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KARYOTYPE COMPARISON IN TWO VARIETIES OF *VIGNA MUNGO* L. AFTER STAINING WITH ORCEIN AND CMA.

SHEIKH SHAMIMUL ALAM* **AND MEHER NIGAR MAHBUB**

Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

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Abstract

Two varieties of *Vigna mungo viz.* Barimash-1 and Barimash-3 were cytologically studied after staining with orcein and CMA. Both the varieties were found to possess 2n =22 metacentric chromosomes. Total length of diploid complements and range of chromosome length were more or less same in the two varieties. The orcein stained interphase nucleus of Barimash-1 had three big heterochromatic blocks, whereas Barimash-3 possessed several small heterochromatins. The orcein stained prophase chromosomes of Barimash-1 was darkly stained throughout the entire length. The gradual staining of prophase chromosomes were found in Barimash-3. The CMA stained interphase nuclei and prophase chromosomes of these two varieties were different. Sixteen entirely CMA banded chromosomes were found in Barimash-1. The percentage of GC-rich areas was 56.20. In Barimash-3, 11 entirely, 4 terminal and 4 centromeric fluoresced banded chromosomes were found. The percentage of GC-rich areas was 63.40. Although the two varieties showed similar conventional karyotypes, mark differences exist in their fluorescent karyotypes and the properties of interphase nuclei and prophase chromosomes. Therefore, these two varieties could be characterized with the help of modern cytological techniques.

Introduction

Vigna mungo L., commonly known as black gram or mash is an important grain legume of our subcontinent. Black gram has been cultivated on marginal lands with minimum inputs (Paroda and Thomas 1987). In Bangladesh black gram is mostly cultivated in the Northern districts. Bangladesh Agriculture Research Institute (BARI) and Bangladesh Institute of Nuclear Agriculture (BINA) have been collected different germplasms of blackgram from different parts of the country (Afzal *et al.* 2004). So far four improved mash varieties namely Barimash-1, Barimash-2, Barimash-3 and Binamash-1 have been realeasd. The different germplasms and released varieties of *Vigna mungo* are characterized on the basis of their morphological features. Due to phenotypic plasticity, same germplasm may show morphological variations. In these case characterization on morphology will create problems like overlapping, misidentification etc. Therefore an authentic identification of these germplasm is necessary.

Karyotype is one of the parameters by which authentic identification of a specimen is possible. Since varieties usually possess same chromosome numbers and major chromosomal differences do not exist among different varieties of a species, it is not possible to characterize these by conventional cytological techniques. Fluorescent chromosome banding is one of the modern cytogenetic methods commonly used for critical karyotype analysis. Two common and effective fluorochromes *viz*. Chromomycin A3 (CMA) and 4-6' Diamidino- 2 phenyl indole (DAPI) have been widely used in Cytogenetics for karyotype analysis (Schweizer 1976, Alam and Kondo 1995, Akter and Alam 2005, Jessy *et al.* 2005, Mahbub *et al.* 2007). The present research programme was undertaken to characterize the karyotypes of two varieties of *Vigna mungo* after staining with orcein and CMA.

*Corresponding author. E-mail:ssalam81@yahoo.com

Materials and Methods

Seeds of two varieties of *Vigna mungo viz*. Barimash-1 and Barimash-3 were collected from Bangladesh Agriculture Research Institute (BARI). The plants were grown and maintained in the Botanical garden, Department of Botany, University of Dhaka.

Healthy roots were collected and pretreated in 0.002 M 8-hydroxyquinoline for 2.20 h at room temperature (25º C) followed by fixation in 45% acetic acid for 15 min at 4ºC. The root tips were hydrolyzed for 6 sec at 60º C in a solution containing 1 N HCl and 45% acetic acid (2:1) and stained with 1% aceto-orcein. For CMA staining, after hydrolysis the root tips were squashed with 45% acetic acid. The cover glasses were removed quickly and air-dried for at least 24 h before study. The slides were incubated in McIlvaine's buffer (pH 7.0) for 10 min followed by Distamycin A treatment (0.1 mg/ml). Slides were mildly rinsed in McIlvaine's buffer supplemented with 5 mM $MgSO_4$ for 10 min. One drop of CMA (0.1 mg/ml) was placed on each slide and kept for 10 min in a humid chamber. The slides were mounted in 50% glycerol and kept overnight at 4ºC. The slides were examined under a Nikon fluorescent microscope with blue-violet filter cassette.

