# Title-Cytological and Phenological Variability in *Dalbergia latifolia* Roxb: A Vulnerable Tree Species

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## Abstract

Forests around the world are an essential part of the ecosystem, and people, particularly in developing countries, rely entirely on forest products for their various kinds of needs. These products also supply timber and non-timber commodities essential for income, nutrition, and health. *Dalbergia latifolia* Roxb., commonly known as Rosewood, is native to low-elevation tropical monsoon forests in eastern India and is classified under the vulnerable category by the IUCN. Phenological and cytological study of this plant has been done on selected trees from its natural populations. The trees ($Tdl_{01}$-$Tdl_{05}$) exhibited meiotic disturbance in wild plants across various geographic locations, significantly impacting the chromosomal architecture during metaphase, anaphase, and telophase stages. Range of different chromosomal abnormalities, viz., stickiness, scattering, unorientation, laggard, and precocious movement, etc., were recorded. During cell division, certain chromosomal anomalies, which result in the acquisition or loss of individual chromosomes, frequently cause stunted growth and deformed traits lead to morphological and genetic variations. The presence of micronuclei in this plant and syncite formation was the key observation irrespective of genotypic diversity. The frequency of micronuclei was 0.37 ± 0.09 % in $Tdl_{04}$, whereas it was 0.39 ± 0.09 % in $Tdl_{05}$ but absent in other selected trees. This study will be very useful in screening the polyploid individuals among the populations as well as mass multiplication of polyploids at the field level. Since the current study aims to undertand the cyto-morphology through the pollen mother cells, including micro sporogenesis and pollen fertility, which can be utilised for tree breeding programs.

Keywords: *Chromosomal abnormalities; Cytology; Dalbergia latifolia; Forests; IUCN; Micronuclei*

Key message: *Dalbergia latifolia* is an important timber species in tropical forests, and due to various biotic and abiotic factors in open forest, it exhibits phenotypic as well as cytotypic variability

## Introduction

A range of ecological services and products, such as the retention of terrestrial biodiversity, regulating the climate, nutrient cycling and habitat for wildlife are all provided by tropical forests, which are of immense ecological significance (Tiwari et al. 2021). India, one of the world's 12 megadiversity countries, accounts for around 8 % of global biodiversity, occupying 2 % of the planet's territory. This is based on the species richness and levels of endemism recorded in a wide range of taxa, including both plants and animals.

*Dalbergia latifolia*, a slow-growing, usually single-stemmed deciduous tree, reaching heights of over 40 m, occurs frequently in Deciduous mixed forests (Nath et al. 2011). This species naturally occurs in India (Jackson 1994), Nepal, Indonesia (Soerianegara; Lemmens 1994), and Malaysia (Praciak et al. 2013). On the other hand, immense plantations of this species have been set up across Kenya, Myanmar, Nigeria, the Philippines and Vietnam (Orwa et al., 2009). *D. latifolia* has a significant economic impact due to its valuable timber (density 850 kg/$m^3$), and the bark and leaves are used in India for ethno-veterinary treatment (Jain et al. 2005; Padal et al. 2010; Selvaraju et al. 2011). Rosewood prices have risen considerably on the global market due to its high timber value and multifarious uses (Troup 1921). According to Thapa (2004), the increasing unavailability of timber, the exponentially rising demand for Rosewood, and the continuous illegal felling activity are the

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ultimate reasons for its overexploitation in its natural range. As a result, the IUCN classified it as "Vulnerable" in 2016 and included it in CITES Appendix II (Arunkumar et al. 2021). For any forthcoming tree breeding effort, studies on reproductive biology and cytology are crucial pieces of research. The natural breeding system regulates the amount of recombination and manages the degree of compatibility, pollination technique and reproductive process. The comprehension of elements, particularly chromosomes and the management of characteristic traits, such as variations in flowering, seed set, seed quality, seed viability, germination and survivability, are crucial for the reproductive system's effectiveness. To implement any improvement program, it is crucial to gather comprehensive information about the species' variability in terms of tree, flower, fruit, seed, and even chromosome characteristics. With these considerations in mind, we designed the current study to comprehensively examine the cyto phenological characteristics of Rosewood. Therefore, once we understand the species variability pattern (Fig. 1), we can take the necessary steps to utilize these variations for tree improvement programs.

## Material and Methods

### *Study site*

The study site is located (between 23005'41.7"N and 79059'23.0"E) in the ICFRE-Tropical Forest Research Institute, Jabalpur, representing the Tropical Forest part in India. There are more than 200 sparsely distributed *D. latifolia* trees on the campus of this institute.

