

Review on *Murdannia*: Cytology, Banding Pattern and Flow Cytometry

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ABSTRACT

This review are useful to Identify Chromosome No on Various *Murdannia* Species also helpful to Study of different Banding Pattern Present in and Flow Cytometry Working. Useful to identify new methodologies for related working. Review provides an updated and comprehensive overview on the Cytology, Banding Pattern and Flow Cytometry

Introduction

Murdannia Royle is a major genus of the family Commelinaceae consisting about 52 species worldwide (Govaerts & Faden, 2012) the genus is well represented in India with 26 species (Nampy & Ancy, 2012; Ancy & Nampy, 2012) of which 23 occur in South India. The genus shows considerable percentage (31%) of endemism in India. *Murdannia* is generally distinguished from related genera in having inflorescences terminal and/or axillary thyrses or variously reduced, flowers actinomorphic to somewhat zygomorphic, fertile stamens 2 or 3, antesepalous, filaments glabrous or bearded, staminodes 3(-4), antepetalous (or 3 antepetalous and 1 antesepalous), antherodes 3-lobed or hastate, capsules trilocular, locules 1-many-seeded and seeds uni- or biseriate. The members of this genus are economically less significant. However, some of them are grown as greenhouse ornamentals or in ethnic herbal medicines. The tuberous roots of *M. edulis* are eaten by tribals. Similarly, *M. loriformis*, has been used in Thailand as traditional medicine to treat malign and benign tumors and chronic bronchitis (Morrogh-Bernard, 2008).

A study by Nandikar et al. (2015) examined the cytology of several species within the genus *Murdannia*, including *Murdannia viscosa* and *Murdannia pauciflora*, both of which are endemic to Maharashtra, India. The study found that these species had diploid numbers of $2n = 30$ and $2n = 16$ chromosomes, respectively. (17)

Chromosome banding refers to alternating light and dark regions along the length of a chromosome, produced after staining with a dye. A band is defined as the part of a chromosome that is clearly distinguishable from its adjacent segments by appearing darker or lighter with the use of one or more banding techniques. (N.B. Spinner, in Brenner's Encyclopedia of Genetics (Second Edition), 2013

In flow cytometry study Plant ploidy levels determination: based on detection of nucleus DNA content, ploidy levels of specific plant species can be calculated with high reliability. Genome size determination: Propidium iodide (PI) staining with flow cytometry is a powerful method for genome sizing because it is relatively fast, works with a wide variety of materials, and provides information on a very large number of nuclei. Measurement of chemical contents: within given plant cells and/or plant tissues, the contents of

subcellular chemical fractions can be measured via dye-labeling, fluorescence tagging or fluorescence emission. Ochatt SJ (2008)(18)

Some Review on *Murdannia* species of cytology, banding pattern and flow cytometry

Che Sulaiman, Intan & Mohamad, Azham & Ahmed, Osumanu. (2021). this review provides an updated and comprehensive overview on the ethnomedicinal use, phytochemistry, pharmacology, and toxicology of *M. loriformis*. Phytochemical analysis of *M. loriformis* revealed that it is composed of phenolics, flavonoids, condensed tannins, chlorophylls, alkaloids, and steroids. Numerous compounds including syringic acid, β -O-D-glucopyranosyl-2-(2'-hydroxy-Z-6'-enecosamide) sphingosine, isovitexin, and 3β -O-D-glucopyranosyl-24 ξ -ethylcholest-5-ene have been identified and isolated from this plant species. The present review attempts to bridge the gap between traditional use and pharmacological studies of *M. loriformis* while improving their existing therapeutic agents and product applications based on this plant (1)

Nair, S.T.K., Nampy, S. (2022). The study examines the karyotype and somatic chromosome numbers of five species of *Murdannia Royle* (Commelinaceae). The karyotype details of the two species namely *M. blumei* and *M. lanceolata* endemic to India are reported for the first time. The karyotype formula of the examined species are: *M. blumei* - $2n(36) = 1M+12m+5sm$; *M. lanceolata* - $2n(20) = 2m+8sm$; *M. crocea* subsp. *ochracea* - $2n(36) = 7m+8sm+3st$; *M. spirata* - $2n(40) = 9m+8sm+3st$ and *M. triquetra* - $2n(40) = 16m+4sm$. Further details on karyomorphology including estimates of asymmetry indices, total form percent, ratio of mean length of short arms to long arms, intrachromosomal / inter chromosomal asymmetry indices, and centromeric indices are provided. The karyomorphological parameters thus analysed suggest that *M. blumei* and *M. lanceolata* fit into the 2A category while *M. crocea* subsp. *ochracea*, *M. spirata* and *M. triquetra* belonged to the 2B category of Stebbins' classification. (2)

