Karyotyping: Basics and application in Forestry

ABSTRACT

Karyotyping is the method of assessing an organism's chromosomal number and structure, is essential for comprehending the genetic composition of species, especially those used in forestry. This method provides important insights into the genetic diversity, evolution, breeding, and conservation of trees and other forest species by examining their chromosomal features. Karyotypes explain how many chromosomes each organism has and how they appear under a light microscope. Length of the chromosome, centromere location, banding pattern, sex-chromosome variations, and other physical traits can also be determined by karyotyping. At traditional level, by using light microscope, we can study chromosomes butto generate chromosomal data, sophisticated methods like as fluorescence in-situ hybridization (FISH) and genomic in-situ hybridization (GISH) can be employed. From taxonomical classification to identify different types of abnormalities, karyotyping is important. There is a lack of study related to karyotyping in forestry species. This study summarizes the basics of karyotypingandthe work related to it, has been done in forestry species.This review article deals with the importance of karyotyping such as how they contribute to the conservation biology of the forestry species, how it helps in the development of taxonomical identification marker and identification in different types of chromosomal abnormalities. It also covers how karyotyping can be done. From traditional methods i.e use of microscopes to the merge of different recent developments in the area of karyotyping helps not only in enrichment of cytogenetics data but also substitute the genomics and transcriptomics in the area of genetic diversity studies.Basics of karyotyping, different types of banding techniques, protocols of some of the techniques, its methodological developments, and its various applications in forestry has also been discussed in this review. Application of karyotyping in different forestry species is limited. With the advancement of imaging techniques and other genetic tools, biodiversity and forests can be managed sustainably.

Keywords: Karyotyping, Forestry species, ddPCR, FISH

1. INTRODUCTION:

Karyotyping consists of the study of both such as chromosome number and morphologyof all living beings (Yoshida and Kitano, 2021). It has vast application starting from studies of species evolution (Moraes *et al.,* 2016), taxonomical classification to analysis of chromosomal abnormalities and mutations.From economically significant wood trees to ecologically significant non-timber plants, forestry species are frequently impacted by human activities and environmental stresses. Significant biodiversity loss and genetic degradation may result from these circumstances.

Karyotyping is useful for identifying genetic abnormalities, identifying hybridization occurrences, and evaluating genetic stability in breeding programs. Using karyotyping, characterization between wild and crop plants is possible in Allium species (Dutta *et al.,* 2015). *Philodendron,* an importantandhighlydiverse genus. Chromosomal diversity using the Giemsa staining procedure identified the Dysploidy i.e the structural rearrangements of the particular chromosomes ((Correia-da-Silva *et al.,* 2014).Karyotypic changes in different cultures such as haploid and diploid cultures have been noticed in *Crepiscapillaris*, compared to the diploid culture, the initially haploid culture had a significantly higher level of polyploidization (Sacristan 1971).

Additionally, it provides a framework for evolutionary research, facilitating the tracking of speciation events and phylogenetic relationships both within and between genera.Considering the initial theory that the present organisms are descended from primitive predecessors, alterations to chromosomes are always important to the evolutionary path of species since they are made up of the genetic material. Because of this, we may identify certain key mechanisms of evolution in the evolutionary history of species (Futuyma 2009). Species evolve in response to biotic and abiotic conditions, habitat instability, and genetic variability, resulting in intra- or interspecific divergence. Thus, every aspect of karyotype analysis contributes to karyotype evolution. Cytaxanomy is the study of all elements of karyotype for species identification and classification, with a particular emphasis on evolutionary processes (de Resende, 2017).

Interspecific variation in terms of banding patterns and GC content in four Cedrus species is analyzed and helped to elucidate the evolutionary links between the four species (Bou Dagher-Kharrat, 2001). Even though eucalypts have a vast number of genetic data available, knowledge of the genus's DNA content is still necessary for a fundamental understanding of the genome's makeup. Comparative karyotype studies revealed no appreciable differences in chromosomal number (2n = 22) or morphology among eucalypt species, despite considerable variances in nuclear DNA content (Carvalho *et al.,* 2017).