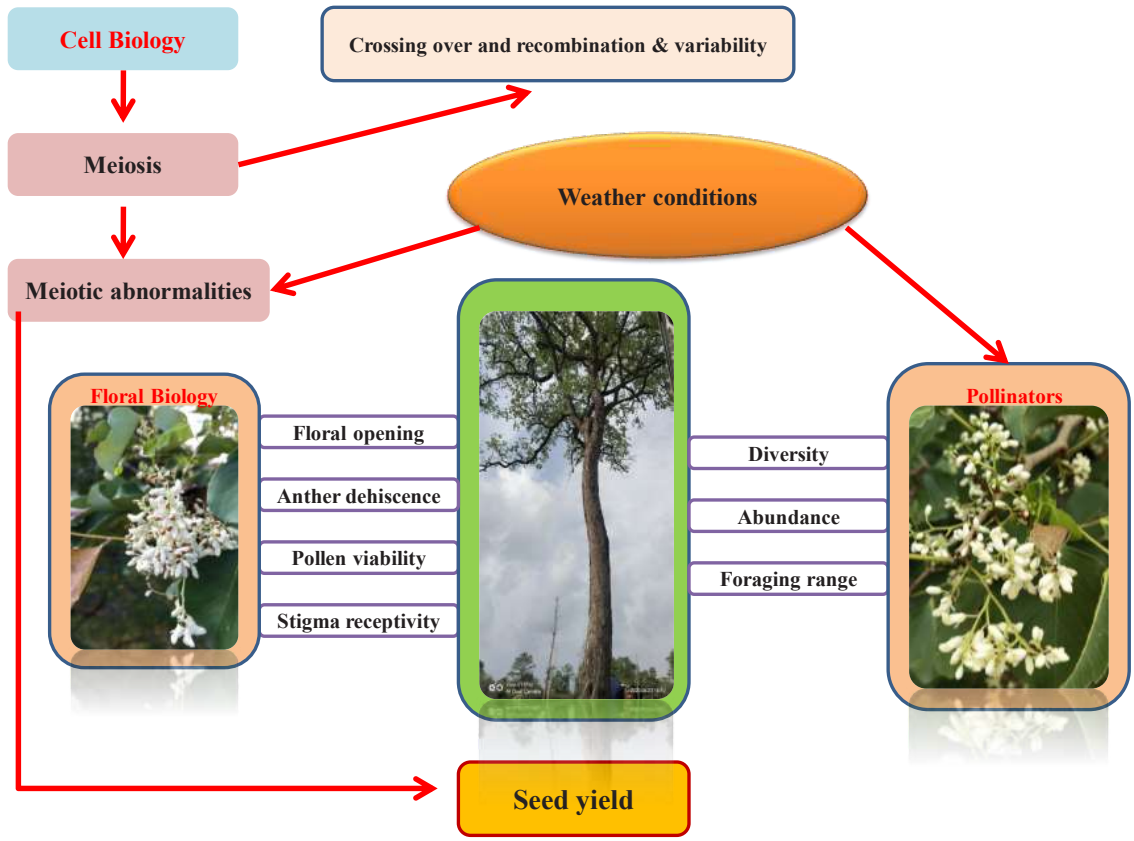


Figure 1
Illustrates the ray diagrams of seed source variations in *Dalbergia Latifolia* trees.

### *Phenology and Floral biology*

For the current investigations, we marked five trees, each representing a different plantation patch, in various locations across the Tropical Forest Research Institute, Jabalpur campus (Table 1). These indicated accessions ($Tdl_{01}$-$Tdl_{05}$) were chosen for a comprehensive study of morphology and floral biology. Over the course of two blooming seasons (2021-2023), morphological observations, Total Height (m), Clear Bole Height (m), Crown Height, Girth at Breast Height (cm), number of primary branches and under phenological events of the tree species (bud break, flowering, fruiting, leaf shedding, fresh leaf emergence, and fruit dispersal) were documented. Every morning during the flowering/fruiting season (September to March), we made observations. Subsequent events, including fruit maturity and dispersal, were observed and documented once a week. A set of randomly tagged blooming branches (n = 5) was used to determine the average number of flowers borne on an inflorescence. Fruit set percentage, fruit retention percentage, fruit % with different seed number, and seed germination percentage were expressed by the following formula:

$$Fruit\ retention\ \% = \frac{Number\ of\ retained\ fruits}{Total\ number\ of\ flower\ per\ inflorecence} X100$$

$$Fruit\ \%\ with\ diffrent\ seed\ number = \frac{Total\ no\ of\ sepecified\ seeds\ per\ pod}{Total\ number\ of\ fruit\ per\ inflorecence} X100$$

$$Germination\ \% = \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ experimental\ seeds} X100$$

### *Cytology*

Floral buds were fixed randomly from selected trees for cytogenetic analysis to determine their meiotic behavior. Immediately after harvesting, we fixed the samples in Carnoy's fixative (3 ethanol: 1 glacial acetic acid) and stored them at -40 C. Meiocyte suspensions were obtained from buds with sizes previously defined through squashing with the 1 % acetic-carmine stain technique. Cell suspension was prepared according to Viccini et al. 2006, and the total abnormality percentage (TAb %) was calculated by using the following formula:

$$Total\ abnormality\ percentage\ (TAb\%) = \frac{No.\ of\ abnormal\ cells}{Total\ no,\ of\ cell\ observed} X100$$

The proportion of pollen fertility was also assessed using the glycero-acetocarmine staining ability. Pollen grains that stained completely were regarded fertile while pollen grains that took partially colored were considered sterile (Mark, 1954).

$$Pollen\ fertility\ \% = \frac{No.\ of\ Fertile\ pollens}{Total\ no.\ of\ pollens} X100$$

Carmine-stained slides were evaluated under the Leica LEITZ DMRBE microscope using LAS EZ software, and views were recorded from each slide. The meiotic process was analysed in 5000 meiocytes per selected tree. The proportion of pollen fertility was also assessed using the glycero-acetocarmine staining ability. The data was analysed by the SPSS 16.0 software (1968). There were three replicates of floral buds from each selected tree for cytological estimations. A one-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT, $P < 0.05$) were performed for mean separation. Actual means and standard errors were calculated.

## Results and Discussion

### *Phenological variability Morphological Observations*

This study revealed that there was a significant variability in each individual's phenotypic traits. Small and large leaves of varying sizes were seen, and the average leaf size was manually measured using a scale (Fig. 2). Leaves are imparipinnate, alternate with swollen base; rachis is 12.8 - 18.6 cms long. Leaflets are 5-7 in number with petiole 6.5 - 9 mm in length. Small leaves have 5.80 ± 0.56 cm length and 5.4 ± 0.45 cm breadth. The average length and breadth of larger leaves were observed to be 9.01 ± 0.61cm and 8.40 ± 0.46 cm, respectively. Stem straightness and other morphological characteristics exhibited a narrow spectrum of variability. Average variations (Table 1) were also seen in total tree height, clear bole height, girth at breast height and crown. Average total tree height was 15.04 ± 2.25 m, Clear bole height 9.60 ± 1.12 m, average ratio of clear bole height to crown height 1.70 ± 0.95, girth at breast height 136.7 ± 2.90 cm and crown thickness ranged from very light to very thick. The variability in bark color (brown to black) was also noticed (Fig. 2 c-e).

### *Floral Observation*

The intensity of flowering varied significantly among *D. latifolia* trees. Additionally, trees with exceptionally dense flowering and very little flowering were noticed. A significant variation was observed in the flower-bearing capacity of trees and pod setting of trees. Along with the onset of the leaves in September, flowering also begins. Weather has an effect on flowering and a rise in temperature accelerates flowering. The inflorescence of *D. latifolia* has an axillary panicle consisting of numerous brief spikes with sub-sessile bisexual flowers. Variation in length of inflorescence was recorded; the range was found to be 5-21.6 cm (Fig. 2b). Flowers of *D. latifolia* were small, pea-shaped, slightly fragrant, with 4 mm long campanulate calyx, lobes shorter than the tube, the lower lobe the longest, and the upper lobes fused; a whitish corolla, with an obviate standard and clawed wings and keel; stamens usually 9, fused into a tube, but free in the upper part; and an ovary superior, with a short style (Fig. 3 a-e).