Rolla S. Rao, R. V. Kammathy, R. Sundara Raghavan (1968) There is much more data to justify the separation of *Murdannia Royle* from *Aneilema* (sensu lato). The evolutionary trend in *Murdannia* seems to follow two different patterns, one with a basic number of $x = 10$ and the other with $x = 6$, both the patterns probably arising from a common extinct ancestor with $x = 5$. Preliminary observations on the Indian species of *Aneilema* suggest a basic number of $x = 14$ (?) in sharp contrast to $x = 13$, widely prevalent in a majority of the African species of *Aneilema*. In *Murdannia* and *Aneilema*, polyploidy and also aneuploidy are quite common and these two genera offer a fertile field for further study. (3)

Kaur, Harpreet. (2018). the work deals with population-based meiotic studies on eight species belonging to four genera of the family Commelinaceae from different regions of Kangra Valley which is well known for its rich floristic diversity. At the world level, different cytotypes for four species such as *Commelina hasskarlii* ($2n = 22, 60$), *C. kurzii* ($2n = 60$), *Murdannia nudiflora* ($2n = 24$) and *M. spirata* ($2n = 24$) have been recorded for the first time at various ploidy levels. Additionally, from India, the new chromosome count for *Tradescantia pallida* ($2n = 24$) has been reported at the tetraploid level. (4)

The course of meiosis has been found to be normal in all the populations of *Commelina benghalensis*, *C. paludosa*, *Murdannia nudiflora* and *M. spirata* while four species, *Commelina hasskarlii*, *C. kurzii*, *Cyanotis cristata* and *Tradescantia pallida* have shown a normal to abnormal meiotic course in different populations. These meiotic abnormalities have revealed a clear effect on the pollen size and pollen fertility. The study of PMCs clearly depicts the presence of 12 bivalents at diakinesis making an additional chromosomal record for the species on a world-wide basis as the species has been previously reported to

exhibit $2n = 20$ – from India (Bhattacharya, 1975; Renugadevi and Sampathkumar, 1986) and from outside India (Jones and Jopling, 1972).

The meiotic studies on PMCs of all the 3 populations exhibit $2n = 24$ through equal distribution of 12: 12 chromosomes at A-I (Fig. 1o). This tetraploid cytotype makes a new chromosomal record for the species. The species has already been reported from India to have other cytotypes with $2n = 18$ (Raghavan and Rao, 1961) and $2n = 20$ (Rao et al., 1970).

Kaur, Navjot, and Raghbir Chand Gupta. (2018) the family Commelinaceae shows a lot of morphological and cytological diversity. Chromosome numbers and male meiosis of 15 populations of 12 species under four genera i.e., *Amischophacelus*, *Commelina*, *Cyanotis* and *Murdannia* of *Commelinaceae* have been studied from Rajasthan, India including the first chromosome report of *Commelina forskoolii* Vahl ($2n=90$) in the world. It includes new euploid cytotypes in six species i.e., *C. attenuata* ($2n=24$), *C. diffusa* ($2n=120$), *C. hasskarlii* ($2n=120$), *C. paludosa* ($2n=30$), *Cyanotis cristata* ($2n=22$) and *Murdannia nudiflora* ($2n=80$) besides new chromosome reports from Rajasthan in three other species i.e., *Amischophacelus axillaris* ($2n=20$), *Co. benghalensis* ($2n=22$) and *Cyanotis fasciculata* ($2n=22$). Meiosis is regular in seven species but has a low frequency of meiotic anomalies like laggards, chromatin bridges and stickiness in five species (*C. albescens*, *C. benghalensis*, *C. forskoolii*, *C. suffruticosa* and *M. nudiflora*). (5)

Faden, Robert B. (1980): The taxonomic and nomenclatural history of *Commelina medica* Lour. and *C. tuberosa* Lour. is outlined and Loureiro's diagnostic characters are presented. The three tuberous-rooted, scapose *Murdannia* species from Indochina and south China are described. A complete synonymy is provided for each one and each in turn compared with Loureiro's descriptions of *C. medica* and *C. tuberosa*. *Murdannia medica*, as recently interpreted, is found to have been correctly associated with *C. medica*. The combinations *M. spectabilis* and *M. edulis* are made for the species usually called *M. loureirii* and *M. scapiflora*, respectively. The former species usually has been associated with *C. tuberosa* Lour., but the latter is shown to be Loureiro's plant. *Aneilema azureum*, *A. loureirii* β . *horsfieldii*, *A. platyphyllum* and *A. multiscaposum* are placed in synonymy for the first time. *Murdannia medica* and *M. spectabilis* are found to be closely related to each other but not to *M. edulis*. The first two are related to *M. vaginata* because of shared unusual inflorescence and floral morphologies. A new section *Vaginatae* is described for these three species. The relationships of *M. edulis* are not established with certainty, but this species must be placed in section *Murdannia*. (6)