Recent developments in cytogenetics, such as genomic in situ hybridization (GISH) and fluorescence in situ hybridization (FISH), have improved the accuracy of karyotypic analysis by making it possible to identify particular chromosomal areas and the functions that go along with them.FISH and GISH identified Narcissus genome composition and showed that chromosome doubling, sexual polyploidization, and distant hybridization all have distinct functions in breeding contemporary Narcissus cultivars (Sun *et al.,* 2024). These developments have broadened the application of karyotyping beyond conventional morphological analyses to include molecular-level research.

With a focus on its importance in genetic research, biodiversity preservation, and breeding tactics, this review attempts to investigate the use of karyotyping in forestry species. We aim to emphasize this field's significance as a crucial instrument for tackling current problems in forestry genetics and conservation biology by emphasizing significant advancements and difficulties in this area.

2. IMPORTANCE:

The importance of karyotyping is discussed below-

2.1 Taxonomical classification:

There are several ways of classification such as morphology based, molecular marker based and cytogenetics based. At the morphology level, classification can be done by analyzing different parameters i.e leaf and mostly by the flowering pattern. For flowering based identification, we have to wait for the flowering period, making it difficult for identification. Karyotyping is the part of cytogenetics, helps to identify at the species and genus level(Jackson 1971 & Badr 2022).

2.2 Assessment of genetic diversity and evolutionary linkages:

Karyotyping is one of the tools by which genetic diversity can be assessed(MesferALshamrani*et al.,* 2022). Variations in the chromosomes either in terms of structure or numbercan provide information about how organisms have changed throughout time to adapt to various environmental circumstances. Karyotype helps to provide idea about evolutionary processes like hybridization and speciation in forest environments. Forest species, for instance, frequently differ in the number of their chromosomes; determining these differences might provide insight on the evolutionary background of various species within a particular forest(Heng & Heng, 2023).

2.3 Chromosomal Abnormalities and Mutations:

Structural and numerical chromosomal aberrations are two types of chromosomal abnormalities. Karyotyping will lead us to identify different types of structural abnormalities such as deletion, duplication, inversion and translocation (Shrestha *et al.,* 2023).By monitoring such irregularities, forestry professionals may ensure healthy tree populations and avoid the development of genetic abnormalities. Karyotyping

can also be used to find spontaneous mutations (Mareschal*et al.,* 2021)that could give some species an evolutionary edge under shifting environmental conditions.

2.4 Conservation:

By studying the chromosomal composition, genetic stability of the species can be determined, ultimately that will help to conserve the vulnerable species.

2.5 Cytogenetics and molecular biotechnology:

Karyotyping frequently forms the basis of molecular cytogenetics, a field in which scientists use molecular methods to conduct more in-depth chromosome studies. By using advanced techniques such as Fluorescencein Situ Hybridization (FISH), we can tag a gene with fluorescence to know its localization and level of expression(Veselinyová*et al.,* 2021).

3. BANDING TECHNIQUE

Banding patterns depends on the differential distribution of the euchromatin and heterochromatin. Chromatin morphology is induced by post fixation treatments which ultimately reformed in the presence of Giemsa stain. Different types of banding technique are discussed below

3.1 G-banding:

Staining of a metaphase chromosome with Giemsa stain is known as G-banding. Differential staining properties of Giemsa stain were 1st observed by Pardue and Gall (1970) in their cytological preparation of mouse chromosomes. G bands appear as lateral striations. Every chromosome has a distinct G-banding patterns since banding depends on GC content which varies from chromosome to chromosome.

3.2 C-banding:

The technique of C-banding originated after the work of Pardue and Gall who reported that constitutive heterochromatin can be stained specifically with Giemsa stain. Each chromosome possesses a different degree of constitutive heterochromatin which enables the identification of individual chromosome. The banding methods is a complex technique that involves several treatments with acid, alkali or increased temperatures. These treatments denature the DNA, then subsequent treatment of Sodium Citrate at 60°C helps to renature the DNA. Repetitive DNA presents in the heterochromatin region renatures but low-repetitive and unique DNA do not renature, which causes the differential staining. The protocols for the C-banding are given below:

Seed germination in petri dishes on moist filter paper for 3-4 days

↓

Fixing of root in 3:1 (95% ethanol:Glacial acetic acid) for at least 24 hours

↓

Softening of the roots using 45% acetic acid or 0.5% acetocarmine (pectinase and cellulose can also be used)