The majority of flower openings occurred between 10:30 am to 14:00 pm, with the peak occurring near 11:00 am to 1:00 pm. Ant her dehiscence started early in the morning while in the bud stage, shortly before the flower opened. During the study, we observed the stigma's receptivity and used a magnifying lens to determine that its shiny and sticky appearance indicated a receptive surface. On both the opened and unopened flowers, details had been collected and found that the stigma was found to become receptive a few hours before to flower opening and was also observed to remain so for a few hours

even after petals had opened. The whole process from bud initiation to flower opening was completed in 14-22 days (Fig. 4f). The flowering process began during the first week of September, reached its peak during the fourth week, and then began to decline during the second week of October. It took 6-7 months from bud initiation to fully ripened pods (Table 2).

### *Carpological observation*

As evidenced by the presence of juvenile pods alongside flowers on the inflorescences, flowering and fruiting are known to occur simultaneously (Fig. 3f). In natural pollinated inflorescences, fruit setting percentage was found to be maximum 44.85 ± 6.86 % in $Tdl_{01}$, but in the case of $Tdl_{05}$ it was noted to be minimum (35.22 ± 5.34 %). Similarly, fruit retention was noted to be maximum (39.91 ± 6.08) in $Tdl_{01}$ and minimum (27.59 ± 4.93) in $Tdl_{05}$ (Table 2). It was also observed that pods with different seed numbers, viz., single-seeded pods to four-seeded pods, Maximum 36.05 ± 5.31 % pods/inflorescence with one seed were found in $Tdl_{02}$, decreasing up to a minimum of 20.36 ± 5.33 % in $Tdl_{05}$. 25.74 ± 6.02 % pods/inflorescence with two seeds was reported maximum in $Tdl_{05}$ and decreased up to 16.41 ± 4.24 in $Tdl_{04}$ (Table 2). 29.73 ± 7.25 % pods/inflorescence with three seeds were found to be the maximum in $Tdl_{05}$, and their minimum was 13.9 ± 5.64 % in $Tdl_{02}$ (Table 2). $Tdl_{04}$ showed maximum 42.28 ± 1.47 % pods/inflorescence with four seeds (Table 2). Five seed pods/inflorescences were rarely found. The average pod length in naturally pollinated inflorescence was found to be 6.9 ± 0.85 cm, while the mean pod breadth was observed to be 1.62 ± 0.11 cm; the differences were found to be significant. The mean of 100 pod weights was recorded, and the range was found to be between 4.39 ± 0.46 to 4.89 ± 0.15 gm in selected trees (Table 2). Seed length was found to be greater in one-seeded pods, having a range of 9.65 ± 0.09 - 9.37 ± 0.15 mm, whereas it declined withincreasing number of seeds per pod up to a minimum of 7.05 ± 0.17 mm within selected trees (Table 2). The germination percentage was very high in seeds, i.e., more than 90 %. However, the mean seed germination percentage was found to be maximum 96.00 ± 1.25 % in one seeded pod (Fig. 4). The genetic makeup of the species, along with the different ages and growing environments of the different trees, could account for this variation.

### *Cytological variability*

A cytological study is a suitable method for mapping genetic diversity and deciphering the mysteries of inheritance. In the present study, cytological work was executed from the collected buds of selected trees of *D. latifolia*. The study displayed that meiotic chromosome number in *D. latifolia* (2n=2x=20) had 10 bivalents at diakinesis along with the nucleus (Fig. 5a). However, the meiotic disturbance in wild plants at different geographic locations was evident in the chromosomal architecture at metaphase, anaphase, and telophase (Fig. 5). We recorded a range of different chromosomal abnormalities, including stickiness, scattering, unorientation, asynchronous anaphase, laggard movement and precocious movement. Among them, stickiness at metaphase (Fig. 5c) and anaphase (Fig. 5f & g) were of serious concern. Metaphasic stickiness ranged from 0.49 ± 0.08 % to 0.89 ± 0.17 %. Accession no. $Tdl_{05}$ had the highest level of stickiness (0.89 + 0.17 %) (Table 3). Along with this, anaphasic stickiness was recorded to be a maximum of 0.90 ± 0.17 % in accession no. $Tdl_{05}$ and a minimum of 0.28 ± 0.15 % in accession no. $Tdl_{04}$. Target proteins cause chromosomal condensation during dynamic cell division stages, which appears to be responsible for stickiness under microclimatic conditions, especially biotic and abiotic ones. Jabee and Ansari (2005) proposed that chromosomal breakage may cause stickiness among the chromosomes. It may also be due to genetic and environmental factors (Rao et al. 1990; Baptista-Giacomelli et al. 2000; Jabee et al. 2008) suggest that the depolymerization of nucleic acid, resulting from partial separation of the nucleoproteins and a change in their association pattern, could cause stickiness. The range of unorientation at metaphase was reported as 0.09 ± 0.09 % to 0.70 ± 0.09 % (Table 3). Microenvironmental conditions generally affect precocious movement in chromosomes, making it a major anomaly compared to others. Maximal precocious movement was detected at 0.09 ± 0.19 % in accession no. $Tdl_{05}$. Agarwal and Ansari (2001), Srivastava and Kapoor (2008), and Kumar and Gupta (2009) suggest that early terminalization or stickiness of chromosomes, abnormal homology for chromosome pairing and movement of chromosomes ahead of the rest during anaphase could have caused precocious movement of chromosomes. Some previous research reports stated that precocious separation of univalents might be responsible for desynapsis or asynapsis (Kumar and Rai 2007). Distal polarity was also one of the chromatic abrasions that were found frequently in $Tdl_{05}$ (0.39 ± 0.09 %) and at a minimum of 0.12 ± 0.012 % in $Tdl_{02}$, whereas it was absent in $Tdl_{01}$ and $Tdl_{03}$. This plant also showed the presence of micronuclei (Fig. 5l p). The frequency of micronuclei was minimum (0.37 ± 0.09 %) in $Tdl_{04}$, whereas it was 0.39 ± 0.09 % in $Tdl_{05}$, but absent in other selected trees (Table 3). Micronuclei can originate from either acentric chromosomal fragments or sometimes the entire chromosome, which is left behind during the anaphasic stage of cell division and cannot be incorporated into the nucleus. Ventura-Camargo et al. (2011) suggested that the micronuclei appear due to the genotoxic effect of clastogen or aneugen and play a significant role in genetic changes. Thus, geographical climate can also induce this type of abnormality. The major problems found were cytomixis and syncite in different samples of cytological studies in the *D. latifolia* plant population.