Tjio JH, Levan A. (1956) they are write a research article on Inherited Chromosome Numbers in Human and introducing G-banding of human chromosome numbers and describing the first protocol for g-banding karyotypes.(19)

K.Kongsuwan and D.R.Smyth (1977) they are studied on Q and C bands in *Lilium* species with various stain methods and shows the comparison of banded pattern of chromosome.(20)

Jiratchariyakul, W., Vongsakul, M., Sunthornsuk, L. et al. (2006) NMR signal reassignments for a cytotoxic glycosphingolipid compound, 2, β -O-D-glucopyranosyl-2-(2'-hydroxy-Z-6'-enecosamide) sphingosine, isolated from an ethanolic extract of the herb *Murdannia loriformis*, have been achieved by use of FAB-MS, and 1D and 2D 1H and 13C NMR. The amount of 2 in the herb juice was quantitatively determined by use of a validated HPLC method (RP-18, MeOH-H₂O, UV detection at 210 nm). The immune modulatory effect of the herb juice and of 2 was proved by means of in vitro cellular immunological assays. Compound 2 at a concentration of 13 nmol L⁻¹ stimulated PBMC proliferation and increased the CD 3,4:CD 3,8 ratio in T lymphocytes. (7)

Keith Jones, Carrol Jopling, 1972 This account of chromosome variation in the *Commelinaceae* is based both on the authors' personal experience with a very large living collection of the family at Kew, and on the numerous reports of earlier workers. There can be few families showing such a wide range of chromosome numbers, karyotype morphology and meiotic behaviour, the description of which may be of interest to cytologists, evolutionists and taxonomists alike. In this present survey the information is presented within the framework of a tentative classification of genera proposed by Brenan, and comments are made throughout on the relevance of cytology to his taxonomic treatment of the family. It is hoped that this account will foster further interest in the *Commelinaceae*, and in particular, encourage more thorough appraisal of intraspecific chromosome variation and interspecific homologies. (8)

Levan, A., Fredga, K., & Sandberg, A. A. (1964). great value if a uniform system for chromosome classification could be obtained and generally accepted. As may be clear from the preceding samples from the literature, there is at present a considerable uncertainty and confusion about terminology of the centromeric position. Different authors (but often also the same author on different occasions) have used different terms for the same chromosome type: IS well as the same term for different chromosome types. In the present paper we suggest a simple system for classification of chromosomes according to relative lengths of their arms. By this system, every chromosome can be classed into one of four groups, 111, sm, st and t, each of which is characterized by a defined amplitude of arm ratios. The ni and t groups, respectively, include the terms M and T for strictly median and terminal positions of the centromere. The system suggested seems to be logical, and is, as far as possible, in agreement with present usage of the terms adopted and with the generally accepted opinions of these terms. (9)

Bashashati, Ali, and Ryan R. Brinkman. (2009). Flow cytometry (FCM) is widely used in health research and in treatment for a variety of tasks, such as in the diagnosis and monitoring of leukemia and lymphoma patients, providing the counts of helper-T lymphocytes needed to monitor the course and treatment of HIV infection, the evaluation of peripheral blood hematopoietic stem cell grafts, and many other diseases. In practice, FCM data analysis is performed manually, a process that requires an inordinate amount of time and is error-prone, non reproducible, non-standardized, and not open for re-evaluation, making it the most limiting aspect of this technology. This paper reviews state-of-the-art FCM data analysis approaches using a framework introduced to report each of the components in a data analysis pipeline. Current challenges and possible future directions in developing fully automated FCM data analysis tools are also outlined. (10)

Doležel, Jaroslav, Sergio Lucretti, and Ingo Schubert. (1994) During the past decade, significant progress has been made in the development of methods for the preparation of plant chromosome suspensions suitable for flow cytometric analysis. In addition to successful classification of chromosomes (flow karyotyping), sorting of single chromosome types with a high degree of purity was reported in several plant species. Sorted chromosomes were used for the establishment of chromosome-specific DNA libraries and for gene mapping. The results confirmed the potential of plant flow cytogenetics and form a solid basis for further progress in this area. This article reviews its current status, analyzes major problems, and assesses future directions. (11)

Jaroslav Doležel, Jan Bartoš, (2005) use of flow cytometry for estimation of DNA quantity in cell nuclei. The method involves preparation of aqueous suspensions of intact nuclei whose DNA is stained using a DNA fluorochrome. The nuclei are classified according to their relative fluorescence intensity or DNA content. Because the sample preparation and analysis is convenient and rapid, DNA flow cytometry has become a popular method for ploidy screening, detection of mixoploidy and aneuploidy, cell cycle analysis

analysis, assessment of the degree of polysomy, determination of reproductive pathway, and estimation of absolute DNA amount or genome size. While the former applications are relatively straightforward, estimation of absolute DNA amount requires special attention to possible errors in sample preparation and analysis. Scope The article reviews current procedures for estimation of absolute DNA amounts in plants using flow cytometry, with special emphasis on preparation of nuclei suspensions, stoichiometric DNA staining and the use of DNA reference standards. In addition, methodological pitfalls encountered in estimation of intraspecific variation in genome size are discussed as well as problems linked to the use of DNA flow cytometry for fieldwork. (12)