↓

Slide preparation will be done using squash method. Cover glass will be removed by dry-ice method (Conger and Fairchild, 1953)

↓

For dehydration,

For dehydration, slides will be treated with 95% to 100% ethanol for 1 hr

↓

After dehydration air-dried slides will be kept in room temperature for overnight (Singh and Robbelen, 1975)

Processing of air-dried slides through BSG (Barium hydroxide/Saline/Giemsa) method

Preparation of fresh saturated solution of Barium hydroxide (5g $Ba(OH)_2.8H_2O$ + 100 ml dH_2O) and filteration of the solution in Coplin jar

Keep coplin jar in waterbath at 50°C -55° C or at RT

Replace the $Ba(OH)_2$ solution with cold water and rinse the slides

Denaturation step

For renaturation, incubation of the slides will be done in 2X SSC (0.3 M NaCl + 0.03 M $Na_3C_6H_5O_7.2H_2O$) at pH 7 to 7.6 at 60–65-degreeCelsius in a water bath or oven for 1 h

For staining, Giemsa stain along with 3 ml of stock solution + 60 ml of Sorenson phosphate buffer ().2 M), pH 6.9 will be kept in slides for 1-2min

After optimal staining, slides will be placed quickly in dH_2O and will be stored in xylene for overnight

3.3 N-Banding:

The technique of N banding was originally described by Matsui and Sasakii 1973. This technique was originallydeveloped to stain nucleolus organizing regions for mammalian and plant chromosomes. The N-bands are generally located at the secondary constriction, satellites,centromeres,telomeresand heterochromatic segments. It is suggested that the N-bands represent certain structural non-histone proteins specifically linked to the nucleolar organizer region of the eukaryotic chromosomes. The protocol of N-banding is given below

Incubate slides at 96±1°C for 15 min in 1N NaH_2PO_4 (pH4.2 ±0.2), adjust pH with 1N NaOH.

Rinse thoroughly in distilled water and stain in Giemsa for 20 min

Rinse slide in tap water and air dry

Incubate air-dried slides in 1 M NaH_2PO_4 (pH 4.15) for 3min at 94 C

Rinse slides in distilled water

Stain for 30min with a solution of 10% Giemsa in 1/15 M Sorensen's phosphate buffer (pH6.0)

Rinse slides in tap water, mount in immersion oil

3.4 Q-Banding:

In Q-banding Quinacrine dye is used. The alkylating dye binds both AT and GC region but only AT-quinacrine-complex fluoresces (Nabil and Sarra, 2017). The dark bands are primarily composed of DNA region rich in AT and the dull bands are rich in Guanine and Cytosine. Q bands are especially useful for distinguishing the human Y chromosome and various chromosome polymorphisms i.e involving satellites and centromeres of specific chromosomes.

3.5 R-banding:

R-banding is the reverse of G banding. The dark regions are euchromatic (guanine-cytokine rich regions) and the bright regions are heterochromatic (adenine-thymine rich regions) (Pai and Thomas, 1980).R banding involves pretreating cells with a hot salt solution that denatures DNA that is rich in adenine and thymine

Features of commonly used banding techniques are summarized in table 1.

Table1: Chromosome banding techniques

Technique	**Procedure**	**Banding pattern**
G-banding	mild trypsin proteolysis, followed by Giemsa staining	Dark bands are AT-rich (Low gene density) Light bands are GC-rich (High gene density)
C-banding (C stands for Constitutive heterochromatin)	Denature with barium hydroxide and then stain with Giemsa	Dark bands contain constitutive heterochromatin
Q-banding (Q stands for Quinacrine)	Stain with Quinacrine mustard	Dark bands are AT-rich Light bands are GC-rich
R-banding	After heat denatures, Giemsa staining is applied. G-banding in reverse, with R denoting reverse	Dark bands are GC-rich Light bands are AT-rich

4. METHODS:

Traditional karyotyping methods involvethe staining of chromosomes with particular stains and then visualization under microscopes (Cheng *et al.,* 2001).In recent years, a number of novel methods and strategies have been employed to carry out karyotypic analysis. The methods are G-banding, FISH, GISH, Next-generation sequencing, Array-based karyotyping and Digital droplet polymerase chain reactions etc.

