Cytogenetical Analysis of Moringa Genome

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Abstract

Advances in cytogenetics have resulted in the development of novel strategies in chromosomal banding patterns that allow the identification of individual chromosomes within a species. Differentiating chromosomes at the basic level is essential to draw essential genetic conclusions with respect to the given plant cells and such efforts have several folds of applications in cytological studies. Recent trends in structural, comparative and functional genomics experiments that supplement, accelerate or enhance the efficiency of Moringa-genome research towards product development are described here. This chapter also provides details on the current status of Moringa cytogenetics experiments and a way forward.

6.1 Importance of Cytogenetics in Crop Improvement

The study of DNA structure and organization that coiled or reduced to form chromosomes within the nucleus during the process of cell division is generally referred to as cytogenetics. The main aim of the cytological experiments is to identify the number and morphology of chromosomes by employing (i) chromosome banding techniques (classical cytogenetics) and (ii) hybridization techniques achieved by means of fluorescently labeled probes (molecular cytogenetics).

Except in the reproductive and liver cells of animals, all the cells of biological systems possess chromosomes with constant numbers and morphological features in the given species. Thus, the number and morphological features are used as an important descriptor of each species. For example, in Moringa, there are 28 (2n = 14) chromosomes and in humans, the chromosomes number are 46 (2n = 23).

In order to more precisely understand the evolution, genetics, genetic recombination and karyotypic stability of the investigated biological species, molecular cytogenetic tools have been employed as a key method in this era of genomics.

As efficient and robust methods of introducing novel variation in crop species, breeders always prefer cytogenetic manipulation of the given chromosome sets and evolve new crop cultivars. Besides, cytogenetics has also shown its utilities in crop improvement as detailed below:

1. Identifying and characterizing chromosome segments or chromosomes that are having various origins (which may be *intra*- and *inter*-specific).

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N. M. Boopathi et al. (eds.), *The Moringa Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-030-80956-0_6

2. Identification and validation of chromosomal markers linked with agronomically and economically important traits that allow plant breeders to efficiently introgress and track those traits in the progenies.
3. Finding the locations of genic and non-genic elements on the DNA sequences along the chromosomes and unravelling (in multi-dimensions) evolution and behaviour of physical chromosome upon addition and/or elimination of chromosomal segments and its impact on cell division and other cellular processes.
4. Cytogenetics has long been used as a key tool to document interspecific hybridization that helped to generate large numbers of commercially important variations in crop plants. Cross-pollination occurs in nature as well as in the controlled hybridization program, among the cultivated crop species and their wild relatives and exchange of chromosomal segments through homoeologous recombination is found widespread. Benavente et al. (2008) have shown that cytogenetic techniques were useful to identify the chromosomal segment from a donor species (i.e. the wild) that has been introgressed into a recipient species (i.e. the crop) by citing several hybrids and their derivatives as examples. As illustrated by them, all the cytogenetical tools (starting from the classical to the trendy molecular tools) can be used efficiently to distinguish the genomes and highlight the chromosomal regions that were exchanged or modified in due course in the investigated crop plants. They have also shown documental evidence that supported interspecific hybrids and fertility restoration by employing various strategies of cytogenetics.

6.1.1 Historical Perspectives of Cytogenetics in Crop Improvement

Revolutionary cellular research on maize (*Zea mays*) by Barbara McClintock has profoundly motivated crop cytogenetics. She undoubtedly laid the foundation for explicit documentation of each chromosome and led to develop several discoveries on the structure and frequent fluctuating behavior of the chromosomal rearrangement in the maize genome. The link between chromosome length and its arm ratio was deliberately established by her research finding that employed carmine-based chromatin staining procedures and she could professionally differentiate diverse chromosomes in a single meiotic nucleus. The same strategy was extended to other crops and cytogenetic maps were developed successfully in rice (*Oryza sativa*), sorghum (*Sorghum propinquum*) and tomato (*Lycopersicon esculentum*).