Table 1
Geographical locations and morphological characterization of selected *Dalbergia latifolia* from TFRI campus.

| Tree No. | GPS Location | | Total Height (m) | CBH (m) | CH (m) | CBH/CH Ratio | GBH (cm) | No. Of primary-Branches | Tree form | Fl / FR |
|---|---|---|---|---|---|---|---|---|---|---|
| | Latitude | Longitude | | | | | | | | |
| $Tdl_{01}$ | 23°05'59.1" | 79°59'21.2" | 21.50 | 16.00 | 05.50 | 2.91 | 165.4 | 5 | Std | P |
| $Tdl_{02}$ | 23°05'44.0" | 79°59'10.0" | 16.00 | 08.50 | 07.50 | 1.13 | 153.2 | 6 | Std | P |
| $Tdl_{03}$ | 23°06'005" | 79°59'11.1" | 14.50 | 09.50 | 05.00 | 1.90 | 149.6 | 4 | Std | P |
| $Tdl_{04}$ | 23°06'06.2" | 79°59'23.5" | 12.00 | 06.50 | 05.50 | 1.18 | 112.5 | 4 | Std | P |
| $Tdl_{05}$ | 23°05'58.3" | 79°59'12.3" | 13.00 | 7.50 | 05.50 | 1.36 | 102.5 | 5 | Std | P |


Figure 2
Morphology of *Dalbergia latifolia*: (a) Individual tree; (b) Flowering branch; (c) Bark pattern in *D. latifolia* tree; (d) Variation in Bark; (e) Inflorescence with new pods; (f) Variation in leaf size; (g) Variation in immature pods with respect to seeds per pod and size; (h) Variation in mature pods with respect to seeds per pod and size.

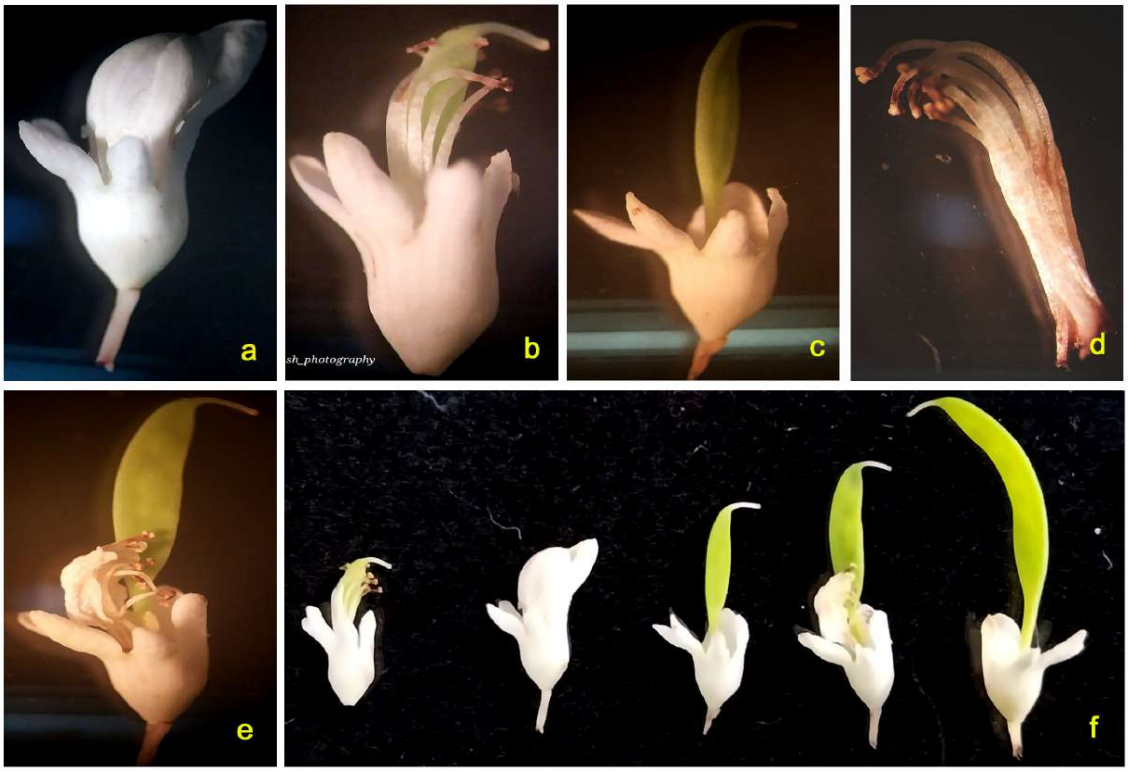


Figure 3
Illustrates the gradual development of flowers in *D. latifolia*: (a) Fully matured unopened flower; (b) Matured opened flower; (c) Fertilised flower; (d) Bunch of stamens; (e) Fertilised flower with stamens residue; (f) Different stages of floral development.

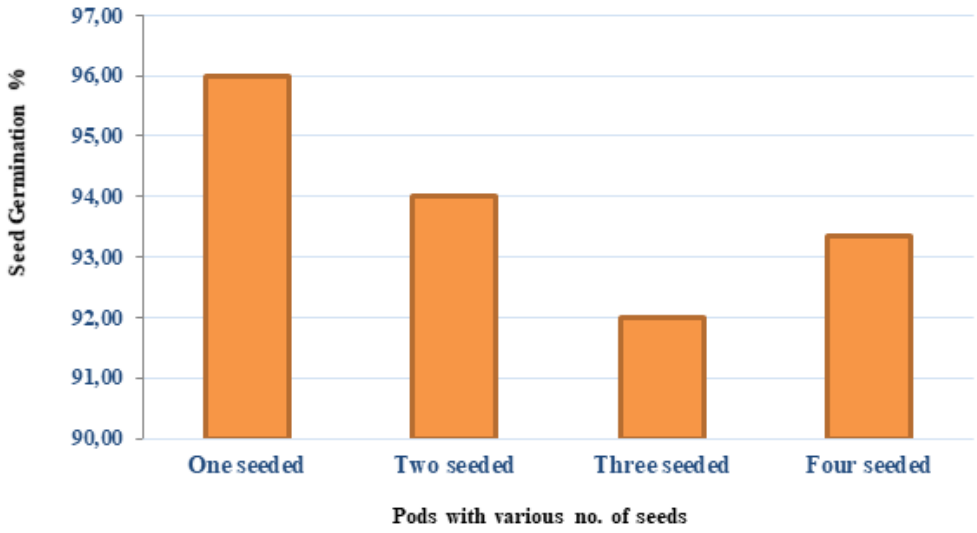


Figure 4
Illustrates how the quantity of seeds in a pod influences seed germination.