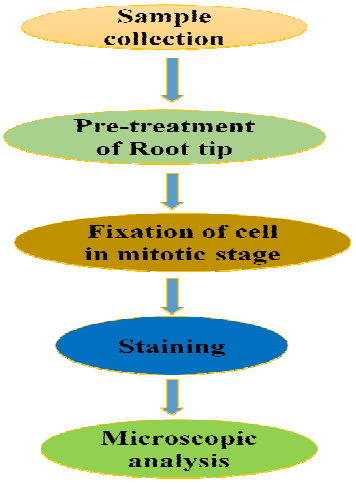

Fig 1: Schematic representation of karyotyping

4.1 G- Banding:

Giemsa banding or G-banding mainly involves the staining of chromosomes with Giemsa stains and analyses of the structure of condensed chromosomes within the nucleus of a cell (Comings *et al.,* 1973).The inherent simplicity, sensitivity, and durability of the material obtained make G-banding of chromosomes the most promising technology for routine chromosomeinterpretation. Previously banding operations have faced several hindrances for its efficiency and consistency of results. As of now, it is feasible to get consistent result with quality banding by performing small modifications to the standard technique (Yunis *et. al*., 1973).

4.2 fluorescence in situ hybridization (FISH):

The fluorescence in situ hybridization (FISH) technology resulted in a new age of molecular cytogenetics (Jiang *et al.,* 2006). By applying the complementary base pairing principle to hybridize fluorescently tagged probes to denatured genomic DNA, FISH allows chromosomes to be identified by counting and arranging their signals under a fluorescence microscope (Zhao *et al*., 2023). There are so many variations of FISH are coming day by day such as genome in situ hybridization (GISH), Multicolor-FISH, BAC (Bacterial Artificial Chromosome)- FISH, Oligo-FISH.

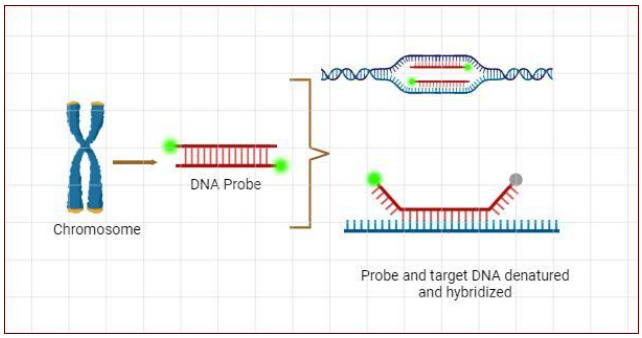

Fig 2: Schematic representation of FISH technique

4.2 GISH:

GISH technique is an advancement in the fluorescence in situ hybridization (FISH) techniquethat allows one to radiolabel parts of genome within the cells.One of the frequently used methods which separate distinct chromosomal groupings using entire genome sequences as probes (Yang *et al.,* 2020;Durnam*et al.,* 1985).

4.3 Multi-color FISH:

It uses polychromatic probes to identify chromosomes (Xiong *et al.,* 2011).The concurrent hybridization of multiple probes results in "multicolor FISH" investigations.Multi-color FISH uses more than two fluorescent dyes for probe identification. Probes of widely different complexity, including repetitive sequences, locus-specific probes (YAC, BAC, P1, cosmids), and whole or partial chromosomal painting probes, can be combined for multicolor FISH techniques. Other examples of multicolor FISH techniques in this manual show how multiple chromosome homologues in the human or murine chromosome complement can be stained with a different color at the same time. (Zitzelsberger*et al*.,2002).

4.4 BAC-FISH:

In BAC-FISH, BAC cloning vectors are used as probes to differentiate chromosomes (Xiong *et al.,* 2011; Jiang *et al.,* 1995).Bacterial artificial chromosome (BAC) library helps to overcome the bottlenecks of molecular analysis due to the large genome size of different species. In large-genome plants, BAC FISH screening is an effective technique for identifying molecular cytogenetic markers.

4.5 Oligo-FISH:

In Oligo-FISH techniquea reference genome is first used to create chromosome-specific oligonucleotides. Oligo-FISH is versatile in its design, doesn't require a lot of library screening, and isn't restricted to specific chromosomal areas (such telomeres, centromeres, and rDNA sites). Additionally, it has a number of benefits over conventionally made probes, such as stable probe quality and reduced preparation time (Zhang *et al.,* 2021; Han *et al.,* 2015). The absence of reliable DNA probes in the majority of plant species, particularly non-model plants, restricts the use of FISH techniques (Jiang and Gill, 1994).

