Karyotyping in Forestry Species: A Vital Tool for Genetic and Conservation Studies

Authors' contributions

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

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. In order to conserve and manage forest tree species sustainably, karyotyping helps to understand genetic diversity, evolutionary histories, ploidy levels, and chromosomal differences. Karyotyping's importance in tackling the issues that forestry species confront, including as habitat loss, climate change, and genetic erosion, is further reinforced by recent developments in cytogenetic procedures.

Keywords: Karyotyping, Forestry species, ddPCR, FISH

1. INTRODUCTION:

Karyotyping, an elementary genetic attribute of all living organism involves studies of both chromosome number and morphological characteristics (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. Additionally, it provides a framework for evolutionary research, facilitating the tracking of speciation events and phylogenetic relationships both within and between genera.

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. 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 various significance of performing karyotyping in a speciesare discussed below-

2.1 Taxonomical classification: Several ways of classifying a species are thereincluding morphology based, molecular marker based and cytogenetics-based classifications. At morphology level, classification can be done by analyzing different structural parameters i.e. leaf and mostly by the flowering pattern. In flowering based identification proximity of flowering period is must, that makes it inefficient for identification. Karyotyping is the part of cytogenetics, helps to identify at the species and genus level by examining genetic diversity.

2.2 Assessment of genetic diversity and evolutionary linkages: Karyotyping is one of the tools by which genetic diversity can be assessed. 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. Karyotypes 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.

2.3 Polyploidy and Hybridization: Polyploidy is condition in plants where they possess multiple sets of chromosomes. Most of the forest trees often exhibits polyploidy and hybridization events like interbreeding between different species. Karyotyping can be used to identify polyploid species among a forest population and understand their role in forest biodiversity, mostly common in *Pinus* (pine) and *Betula* (birch).

2.4 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.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 that could give some species an evolutionary edge under shifting environmental conditions.

2.5Conservation: By studying the chromosomal composition, genetic stability of the species can be determined, eventually will help to conserve the vulnerable species. Karyotyping can also be used to evaluate the genetic diversity present within a population, which is an important step in conservation biology. Populations with unique genetic traits crucial for the survival of the species and are prioritized for conservation biology.

2.6Cytogenetics 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.

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3.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. 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 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. One of the frequently used methods is GISH, which separates distinct chromosomal groupings using entire genome sequences as probes (Yang *et al.,* 2020; Durnam*et al.,* 1985). Multi-color FISH uses polychromatic probes to identify chromosomes (Xiong *et al.,* 2011). In BAC-FISH, BAC cloning vectors are used as probes to differentiate chromosomes (Xiong*et al.,* 2011; Jiang *et al.,* 1995). Oligo-FISH,in which a reference genome is used to first 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). With the advancements of technologies, high resolution techniques are coming to overcome the barriers of traditional methods. One such example is Next-generation sequencing based karyotyping (Tamura *et al.,*2021). Because it includes cell culture, the use of NGS for karyotyping 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 (Mareschа*et al.,* 2021).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. Digital droplet PCR (ddPCR) is a recent advancement in the technologies in which chromosomal abnormalities are analyzed (Codner *et al.,* 2016).

4. APPLICATIONS:

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

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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). Karyotypes give a physical map, chromosomal counts, and cytological traits of Fabaceae species and also help to create the Oligo-FISH barcode and provide molecular cytogenetics information for Fabaceae species (He *et al.,* 2022).

5. CONCLUSION:

Karyotyping is aneffective technique that improves knowledge of the genetic diversity, structure, and evolution of forestry species. 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 as biodiversity conservation, phylogenetic analysis, genetic enhancement, and species identification. Karyotyping advances our knowledge of genome organization and evolutionary relationships among forestry species 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.

6. COMPETING INTERESTS

Authors have declared that no competing interests exist.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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