Table 2:
Carpological indices and time scale.

| S.No. | Trees | Flowering Initiation | Fruit maturation | | Fruit set % (Mean± SE) | Fruit retention % (Mean± SE) | Pod% with different numbers of seeds and seed length (mm). (Mean± SE) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mature Green | Mature Brown | | | One seeded | Two seeded | Three seeded | Four seeded |
| 1 | $Tdl_{01}$ | September First week | February First week | Mid-March | 44.85±6.86 | 39.91±6.08 | 27.55±3.02 | 21.37±3.63 | 27.56±4.33 | 23.52±3.38 |
| | | | | | | | 9.50±0.15 | 8.64±0.16 | 7.89±0.09 | 7.27±0.11 |
| 2 | $Tdl_{02}$ | September First week | February First week | Mid-March | 39.07±6.26 | 31.90±5.28 | 36.05±5.31 | 18.15±3.69 | 13.95±1.64 | 31.84±4.08 |
| | | | | | | | 9.65±0.09 | 8.04±0.15 | 7.63±0.17 | 7.05±0.17 |
| 3 | $Tdl_{03}$ | September First week | February First week | Mid-March | 41.24±6.60 | 32.26±4.57 | 27.98±2.01 | 19.84±4.28 | 20.53±4.54 | 31.66±6.86 |
| | | | | | | | 9.42±0.09 | 8.66±0.17 | 7.90±0.22 | 7.13±0.16 |
| 4 | $Tdl_{04}$ | September First week | February First week | Mid-March | 42.13±6.24 | 33.65±5.23 | 21.90±3.78 | 16.41±4.24 | 19.41±3.82 | 42.28±1.75 |
| | | | | | | | 9.45±0.19 | 8.57±0.14 | 7.96±0.07 | 7.11±0.16 |
| 5 | $Tdl_{05}$ | September First week | February First week | Mid-March | 35.22±5.34 | 27.59±4.93 | 20.36±5.33 | 25.74±6.02 | 29.73±7.25 | 24.17±2.43 |
| | | | | | | | 9.37±0.15 | 8.60±0.19 | 7.72±0.17 | 7.36±0.16 |

Significant frequencies of PMCs in the majority of cells (Fig. 6o) showed cytomictic flow between 3 and 4 cells in the accessions $Tdl_{01}$, $Tdl_{03}$, and $Tdl_{05}$. Kornicke (1901) first observed cytomixis, which is clearly defined as the migration of chromatin between adjacent cells through cytoplasmic connection channels, in PMCs of Crocus sativus. In the early prophase, Kornicke (1901) observed a seemingly doubled amount of chromatin and the flow of two nucleoli, suggesting that cell fusion or cytomixis during premeiotic conditions led to the formation of larger PMCs. Pagliarini et al. (2000) observed and reported that a disorder in cytokinesis during premeiotic situations, coupled with abnormal spindle formation, serves as the forceful mechanism for the formation of syncytes. Total abnormality percentage was recorded as a maximum of 6.36 ± 0.12 % in accession no. $Tdl_{05}$ and a minimum of 2.05 ± 0.04 % in $Tdl_{01}$. The frequency of TAB % increased significantly, exhibiting a high proportion of stickiness, precocious movement, and laggards. Reddy and Munirajappa (2012) were of the opinion that abnormal cell division, where the spindle fibers fail to carry the respective chromosomes to the polar regions, causes the variation among and between the populations. It implies that the microclimatic condition may have brought some alterations in the pattern of organisation of chromosomes to achieve variations (Shah et al. 2006). Size and structure of pollen grains (Fig. 6) from different flowers were measured with the help of a microscope using a micrometer. Normal pollen grains were spherical in shape, having a size of 21.05 ± 0.22 µm, along with normal pollen abnormalities in pollens that were also reported, viz., oval, elliptical, kidney-shaped, mega pollens, micro pollens etc. (Fig. 6a-q). Pollen fertility standards were likewise changed in accordance with evaluating the PMCs of different genotypes on the viability of pollens. In the case of fertile pollen that was found to be a conspicuously stained spherical nuclear substance, while sterile pollen was insufficiently stained and contracted. Most elevated pollen fertility (91.08 ± 0.98 %) was recorded in $Tdl_{01}$, which went for a decay (79.09 ± 0.76) in $Tdl_{05}$. According to Kumar and Rai (2007), having more chromosomal abnormalities had a big effect on microsporogenesis because they caused nonviable gametes to form, which made pollen much less fertile.

## Conclusion

Phenological and meiotic analyses, including data on chromosomal abnormalities and pollen fertility, did shed some light on the probable genetic diversity available in *D. latifolia*. Phenological changes in morphology and structural changes in chromosomes appear to have contributed minimally to the species diversification of the genus Dalbergia, while *D. latifolia* appears to have an average contribution to genetic divergence. The present work supports x=10 as the basic number of chromosomes for the *D. latifolia* tree. The selected tree with accession number $Tdl_{01}$ exhibited the lowest percentage of chromosomal abnormalities, while $Tdl_{05}$ displayed a higher percentage. The stickiness of chromosomes during both metaphase and anaphase, as well as the scattering, unorientation, precocious movement of chromosomes, and the anaphasic bridge stage, suggest significant cytological abnormalities in the trees at this geographical location, which contribute to genetic variation. The amount of TAB % wasn't too high in the wild trees. The abnormality ranged from 2.50 ± 0.04 to 4.41 ± 0.11 % in four trees, but one tree (accession number $Tdl_{05}$) had a high abnormality that was outside this range. This could be because of changes in the soil or the plant's physiology. During cell division, certain chromosomal anomalies such as stickiness, laggards, and bridges can cause unequal chromosome segregation, leading to the gain or loss of individual in stunted chromosomes. This, in turn, often results growth and distorted traits. Cytokinesis (the movement of chromatin between cells)