4.6 Next-generation sequencing based karyotyping:

With the advancements of technologies, newly developedhigh resolution techniques are able to overcome varrious barriers of traditional methods. One such example is Next-generation sequencing based karyotyping (Tamura *et al.,*2021). The NGS technique for karyotyping includes cell culturethat can be very effective, but it is frequently prohibitively expensive when compared to conventional laboratory procedures. NGS is further limited by the fact that minor fragmentations in WGS obscure any significant structural alterations in the genome that can be found using other techniques like G-banding (Mareschaet *al.,* 2021).

4.7 Array-based karyotyping:

For the precise identification of chromosomal abnormalities, array-based karyotyping provides the same whole-genome coverage as G-band karyotyping. Because conventional approaches like G-banding and FISH procedures had restricted resolution by the microscopes, this necessitates the development of array-based karyotyping systems. Reduced resolution is a major drawback of array-based systems.

4.8 Digital droplet PCR (ddPCR):

It is a recent advancement in the technologies in which chromosomal abnormalities are analyzed (Codner*et al.,* 2016).In current scenario commercial platform pairing of dPCR with microfluidic

technologies should result in a crucial tool for cancer patient management. Cancer research applications includethe examination of various bodily fluids and tumor heterogeneity. In fact, droplet-based dPCR is especially well suited for the newly developing field of liquid biopsy analysis.

5. KARYOTYPING STUDY IN FORESTRY:

Karyotypes provide a physical map, chromosomal counts, and cytological features of Fabaceae species. In addition to that karyotype study also helps to construct the Oligo-FISH barcode and giving molecular cytogenetics information for Fabaceae species (He *et al.,* 2022).Cytological data of the Acacia species such as *Acacia mangium* and *Acacia auriculiformis* and their F_1 and F_2 has been revealed that they possessed the somatic chromosome 2n=2x=26 (Shukor*et al.,* 1994). Using hapten- or fluorochrome-labeled probes for the plant telomere repeat, centromeric repeat (PCSR), and rDNA, chromosomal landmarks in four *Pinus* species—*P. densiflora, P. thunbergii, P. sylvestris*, and *P. nigra*—were detected by fluorescence in situ hybridization (FISH) (Hizume 2002). Karyotype analysis of four Alnus species such as *A. mandshurica*, *A. pendula*, *A. sibirica*, and *A. sieboldiana*categorizedinto three groups according to ploidy levels or chromosome numbers: 2n = (4x) = 28, 2n = (8x) = 56, and 2n = (16x) = 112. Natural polyploidization may have caused the variations in chromosome count and karyotype characteristics, both within and between *Alnus* species (Jun et al., 2010). Three significant aromatic *Cinnamomum* L. species' karyotypes are characterized using reversible chromosomal banding techniques (Firdausi *et al.,* 2018). Usingsequential fluorescence *in situ* hybridization, five species of *Populus*have been karyotyped. It showed the synteny of the Populus chromosome after 14 years of divergence (Xin *et al.,* 2020). Because of their morphological similarities, *Taxus* species are difficult to identify. Oligo-FISH of five *Taxus* species gave insight on the evolution of the chromosomes of the *Taxus* species, which ultimately helped in enriching the molecular cytogenetics data of the species (He *et al.,* 2022).Cytogenetics study of the five *Lantana* species showed notable variations in karyotype, chromosome count, and nuclear DNA content between three native and two invasive *Lantana* species, which will aid in the identification, conservation, and utilization of native *Lantana* species (Parrish *et al.,* 2021).

6. CHALLENGES AND FUTURE DIRECTIONS:

Karyotyping has many advantages, but it also has drawbacks, including the requirement for trained technicians, the difficulty of detecting minute genetic alterations, and the necessity for extensive preparation. These restrictions may be overcome with the help of developments in molecular cytogenetics and the combination of genomic technologies and karyotyping. The use of high-resolution karyotyping techniques in forestry may eventually result in the creation of more resilient and fruitful tree species, improving biodiversity conservation and forest sustainability.