Results and Discussion

Both the varieties of *Vigna mungo* were found to possess 2n = 22 metacentric chromosomes (Figs. 3, 6) indicating that in respect of centromeric position both the varieties are symmetrical. Total length of 2n chromosome complement and range of individual chromosome length were more or less same in these varieties (Table 1), indicating conventional method do not show any remarkable difference between these two varieties. Therefore, fluorescent banding technique methods was used.

Table 1. Comparative orcein and CMA-karyotypes in two varieties of *Vigna mungo*.

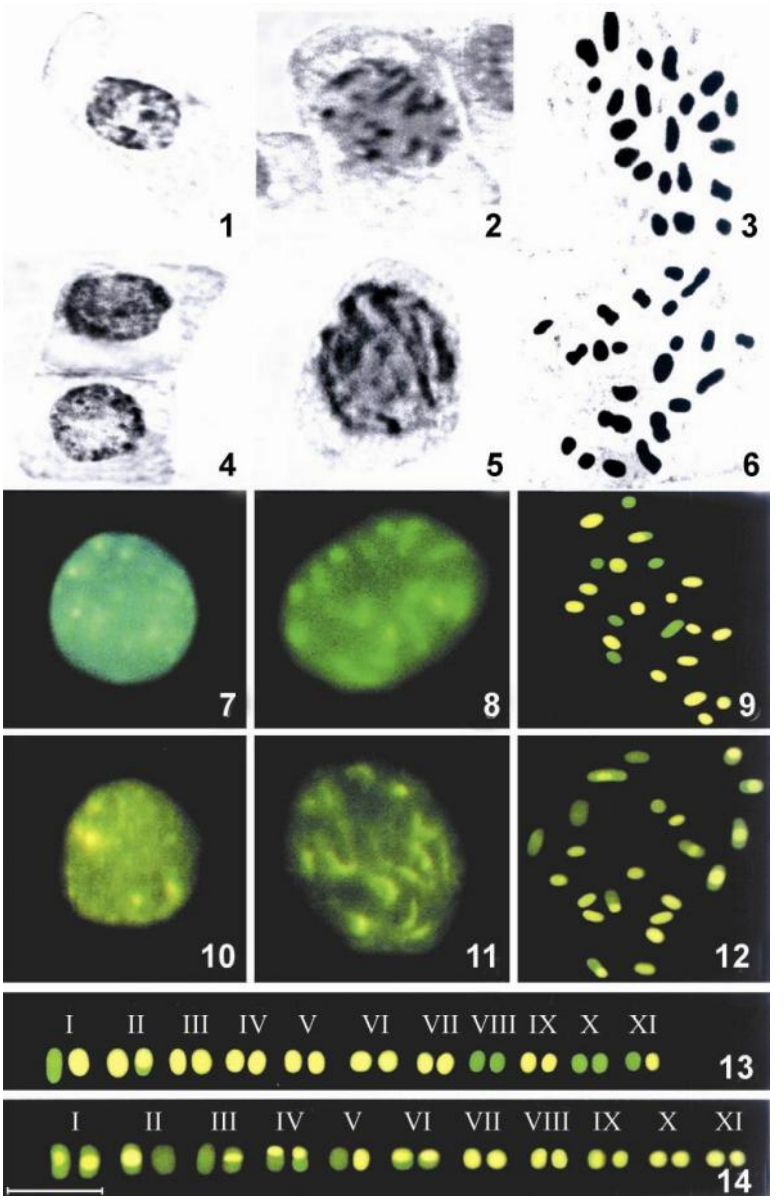
Varieties	2n	Range of chromosome length (µm)	Total length of 2n chromosome complements (µm)	No. of CMA-positive bands	Total CMA-banded length (µm)	% of GC-rich area	Orcein Karyo-typic formulae	CMA-banded Karyotypic formulae
Barimash-1	22	2.00- 4.90	70.30	16	39.60	56.20	22m	16θ +6φ
Barimash-3	22	2.00- 4.80	68.60	19	39.70	63.40	22m	11θ + 4α + 4β + 3φ