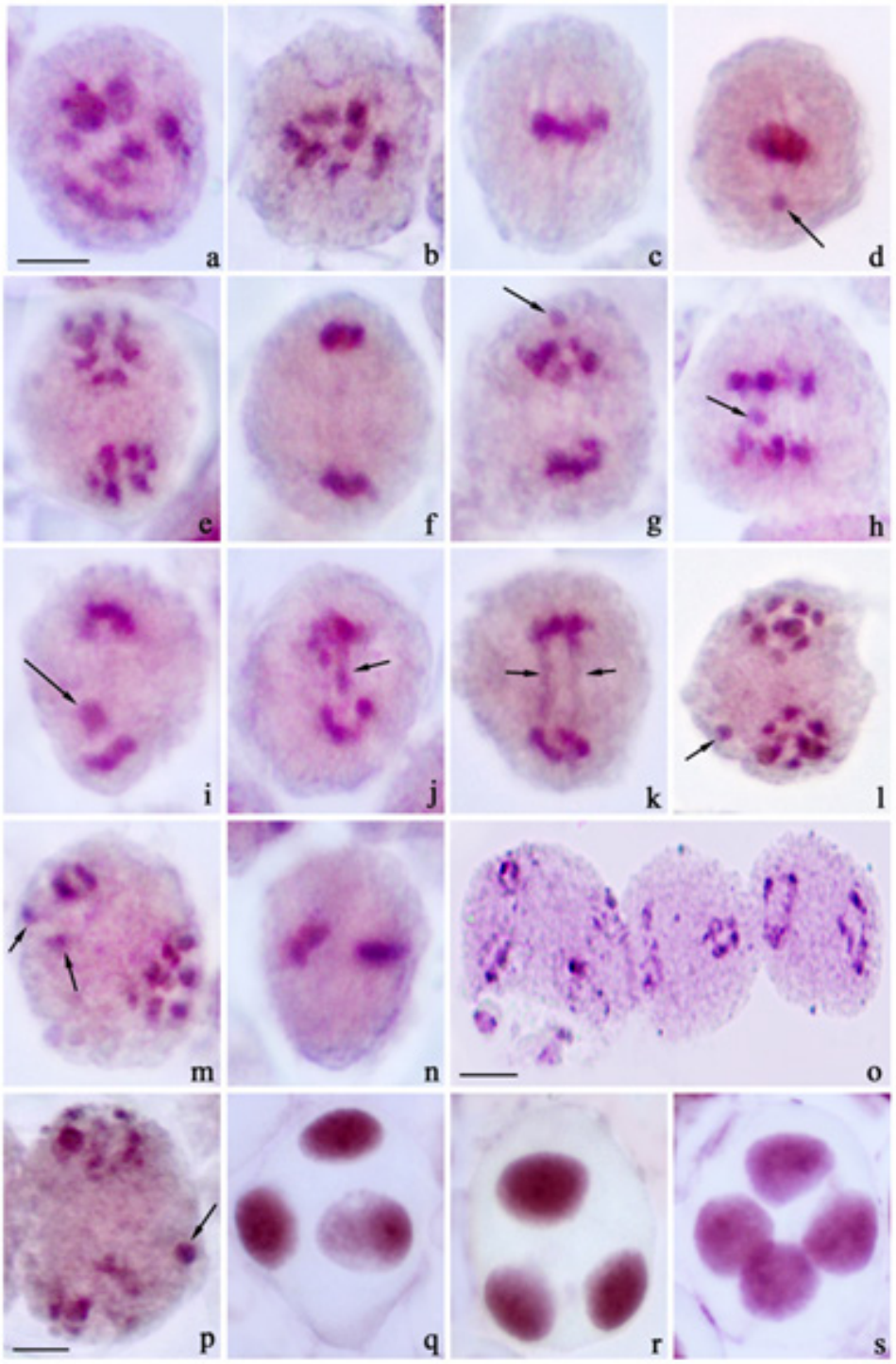


Figure 5
Various chromosomal abnormalities observed in *Dalbergia latifolia*. (a) Prophase I; (b) Metaphase I; (c) Stickiness at Metaphase I; (d) Precocious movement at Metaphase I ; ( e) Anaphase I; (f) Stickiness at Anaphase I; ( (g) Forward movement at sticky Anaphase I; (h) Anaphase I with laggard chromosome; (i) Anaphase I with laggard chromosome (j) Laggards with broken bridge at Anaphase I; (k) Anaphase I with double bridge; (l,m,) Laggard at Anaphase I; (n) Unorientation at Metaphase II; (o) Cytomixis between cells (on the right) - Metaphase II, (in the center) - Metaphase II, (on the left) - apparently, metaphase II; (p) Dyad with micronuclei; (q, r) Triad; (s) Tetrad ; Scale bar= 10 µm.