7. CONCLUSION:

Aneffective technique that improves knowledge of the genetic diversity, structure, and evolution of species is known askaryotyping. With its ability to provide important insights on chromosomal form, quantity, and behavior, karyotyping has emerged as a crucial tool in forestry genetics and conservation. It is used in wide range of fields, including biodiversity conservation, phylogenetic analysis, genetic enhancement, and species identification. Karyotyping advances our knowledge of genome organization and evolutionary relationships among differentspecies by making it possible to detect chromosomal differences, hybridization events, and polyploidy. Karyotyping is a crucial part of modern forestry science because of its capacity to identify chromosomal abnormalities, assist breeding operations, and aid in conservation initiatives.

Even while using this method on complex forest genomes presents difficulties, the developing science of molecular cytogenetics holds promise for removing these obstacles and improving our capacity to manage and safeguard forest species in a time of environmental change. Experts in forestry can guarantee the long-term viability and well-being of forest ecosystems across the globe by using these genetic technologies. Karyotyping has a lot of promise for directing conservation efforts and sustainable forest management in the face of growing concerns such as habitat loss, climate change, and overexploitation. It aids in the preservation and restoration of forestry species by offering a strong basis for comprehending genetic diversity and stability.

Karyotyping's usefulness will be further enhanced as the discipline develops by combining it with cutting-edge technologies like genome sequencing and bioinformatics. Karyotyping will continue to be an essential and developing tool for enhancing conservation science and forestry genetics as long as there is ongoing study and cooperation in this area.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript

---

8. DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

9. COMPETING INTERESTS

The authors have declared that no competing interests exist.

10. REFERENCES:

1. Badr, A. (2022). Chromosomes as Sources of Taxonomic Information for Plant Systematics and Evolution. *Taeckholmia*, *41*(1), 70-90.
2. Bou Dagher-Kharrat, M., Grenier, G., Bariteau, M., Brown, S., Siljak-Yakovlev, S., &Savouré, A. (2001). Karyotype analysis reveals interspecific differentiation in the genus Cedrus despite genome size and base composition constancy. *Theoretical and Applied Genetics*, *103*, 846-854.
3. Carvalho, G. M. A., Carvalho, C. R., & Soares, F. A. F. (2017). Flow cytometry and cytogenetic tools in eucalypts: Genome size variation× karyotype stability. *Tree Genetics & Genomes*, *13*, 1-11.
4. Cheng, Z., Buell, C. R., Wing, R. A., Gu, M., & Jiang, J. (2001). Toward a cytological characterization of the rice genome. *Genome research*, *11*(12), 2133-2141.
5. Codner, G. F., Lindner, L., Caulder, A., Wattenhofer-Donzé, M., Radage, A., Mertz, A., ... &Teboul, L. (2016). Aneuploidy screening of embryonic stem cell clones by metaphase karyotyping and droplet digital polymerase chain reaction. *BMC Cell Biology*, *17*, 1-13.
6. Comings, D. E., Avelino, E., Okada, T. A., &Wyandt, H. E. (1973). The mechanism of C-and G-banding of chromosomes. *Experimental cell research*, *77*(1-2), 469-493.
7. Conger, A. D., & Fairchild, L. M. (1953). A quick-freeze method for making smear slides permanent. *Stain technology*, *28*(6), 281-283.
8. Correia-da-Silva, M., Vasconcelos, S., Soares, M. D. L. D. C., Mayo, S. J., & Benko-Iseppon, A. M. (2014). Chromosomal diversity in Philodendron (Araceae): taxonomic significance and a critical review. *Plant Systematics and Evolution*, *300*, 1111-1122.
9. de Resende, K. F. M. (2017). Karyotype evolution: concepts and applications. *Chromosome structure and aberrations*, 181-200.
10. Durnam, D. M., Gelinas, R. E., & Myerson, D. (1985). Detection of species specific chromosomes in somatic cell hybrids. *Somatic cell and molecular genetics*, *11*, 571-577.

11. Dutta, M., Negi, K. S., & Bandyopadhyay, M. (2015). Novel cytogenetic resources of wild Allium (Amaryllidaceae) from India. *The Nucleus*, *58*, 15-21.