m = metacentric, θ = entirely fluoresced banded chromosomes, α =band in centromeric region, β = band in terminal region, φ = no band.

In Barimash-1, three big orcein stained heterochromatic blocks were found in the interphase nuclei (Fig. 1), whereas 10-11 less prominent small chromocenters were observed in Barimash-3 (Fig. 4). It suggests the presence of more heterochromatins in Barimash-1. The prophase chromosomes stained darkly throughout the entire length in Barimash-1 (Fig. 2), whereas in Barimash-3 one end was stained darker than another gradual staining was observed (Fig. 5). Prophase chromosomes of two varieties had different orcein staining properties and helped in characterizing the two varieties.

The interphase nucleus of Barimash-1 showed many prominent dots-like CMA-positive bands (Fig. 7). The prophase chromosome of this variety had six bright CMA-positive bands (Fig. 8). Four prominent and many dots-like CMA-positive bands were found in the interphase nuclei of Barimash-3 (Fig. 10). The prophase chromosome of this variety showed five bright CMA-positive bands (Fig. 11). The nature of CMA-stained interphase nuclei and prophase chromosomes are

useful for characterization. CMA- staining of metaphase chromosomes in these two varieties showed two common features, (i) many entirely fluorescent banded chromosomes and (ii) bands were thick, bright and prominent (Figs. 9 and 12).



Figs. 1-14. Different mitotic phases in two varieties of *Vigna mungo* Barimash-1 and Barimash-3, after staining with orcein and CMA.

Orcein stained 1. interphase nucleus of Barimash-1, 2. prophase chromosomes of Barimash-1, 3. metaphase chromosomes of Barimash-1, 4. interphase nucleus of Barimash-3, 5. prophase chromosomes of Barimash-3, 6. metaphase chromosomes of Barimash-3;

CMA-stained 7. interphase nucleus of Barimash-1, 8. prophase chromosomes of Barimash-1, 9. metaphase chromosomes of Barimash-1, 10. interphase nucleus of Barimash-3, 11. prophase chromosomes of Barimash-3, 12. metaphase chromosomes of Barimash-3, 13. karyotype of Barimash-1, 14. karyotype of Barimash-3. Bar = 10 µm.

The two varieties of *V. mungo* showed mark differences in CMA banding pattern.

In Barimash-1, 16 entirely fluoresced banded chromosomes were found. The rest 6 chromosomes did not show any band (Fig. 9). The total CMA-positive banded region was 39.60 µm, total GC-rich region was 56.20% and the karyotype formula of this variety was 16θ + 6φ (Table 1).

In Barimash-3, 19 different CMA- positive bands were observed. Out of 19 bands, 11 were entirely, 4 terminal and 4 centromeric banded chromosomes (Fig. 12). In this variety, the total CMA-positive banded region was 39.70 µm. GC rich region was 63.40%. The karyotypic formula of this variety was 11θ+4α+4β+3φ (Table 1).

The difference in the number of CMA-positive bands and the amount of GC-rich areas may be related with their karyotypic diversification.

Polymorphisms regarding CMA banding were found in pair I and XI of Barimash-1 (Fig.13) and pair II, III and V of Barimash-3 (Fig. 14). Here one member of the pair showed CMA banding and another did not. The polymorphism of CMA-positive banding pattern of two varieties indicates the probable occurrence of minute structural aberration and presence of different heterochromatins. The banded chromosomes were stable and made each karyotype unique. Therefore, fluorescent banding technique could help in differentiating the two varieties of *V. mungo* used during this investigation.

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