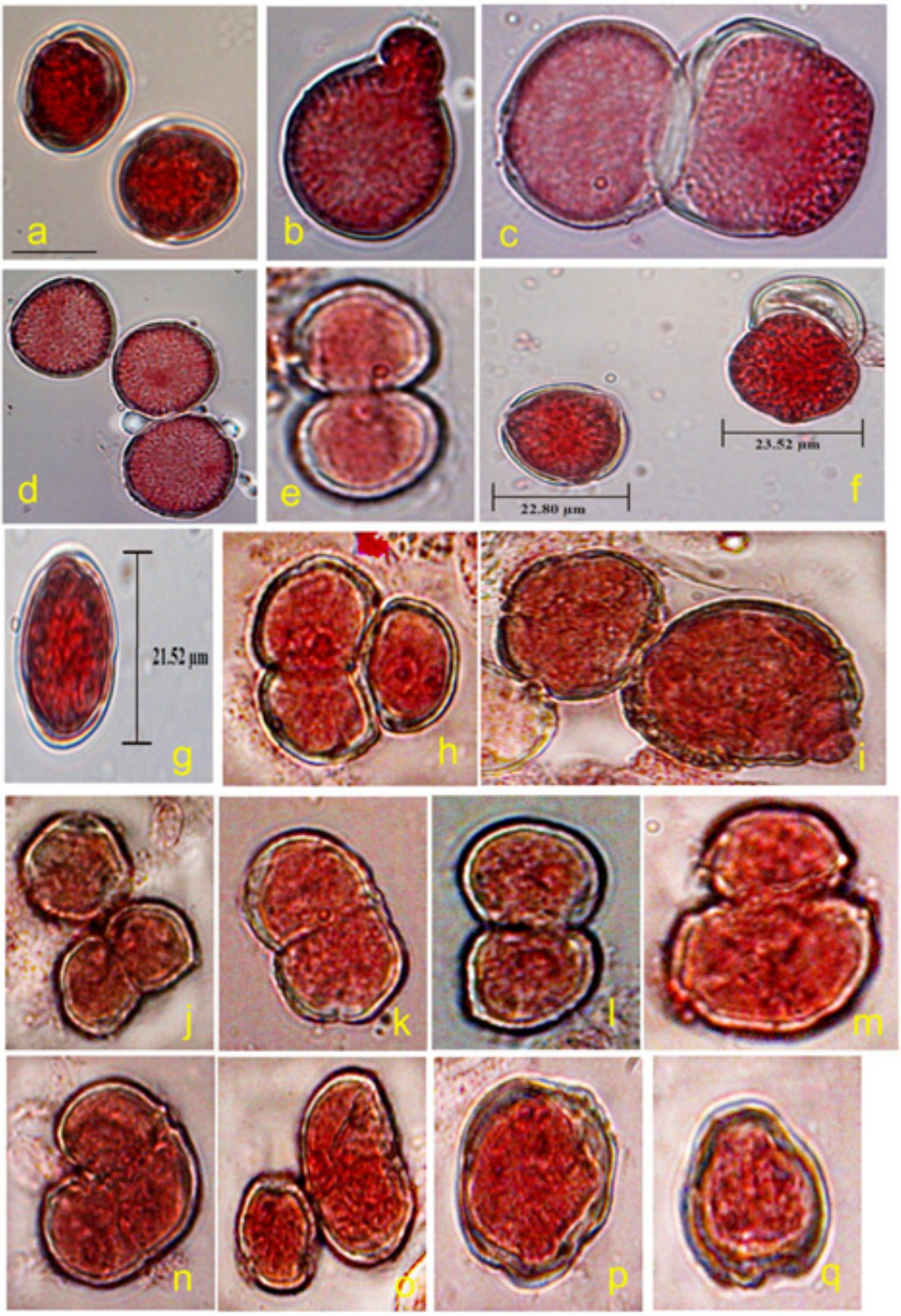


Figure 6
Pollen grain variability observed in *Dalbergia latifolia*. (a) Fertile pollen (Polar orientation); (b) Budded pollen; (c) Normal and rectangular fertile pollen; (d) Angular and circular pollen; (e) Dumbly shaped pollen; (f) Tricolpate and exposed pollen; (g) Elliptical pollen (equatorial orientation); (h) Abnormal Synpollen and micro pollen; (i) Mega pollens with thick exine; (j) Penta angular and asepted pollen; (k, l) Fused pol-len; (m) Synmega pollen; (n) Cordate shaped pollen; (o) Micro and mega pollen; (p) Mega pollen with irregular exine; (q) Tri-colpate unoriented pollen; Scale bar= 10 µm.

Table 3
Total Abnormality Percentage (TAB%), various Chromosomal Abnormality and Pollen fertility percentage in different tree of *Dalbergia latifolia*. (Abbreviations: St- Stickiness; Sc- scattering; Un- Unoriantation; Pr- Precocious movement; Ast- Anaphasic stickiness; Asc- Anaphasic scattering; Lg- Laggard; Dp- Distal polarity; MN-*Micronuclei*; Oth- Other; TAB- Total abnormalities percentage.

SE- Standard error

| Doses | No. of PMCs Observed | Metaphasic Abonormality (Mean±SE) | | | | Anaphasic Abnormality (Mean±SE) | | | | Telophasic Abnormality (Mean±SE) | | Oth (Cytomixis) | TAB% | Pollen fertility% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | St | Sc | Pr | Un | Asc | Ast | Lg | Un | Dp | Mn | | | |
| Tdl01 | 306.00 | 0.49±0.08 | 0.00±0.00 | 0.35±0.07 | 0.14±0.14 | 0.00±0.00 | 0.35±0.07 | 0.49±0.08 | 0.49±0.08 | 0.00±0.00 | 0.00±0.00 | 0.21±0.21 | 2.50±0.04 | 91.08±0.98 |
| Tdl02 | 275.00 | 0.85±0.11 | 0.24±0.12 | 0.74±0.23 | 0.60±0.11 | 0.13±0.13 | 0.48±0.12 | 0.62±0.12 | 0.62±0.12 | 0.12±0.12 | 0.00±0.00 | 0.00±0.00a | 4.41±0.11 | 84.11±0.81 |
| Tdl03 | 367.00 | 0.54±0.14 | 0.09±0.09a | 0.71±0.22 | 0.18±0.09 | 0.19±0.09 | 0.46±0.10 | 0.54±0.14 | 0.55±0.15 | 0.00±0.00 | 0.00±0.00 | 0.18±0.09a | 3.43±0.52 | 88.02±0.43 |
| Tdl04 | 355.00 | 0.57±0.15 | 0.20±0.10a | 0.55±0.14 | 0.09±0.09 | 0.11±0.11 | 0.28±0.15 | 0.48±0.11 | 0.46±0.17 | 0.20±0.10 | 0.37±0.09 | 0.00±0.00a | 3.30±0.30 | 88.90±0.56 |
| Tdl05 | 336.00 | 0.89±0.17 | 0.20±0.10a | 0.90±0.19 | 0.70±0.09 | 0.10±0.10 | 0.90±0.17 | 0.89±0.15 | 0.90±0.19 | 0.39±0.09 | 0.39±0.09 | 0.10±0.10 | 6.36±0.12 | 79.09±0.76 |

and other problems during meiosis can cause genetic diversity as well as polyploidy in diploid populations. Overall, chromosomal errors reshape plant form and development, contributing to both beneficial adaptations and detrimental deformities (Kaur et al. 2019). This study will be highly beneficial in identifying best cyto type individuals within the population and promoting their mass multiplication at the field level. Through this study, it can be concluded that *D. latifolia* is a highly suitable tree species for this geographical region. The work requires continuous amplification by analyzing cytotypes and phytophysiological changes in different agroclimatic zones for *D. latifolia*

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## Author contributions

All authors contributed to the study conception and design. Data collection and analysis were done by Dr. Kaushal Tripathi, Dr. Moni Mishra. Material preparation and cytological plate analysis were performed by Dr. Kaushal Tripathi and Miss. Jyoti Yadav. The first draft of the manuscript was written by Dr. Kaushal Tripathi and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

## Conflict of interest

The authors of this manuscript declare that they have no conflict of interest.