But later it was noticed that for the similar-sized chromosomes, more refined technological advancements are needed to improve the cytogenetic resolution and karyotyping (which is an artificial diagram of the genome in the form of chromosomes by pairing and ordering all the chromosomes of an organism). To this end, a fluorescent dye, quinacrine, was employed in 1968 at the Caspersson laboratory, to develop supplementary banding patterns on plant chromosomes and such bands were called as Q-bands. Q-bands provided additional resolution of alike sized chromosomes. Four years later, Giemsa C-banding was developed by Vosa and Marchi and it was compared with Q-banding that was generated with the chromosomes of bean (*Viciafaba*), keeled garlic (*Allium carinatum*) and maize.

Such developments in chromosome-banding methods greatly advanced the practicality of utilizing the information on somatic chromosomes as it was found to be relatively simpler to obtain the information than meiotic chromosomes. Further advancement (such as cold pretreatments of the plant tissue samples) was also found to be useful for improved and better visualization of chromosomes when combined with a different modified procedure that employs various concentrations of carmine that were optimized for several economically important crops. Despite the considerable progress in chromosome characterization, development of in situ hybridization (ISH) techniques has made a

quantum leap in cytogenetics as it allowed superior imaging of specific DNA sequences on chromosomes.

ISH utilized radioactive tracer or modified nucleotides (a chemical moiety (such as biotin, digoxigenin, or fluorescent moieties) that is attached to the nucleotide) which binds to the complementary sequences in cells/nuclei and/or on individual chromosomes. Such binding facilitates easy visualization of the ISH probes and their localization on the chromosomes.

Langer-Safer et al. (1982) have first reported the Fluorescence in situ hybridization (FISH) and since then it has been largely applied in several crops and of late, after the attachment of CCD camera and imaging process of FISH signals, its applications in cytogenetical studies are widespread. In addition to that, there are continuous advancements in FISH and it led to the introduction of several novel improvements such as multi-colour FISH (McFISH), genomic in situ hybridization (GISH), chromosome image analyzing system (CHIAS), flow cytometry, pulse-field gel electrophoresis (PFGE), chromosomal microdissection and microcloning. Such advancements have greatly increased the visualization of not only the individual chromosomes with high resolution but also efficient physical localization of target DNA fragments on individual chromosomes with high precision (Gupta and Dhar 2004). Besides, FISH has also been extensively employed in mapping repetitive DNA sequences, low-copy-number sequences, chromosome recognition in a cocktail and establishing genomic relationships among chromosomes in the polyploid organisms or ecotypes with high similarity.

The key application of today's cytogenetic experiments is precisely identifying the locus and order of two or more DNA fragments (usually referred to as molecular markers) on the given chromosome. The capacity to discriminate the positions of two loci as proximal or distal to the centromere is generally defined as axial resolution and it is indicated in either base pairs or spatial unit (centi McClintock, cMC; (Figueroa and Bass 2010). Several studies have reported axial resolutions ranging from <0.7 kb in fiber-FISH to 10,000 kb in some metaphase(M)-FISH and helped to make clear differences among the DNA fragments on the chromosomes and it has been predicted that an axial resolution limit of ~600–700 bp can be obtained with the current epifluorescence microscopy (Figueroa and Bass 2010). Such efforts are particularly useful in high-density genetic map construction using molecular markers (Boopathi 2020).

FISH has been used to unravel the unearthed information on individual chromosomes such as their structure, mutation and evolution and differentiating closely related species based on their chromosome organization, besides characterizing each chromosome by localizing satellites, chromosome arms and centromeres. These studies would have implications in designing novel probes for RFLPs and mapping of agronomically important traits and characterizing plant genomes for gene duplication, chromosome synteny and evolution (Gupta and Dhar 2004). Further, FISH mapping has also been useful in the construction of physical maps and joining the linkage maps and physical maps.

The most substantial recent advance in FISH, in the above lines, is the use of synthetic oligonucleotides (oligos) to prepare probes. Oligos can be artificially synthesized to mimic repetitive DNA sequence or a specific chromosomal region or even an entire chromosome and labeled with fluorescent dyes. Such probes can be used for extensive comparative cytogenetic mapping among genetically related species (Jiang 2019) and their genetic improvement by translating the genetic information identified in one species to the other.