12. Firdausi, J., Dash, C. K., Rashid, M. H., & Sultana, S. S. (2022). Karyotype characterization of three important aromatic Cinnamomum L. species with special emphasis on reversible chromosome banding. *Journal of Applied Research on Medicinal and Aromatic Plants*, *31*, 100430.

13. Futuyma, D. J. (2015). Can modern evolutionary theory explain macroevolution?. *Macroevolution: Explanation, interpretation and evidence*, 29-85.

14. Han, Y., Zhang, T., Thammapichai, P., Weng, Y., & Jiang, J. (2015). Chromosome-specific painting in Cucumis species using bulked oligonucleotides. *Genetics*, *200*(3), 771-779.

15. He, Z., Luo, X., Lei, Y., & Zhang, W. (2022). Five species of Taxus karyotype based on oligo-FISH for 5S rDNA and (AG3T3) 3. *Genes*, *13*(12), 2209.

16. He, Z., Zhang, W., Luo, X., & Huan, J. (2022). Five Fabaceae karyotype and phylogenetic relationship analysis based on oligo-FISH for 5S rDNA and (AG3T3) 3. *Genes*, *13*(5), 768.

17. Heng, J., & Heng, H. H. (2023). Karyotype as code of codes: An inheritance platform to shape the pattern and scale of evolution. *Biosystems*, *233*, 105016.

18. Hizume, M., Shibata, F., Matsusaki, Y., &Garajova, Z. (2002). Chromosome identification and comparative karyotypic analyses of four Pinus species. *Theoretical and Applied Genetics*, *105*, 491-497.

19. Jackson, R. C. (1971). The karyotype in systematics. *Annual Review of Ecology and Systematics*, 327-368.

20. Jiang, J., & Gill, B. S. (1994). Nonisotopic in situ hybridization and plant genome mapping: the first 10 years. *Genome*, *37*(5), 717-725.

21. Jiang, J., & Gill, B. S. (2006). Current status and the future of fluorescence in situ hybridization (FISH) in plant genome research. *Genome*, *49*(9), 1057-1068.

22. Jiang, J., Gill, B. S., Wang, G. L., Ronald, P. C., & Ward, D. C. (1995). Metaphase and interphase fluorescence in situ hybridization mapping of the rice genome with bacterial artificial chromosomes. *Proceedings of the National Academy of Sciences*, *92*(10), 4487-4491.

23. Jun, L., Bao-Qing, R., Peigao, L., &Zhenglong, R. (2010). Karyotype analysis of Alnus Mill.(Betulaceae) species originating from northeastern Asia. *SilvaeGenetica*, *59*(1-6), 219-223.

24. Mareschal, S., Palau, A., Lindberg, J., Ruminy, P., Nilsson, C., Bengtzén, S., ... & Lehmann, S. (2021). Challenging conventional karyotyping by next-generation karyotyping in 281 intensively treated patients with AML. *Blood Advances*, *5*(4), 1003-1016.

25. Mareschal, S., Palau, A., Lindberg, J., Ruminy, P., Nilsson, C., Bengtzén, S., ... & Lehmann, S. (2021). Challenging conventional karyotyping by next-generation karyotyping in 281 intensively treated patients with AML. *Blood Advances*, *5*(4), 1003-1016.

26. Matsui, S. I., & Sasaki, M. (1973). Differential staining of nucleolus organisers in mammalian chromosomes. *Nature*, *246*(5429), 148-150.

27. MesferALshamrani, S., Safhi, F. A., Alshaya, D. S., Ibrahim, A. A., Mansour, H., & Abd El Moneim, D. (2022). Genetic diversity using biochemical, physiological, karyological and molecular markers of Sesamum indicum L. *Frontiers in Genetics*, *13*, 1035977.

28. Moraes, A. P., Olmos Simões, A., Ojeda Alayon, D. I., De Barros, F., &Forni-Martins, E. R. (2016). Detecting mechanisms of karyotype evolution in Heterotaxis (Orchidaceae). *PLoS One*, *11*(11), e0165960.

29. Nabil, A., &Sarra, F. (2017). Q-Banding. *Reference Module in Life Sciences; Elsevier: Oxford, UK*, 1-3.

30. Pai, G. S., & Thomas, G. H. (1980). A new R-banding technique in clinical cytogenetics. *Human genetics*, *54*, 41-45.

