vigna radiata* L. 1) CMA-stained mitotic metaphase of Barimung-2, 2) CMA-stained mitotic metaphase of Barimung-3, 3) CMA-stained mitotic metaphase of Barimung-5, 4) DAPI-stained mitotic metaphase of Barimung-2, 5) DAPI-stained mitotic metaphase of Barimung-3, 6) DAPI-stained mitotic metaphase of Barimung-5, 7) CMA-stained karyotype of Barimung-2, 8) DAPI-stained karyotype of Barimung-2, 9) CMA-stained karyotype of Barimung-3, 10) DAPI-stained karyotype of Barimung-3, 11) CMA-stained karyotype of Barimung-5, 12) DAPI-stained karyotype of Barimung-5. Bar=10 μm.
negative banded area showed bright DAPI-positive bands (Figs. 4, 8 arrowhead). This reversible banding pattern (CMA-negative and DAPI-positive) made these 2 chromosomes unique. A member of pair V in Barimung-5 fluoresced entirely with both CMA and DAPI revealed the tandem presence of GC- and AT-rich repeats (Figs. 3, 6, 11, 12 thin arrows). This kind of chromosome i.e. entirely fluoresced with both CMA and DAPI was absent in other varieties. The other member of this pair showed a terminal deep DAPI-negative band and a bright DAPI-positive band at the interstitial region of long arm (Figs. 6, 12 arrow, arrowhead). Presence of DAPI-negative and positive band in the same chromosome was not observed in any other chromosomes of theses varieties. Therefore, with the help of marker chromosomes it was possible to characterize authentically the karyotypes of 3 varieties in *V. radiata*.

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References