Thus, FISH has shown its widespread applications in structural, comparative and functional genomics and accelerated the crop-genome research towards efficient improvement of crop plants in this genomics era. Further involvement of other advanced strategies such as molecular and immune-chromatin cytology (for example, combining FISH, chromatin immunoprecipitation and

immuno-cytochemistry), novel chromosome preparation methods and high-resolution imaging would offer novel insights in chromosome organization at several dimensions.

6.2 Cytogenetics of Moringa Genome

Initial cytological studies have discovered that *Moringa oleifera* Lam., possess 2c genome size of 1.2 pg (Ohri and Kumar 1986) and its diploid number (2 N) was 28 (Mendior et al. 2004). Totally, 14 bivalents in diakinesis were found as the meiotic behaviour of *M. oleifera*. Further studies have revealed that there were more than 88% pollen viability and rare meiotic abnormalities in which the most affected cell cycle was metaphase I. It was additionally established that there was a development of unreduced gametes due to tripolar spindles in metaphase II (Silva et al. 2011).

In another study (Anwar 2016), cytological features (such as cell shape and size, mitotic chromosomes and nucleolus appearance) of *M. oleifera* and *M. stenopetala* were explored and he reported that active meristematic cells could be categorized into three types: Type I (including dividing cells), Type II (meristematic cells with diffused chromatin) and Type III (cylindric cells with diffused chromatin) and further reported the percentages of dividing cells as the mitotic index.

It was also evident from that study that mitotic index was considerably high at cells of *M. oleifera* than that of *M. stenopetala*. The scored diploid number (2 N) of metaphase plates of the plants of the two Moringa species was mostly 28 and they were small-sized chromosomal sets. However, large nucleolus at metaphase and even at anaphase or telophase appeared only with *M. stenopetala* whereas no nucleolus body was detected in mitotic cells of *M. oleifera* except at interphase and in some cases at prophases (Anwar 2016). Nucleolus body appeared in metaphase and anaphase/telophase cells of *M. stenopetala* may be due to the species-specific difference and it may be used as a cytological marker to distinguish the plants of these two Moringa congeneric species.

6.3 Way Forward

In scientific history, a handful number of techniques have dominated the research field for more than four decades and FISH is one such technique that has been successfully used in cytogenetic research since the 1980s. On the other hand, due to the unavailability of robust DNA probes in many crops, especially in non-model crops, the application of FISH techniques has been limited down since the dawn of the twentieth century. To alleviate this limitation, synthetic oligo probes were introduced. In addition, several exciting new developments were also introduced recently. For example, exploration of CASFISH (employing fluorescently labelled nuclease-deficientCas9 (dCas9) proteins to detect chromosomal regions without employing DNA denaturing process (otherwise it would interrupt the organization of genome in the fixed or living cells) and single-molecule RNA-FISH (smRNA-FISH) techniques (employed to quantify the transcription of multiple genes or non-coding RNAs within single cells) would advance high-end FISH applications in plants.

With respect to cytogenetics studies in Moringa, a lot more has to be done starting from karyotyping to merging of physical mapping with chromosomal mapping. It is sincerely hoped that advances in molecular cytogenetics tools and strategies, would pave new ways and means to fill these cytogenetics research gaps in Moringa. Although not yet reported in *M. oleifera*, cytogenetics-based manipulation of Moringa chromosomes can be used to induce polyploidy as it has more impact on the improvement of nutritional and the medicinal properties of this species. Moringa molecular cytogenetics can also be combined with genomics, reverse genetics and comparative genomics strategies which will eventually lead to the discovery and isolation of

novel genes (that are agronomically, economically and nutritionally important). Such cytogenetic information will be extensively utilized in precision breeding strategies that help to genetically improve Moringa. Identification of such novel genes is not possible with the conventional Mendelian genetic analysis.

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