Sliwinska, E., Loureiro, J., Leitch, I. J., Šmarda, P., Bainard, J., Bureš, P., Chumová, Z., Horová, L., Koutecký, P., Lučanová, M., Trávníček, P., & Galbraith, D. W. (2022). Flow cytometry (FCM) is currently the most widely-used method to establish nuclear DNA content in plants. Since simple, 1-3-parameter, flow cytometers, which are sufficient for most plant applications, are commercially available at a reasonable price, the number of laboratories equipped with these instruments, and consequently new FCM users, has greatly increased over the last decade. This paper meets an urgent need for comprehensive recommendations for best practices in FCM for different plant science applications. We discuss advantages and limitations of establishing plant ploidy, genome size, DNA base composition, cell cycle activity, and level of endo reduplication. Applications of such measurements in plant systematics, ecology, molecular biology research, reproduction biology, tissue cultures, plant breeding, and seed sciences are described. Advice is included on how to obtain accurate and reliable results, as well as how to manage troubleshooting that may occur during sample preparation, cytometric measurements, and data handling (13)

Göttlinger, C., Mechtold, B., Radbruch, A. (1992). Biomedical research, on the way from the organism to the molecule and back again, requires powerful tools to analyze the functional status of the individual cell, the unit of organization of life. Cells were detected by microscopy, and microscopy, in combination with powerful staining technologies, continues to be the main instrument for obtaining direct information on their state of activation, proliferation, and differentiation. The drawback of microscopy is that the data generated are mainly „visual impressions “and not exact numbers. Today, systems are available that allow quantification of the light intensity of microscopic objects, but these systems are still too slow to allow analysis of enough objects to obtain good statistics. One could say that microscopy generates too much information in cases where information only on the amount of a particular stain per cell is desired. (14)

Barbara Vilhar, Johann Greilhuber, Jasna Dolenc Koce, Eva Maria Temsch, Marina Dermastia, (2001) To test the reliability of DNA image cytometry for the measurement of nuclear DNA content in plant material, we conducted independent experiments in two laboratories using different image analysis instruments for densitometric measurement of nuclear DNA amount in Feulgen-stained squash preparations of root tips. The 2C nuclear DNA content of the nine species studied spanned a 100-fold range (approx. 0.3 ± 33 pg). The estimates of nuclear DNA content measured with image cytometry methods were comparable to values obtained previously using both photometric cytometry and flow cytometry. Image cytometry methods showed little variation among repeated experiments within each laboratory or among different operators using the same instrument. Furthermore, the interphase-peak method (measurement of several hundred interphase nuclei per slide) was comparable to the classical prophase/telophase approach (measurement of ten early prophase and ten late telophase nuclei per slide). Hence, DNA image cytometry gives accurate and reproducible results and may be used as an alternative to photometric cytometry in plant nuclear DNA content measurements. In the present study, we propose that two standards for quality

control of nuclear DNA content measurement are used in plant DNA image cytometry: (1) the coefficient of variation of the peak should be lower than 6%, and (2) the 4C/2C ratio should be between 1.9 and 2.1. (15)

Galbraith, D., Loureiro, J., Antoniadi, I., Bainard, J., Bureš, P., Cápál, P., Castro, M., Castro, S., Čertner, M., Čertnerová, D., Chumová, Z., Doležel, J., Giorgi, D., Husband, B.C., Kolář, F., Koutecký, P., Kron, P., Leitch, I.J., Ljung, K., Lopes, S., Lučanová, M., Lucretti, S., Ma, W., Melzer, S., Molnár, I., Novák, O., Poulton, N., Skalický, V., Sliwinska, E., Šmarda, P., Smith, T.W., Sun, G., Talhinhos, P., Tárnok, A., Temsch, E.M., Trávníček, P. and Urfus, T. (2021) Flow sorting of higher plant chromosomes has provided in valuable information regarding the organization of DNA sequences within plant species. It has also greatly facilitated the process of whole-genome sequencing by permitting subdivision of large genomes into samples comprising entire chromosomes or chromosome arms. 5FCS methods applied to wall-less cells (protoplasts) expressing fluorescent proteins (FPs) in a cell type-specific manner have allowed elucidation of patterns of co-regulated gene expression and plant hormone gradients identification^{6,7} within organized tissues, such as roots. (16)

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