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SHORT RESEARCH ARTICLE

Chromosome identification and karyotype analysis of rice bean [*Vigna umbellata* (Thunb.) Ohwi et Ohashi] using fluorescence in situ hybridization

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Abstract

Rice bean (*Vigna umbellata*), an underutilized climate-resilient legume, includes the high-yielding, leaf spot resistance variety VRB-3 (Him Shakti). In the present study, using fluorescence in situ hybridization, one pair each of 45S and 5S rRNA gene loci was localized to the terminal region of the chromosomes and the estimated genome content for VRB-3 is 1.35pg against the reference standard *Solanum lycopersicum* cv. *Stupicke*.

Keywords: Flow cytometry, fluorescence *in-situ* hybridization, genome size, *Vigna umbellata*

Rice bean [*Vigna umbellata* (Thunb.) Ohwi et Ohashi] is a traditional but underutilized crop, native to India and central China, later spreading to Africa, the Indian Ocean Islands, Australia, and America. It is a nutrient-dense food and a potential source of genes for biotic and abiotic stress tolerance, including drought resistance, soil acidity tolerance, and resistance to pest infestation during storage. The entire plant is edible and used in various food preparations, green manure and fodder (Tomooka et al., 2002). In the present study, we have mapped 45S and 5S ribosomal regions on *V. umbellata* (VRB-3) chromosomes using fluorescence in situ hybridization (FISH) and estimated genome size using a flow cytometer. The central sub-committee on crop standards and release of varieties, India, released and notified VRB-3 (Him Shakti), a high-yielding rice bean variety, for cultivation in the mid-hills of the Indian states of Himachal Pradesh, Uttarakhand, Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, and Tripura. VRB-3 was evaluated for three years at 17 different sites, demonstrating optimal performance with an average yield of 17.08 q/ha as compared to check varieties, PRR2, PRR1 and RBL6. It exhibits resistance to leaf spot disease caused by the fungus *Cercospora canescens* (Rana et al. 2014). Previous researchers have used cytogenetic approaches based on karyomorphology and FISH to demonstrate chromosomal variation at the interspecific level within the genus *Vigna*. Consequently, determining the chromosomal and genomic variation in VRB-3 is highly significant and of considerable research interest.

The karyotype of *V. umbellata* (VRB-3) is 2n = 2x = 22 = 22m.The metaphase chromosomes were small and metacentric with lengths ranging from 93.25 ± 10.18 to 34.85 ± 5.02 pixels. FISH analysis using 45S rDNA probe revealed two fluorochrome bands (Fig. 1, a-d) on chromosomes 1 and 2 (Fig. 1 i). Similarly, two 5S rDNA signals (Fig. 1, e-h) were detected on chromosomes 5 and 7 in the terminal region (Fig. 1 j). Both loci were observed in interphase and metaphase spreads. Previous studies have also described *V. umbellata* chromosome as small and metacentric (She et al. 2015), although karyomorphological analysis of ten accessions of *V. umbellata* revealed a higher degree of karyotype asymmetry (Shamurailatpam et al. 2015).