31. Pardue, M. L., & Gall, J. G. (1970). Chromosomal localization of mouse satellite DNA. *Science*, *168*(3937), 1356-1358.

32. Parrish, S. B., Qian, R., & Deng, Z. (2021). Genome size and karyotype studies in five species of Lantana (Verbenaceae). *HortScience*, *56*(3), 352-356.

33. Sacristan, M. D. (1971). Karyotypic changes in callus cultures from haploid and diploid plants of Crepiscapillaris (L.) Wallr. *Chromosoma*, *33*(3), 273-283.

34. Shrestha, S., Koo, D. H., Evers, B., Wu, S., Walkowiak, S., Hucl, P., ... & Poland, J. (2023). Wheat doubled haploids have a marked prevalence of chromosomal aberrations. *The Plant Genome*, *16*(2), e20309.

35. Shukor, N. A., Rashid, M. A., &Itam, K. (1994). Karyotypic comparison of Acacia mangiumWilld., A. auriculiformis A. Cunn. Ex Benth and their F1 and F2 hybrids.

36. Singh, R. J., &Robbelen, G. (1975). Comparison of somatic Giemsa banding pattern in several species of rye.

37. Sun, Y., Zeng, J., Liu, S., & Zhou, S. (2024). FISH and GISH reveal genome composition of popular Narcissus cultivars and the possible ways of their origin. *Euphytica*, *220*(6), 1-12.

38. Tamura, Y., Santo, M., Araki, Y., Matsubayashi, H., Takaya, Y., Kitaya, K., ... & Ishikawa, T. (2021). Chromosomal copy number analysis of products of conception by conventional karyotyping and next-generation sequencing. *Reproductive Medicine and Biology*, *20*(1), 71-75.

39. Veselinyová, D., Mašlanková, J., Kalinová, K., Mičková, H., Mareková, M., &Rabajdová, M. (2021). Selected in situ hybridization methods: principles and application. *Molecules*, *26*(13), 3874.

40. Xin, H., Zhang, T., Wu, Y., Zhang, W., Zhang, P., Xi, M., & Jiang, J. (2020). An extraordinarily stable karyotype of the woody Populus species revealed by chromosome painting. *The Plant Journal*, *101*(2), 253-264.

41. Xiong, Z., & Pires, J. C. (2011). Karyotype and identification of all homoeologous chromosomes of allopolyploid Brassica napus and its diploid progenitors. *Genetics*, *187*(1), 37-49.

42. Xiong, Z., Gaeta, R. T., & Pires, J. C. (2011). Homoeologous shuffling and chromosome compensation maintain genome balance in resynthesized allopolyploid Brassica napus. *Proceedings of the National Academy of Sciences*, *108*(19), 7908-7913.

43. Yang, Y., Yan, G., Li, Z., Yuan, J., Wei, X., Wei, F., ... & Cao, G. (2020). Cytological atlas at meiosis reveals insights into pollen fertility in synthetic Brassica allotriploids between allotetraploid B. carinata and diploid B. rapa. *Plant Physiology and Biochemistry*, *148*, 237-245.

44. Yoshida, Kohta, and Jun Kitano. "Tempo and mode in karyotype evolution revealed by a probabilistic model incorporating both chromosome number and morphology." *PLoS genetics* 17.4 (2021): e1009502.

45. Yunis, J. J., & Sanchez, O. (1973). G-banding and chromosome structure. *Chromosoma*, *44*(1), 15-23.

46. Zhang, T., Liu, G., Zhao, H., Braz, G. T., & Jiang, J. (2021). Chorus2: design of genome-scale oligonucleotide-based probes for fluorescence in situ hybridization. *Plant Biotechnology Journal*, *19*(10), 1967-1978.

47. Zhao, K., Bai, Y., Zhang, Q., Zhao, Z., Cao, Y., Yang, L., ... & Xiong, Z. (2023). Karyotyping of aneuploid and polyploid plants from low coverage whole-genome resequencing. *BMC Plant Biology*, *23*(1), 630.

48. Zitzelsberger, H., O'Brien, B., & Weier, H. U. (2002). Multicolor FISH techniques for the detection of inter-and intrachromosomal rearrangements. In *FISH Technology* (pp. 408-424). Berlin, Heidelberg: Springer Berlin Heidelberg.