## References

Agarwal R, Ansari MYK (2001) The effect of Aniline on root tip cells of Vicia faba L. J Cytol Genet 2(NS): 129-134.

Arunkumar AN, Warrier RR, Kherand MM, Teixeira da Silva JA (2022) Indian rosewood (Dalbergia latifolia Roxb.): biology, utilisation, and conservation practices. Trees, 36(3):883-898. https://doi.org/10.1007/s00468-021-02243-3

Baptista-Giacomelli FR, Pagliarini MS, de Almeida JL (2000) Meiotic behavior in several Brazilian oat cultivars (Avena sativa L.). Cytologia 65(4): 371-378.

Bhat TA, Khanand, AH Parveen S (2006) Effect of gamma rays on certain cytomorphological parameters in two varieties of Vicia faba (L.). Advances in Plant Sciences 19(1): 227. https://doi.org/10.3923/ajps.2007.558.561

Jabee F, Ansari MYK (2005) Mutagenic effectiveness and efficiency of hydrazine sulphate (HS) in inducing cytomorphological mutations in Cicer arietinum L. var. K-850. J Cytol Genet 6:161-166.

Jabee F, Ansari MYK, Shahab D (2008) Studies on the effect of maleic hydrazide on root tip cells and pollen fertility in Trigonella foenum-graecum L. Turkish Journal of Botany 32(5): 337-344.

Jackson JK (1994) Manual of afforestation in Nepal. Forest Research and Survey Centre, Ministry of Forest and Soil Conservation, Kathmandu.

Jain A, Katewa SS, Galavand PK, Sharma P (2005) Medicinal plant diversity of Sitamata wildlife sanctuary, Rajasthan, India. Journal of ethnopharmacology 102(2): 143-157. https://doi.org/10.1016/j.jep.2005.05.047

Kaur D, Singhal VK (2019) Meiotic abnormalities affect genetic constitution and pollen viability in dicots from Indian cold deserts. BMC Plant Biol 19, 10.
Kornicke M (1901) Über Ortsveränderung von Zellkernen. SB Niederrhein. Gas. Natur. 14-25. https://doi.org/10.1186/s12870-018-1596-7
Kumar G, Gupta P (2009) Induced karyomorphological variations in three phenodeviants of Capsicum annuum L. Turkish Journal of Biology 33(2): 123-128. https://doi.org/10.3906/biy-0807-29
Kumar G, Rai PK (2007) EMS induced karyomorphological variations in maize (Zea mays L.) inbreds. Turkish Journal of Biology 31(4): 187-195.
Marks GE (1954) An aceto-carmine glycerol jelly for use in pollen-fertility counts. Stain technology 29(5): 277-277. https://doi.org/10.3109/10520295409115483
Nath CD, Pélissier R, Rameshand BR, Garcia C (2011) Promoting native trees in shade coffee plantations of southern India: comparison of growth rates with the exotic Grevillea robusta. Agroforestry Systems 83: 107-119. https://doi.org/10.1007/s10457-011-9401-8
Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A (2009) Agroforestree Database: a tree reference and selection guide. Version 4.
Padal SB, Murty PP, Raoand DS, Venkaiah M (2010) Ethnomedicinal plants from Paderu division of Visakhapatnam district, AP, India. Journal of Phytology 2(8).
Pagliarini MS (2000) Meiotic behavior of economically important plant species: the relationship between fertility and male sterility. Genetics and Molecular biology 23:997-1002. https://doi.org/10.1590/s1415-47572000000400045
Praciak A, Pasiecznik N, Sheil D, van Heist M, Sassen M, Sousa C, Correia, Dixon C, Fyson G, Rushforthand K, Teeling C (eds) (2013) The CABI encyclopedia of forest trees. CABI, Oxfordshire, UK https://doi.org/10.1079/9781780642369.0000
Rao PN, Ranganadhamand P, Nirmala A (1990) Behaviour of a 'sticky-desynaptic'mutant in pearl millet. Genetica 81(3): 221-227. https://doi.org/10.1007/bf00360869
Muniswamy RPM (2012) Gamma ray induced meiotic abnormalities IN S13 Mulberry. International Journal of Science and Nature 3(1):170-172.
Selvaraju A, Ayyanar M, Rathinakumarand SS, Sekar T (2011) Plants used in ethno-veterinary medicine by malayali tribals in Salem district of Tamil Nadu, India. Medicinal Plants-International Journal of Phytomedicines and Related Industries 3(3): 209-215. https://doi.org/10.5958/j.0975-4261.3.3.034
Shah TM, Mirza JI, Haq HA, Atta BM (2006) Induced genetic variability in chickpea (Cicer arietinum L.). I. Frequency and spectrum of chlorophyll mutations. Pakistan Journal of Botany 38(4):12-17.
Soerianegara I, Lemmens RH MJ (1994) Plant resources of South. East Asia, 5(1): 95-102. https://doi.org/10.2307/1224176
Srivastava A, Kapoor K (2008) Seed yield is not impaired by chromosome stickiness in sodium azide treated Trigonella foenum-graecum. Cytologia 73(2):115-121. https://doi.org/10.1508/cytologia.73.115
Thapa HB (2004) Early growth, thinning yield and estimated biomass of standing trees of Dalbergia latifolia Roxb. in the eastern Terai, Nepal. Banko Janakari 14(1):31-40.
Tiwari S, Ghosh BG, Vaidya SN, Rahaman SK, Khatun M, Garai S, Saikiaand P, Kumar A (2021) Modelling potentially suitable lac cultivation zones of Butea monosperma to promote livelihood security in rural India. Vegetos 34:630–637 https://doi.org/10.1007/s42535-021-00222-0
Troup RS (1921). The silviculture of Indian trees (Vol. 1). Clarendon Press. https://doi.org/10.5962/bhl.title.24363
Troup RS, Joshi HB (1983) Troup's The Silviculture of Indian Trees. Vol IV Leguminosae Delhi, India.
Ventura-Camargo BDC, Maltempi PP, Marin-Morales MA (2011) The use of the cytogenetic to identify mechanisms of action of an azo dye in Allium cepa meristematic cells. J Environ Anal Toxicol, 1(3):1- https://doi.org/10.4172/2161-0525.1000109