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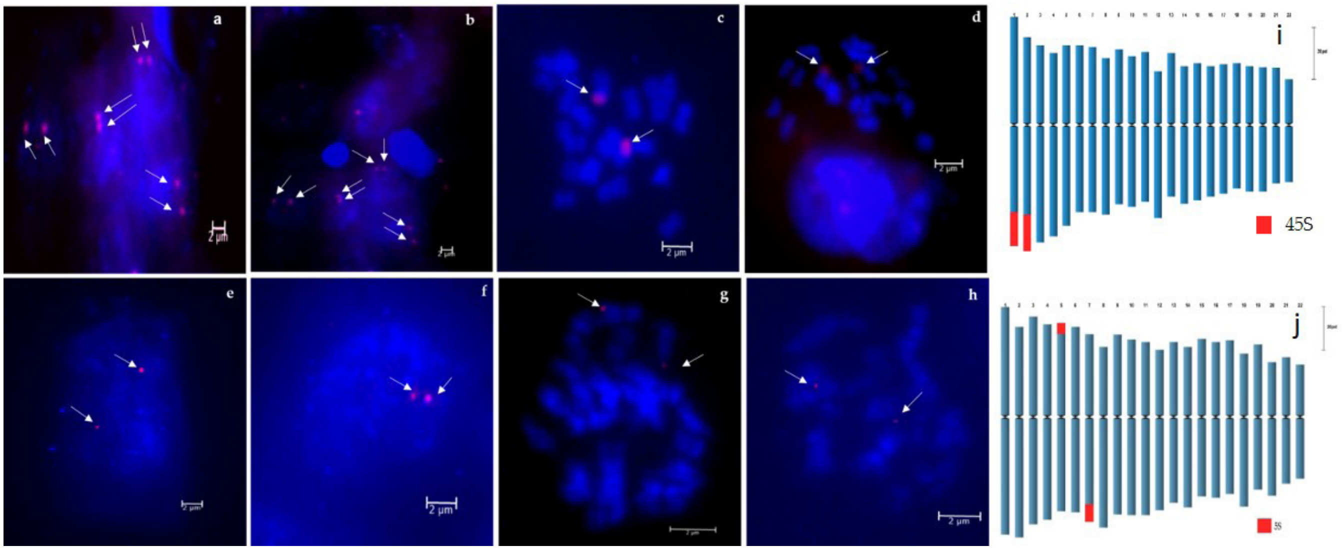

Fig. 1. FISH based localization of two rDNA sites in *V. umbellata* (VRB-3): a-b) Interphase with 45S rDNA signals; c-d), Metaphase chromosomes with 45S rDNA signals; e-f) Interphase with 5S rDNA signals; g-h) Metaphase chromosomes with 5S rDNA signals; i-j) Karyo-Ideogram showing the karyotype of *V. umbellata* (VRB-3) chromosome and the position of hybridization signals from 45S and 5S region respectively.

Such variation and asymmetry may result from structural rearrangements, including duplications, deletions, interchanges, and inversions in chromosomes.

In *Vigna* subgenera *Ceratotropis* and *Vigna*, rDNA shows random distribution, with45S/35S and 5S rDNA exhibiting variation in position (proximal to terminal) and copy numbers(Dias et al., 2024). A considerable difference in the number of rDNA sites for 45S rRNA and 5S rRNA in *V. umbellata* was reported by She et al. (2015) as compared to our study, with eight (four pairs) and two (one pair) sites for each locus, respectively, which could be attributed to differences in the material analyzed or genome rearrangement. Transposons, homologous or non-homologous unequal crossing over, inversion, translocation, and locus duplication or deletion contribute to changes in the number and location of rDNA sites at the intraspecific and interspecies levels. Inversions and translocations are the primary chromosomal rearrangements that play a significant role in the evolution of *Vigna* subgenera (Dias et al. 2024). The genomic content of VRB-3 was estimated using *Solanum lycopersicum* cv. *Stupicke* (2C=1.96 pg) as the reference standard, yielding a 2C content of 1.35 ± 0.051 pg, and genome size (1C) of 660 Mbp. Earlier reports estimated the genome size of rice bean landrace FF25 as 475.64 Mbp, based on Illumina genome sequencing and de novo assembly, covering 90.49% of the estimated genome size (Guan et al. 2022). For VRB-3, a de novo assembly yielded a genome size 414 Mbp, withidentification of 31,276 high-confidence index genes across 15,521 scaffolds (Kaul et al., 2022). This study cytogenetically characterized *V. umbellata* (VRB-3), a high-yielding rice bean variety. These findings have enhanced our understanding of the cytological features and genomic content of this species.

Authors' contribution

Conceptualization of research (RS, DPW); Designing of the experiments (RS); Contribution of experimental materials (G); Execution of field/lab experiments and data collection (PP); Analysis of data and interpretation (PP); Preparation of the manuscript (RS, PP).

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