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Somatic and gametic chromosomal characterization with fluorescence banding of Giloy (*Tinospora cordifolia*): A berberine synthesizing important medicinal plant of India

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Abstract. Giloy [*Tinospora cordifolia* (Willd.) Miers.] has been a potential medicinal plant since ancient times; even today, it has great economical values; however, it still receives less attention in cytogenetic study. Detailed baseline data of chromosomes in mitotic and meiotic cell division remain very important for genetical characterization and evaluation of the reproductive potentiality of a species. Cytogenetic characterization with the aid of a chromosome banding technique is beneficial in searching chromosomal landmarks and constructing an accurate karyotype, which is completely lacking in the genus *Tinospora*. Hence, this is the first attempt of the detailed karyological study with fluorescence banding after enzymatic degradation of the cell wall. Chromosomes of *T. cordifolia* are small (1.8–2.4 μm) and $2n=26$ having a symmetric karyotype. The secondary constriction of two submetacentric pairs has DAPI negative /CMA positive bands. The meiosis studies of male flowers show the presence of 13 bivalents having secondary associations among themselves. Meiotic abnormalities such as precocious movement (7.56%) and laggard (2.83%) were recorded, and the pollen viability was estimated to be 47.17%. The berberine content produced in the stem of *T. cordifolia* has been quantitatively measured by high-performance liquid chromatography and found to be 0.424±0.02% on dry weight (DW) basis. In this study, precise karyological profiling by differential banding has been constructed and linked with the medicinal quality of the genotype, thus considered to be beneficial in the selection, cultivation, management, and improvement program of this species.

Keywords: *Tinospora cordifolia*, fluorescence banding, DAPI, CMA, karyotype, meiosis, berberine.

INTRODUCTION

An important medicinal plant *Tinospora cordifolia* (Willd.) Miers. commonly known as "Giloy," "Guduchi," or "Amrita," belonging to the Menispermaceae family. It is widely distributed ranging from the Himalayas to the southern part of India and also found in Southeast Asian as well as African countries (Lade *et al*. 2018). It is a large deciduous climber with aerial root,

fleshy stem, heart-shaped leaves, and unisexual flowers. Although different parts of the plants such as shoot, leaf, root, fruit, and seed have utility in herbal medicine, but maximum activities are found in stems (Bala *et al*. 2015). This plant has extensive use in the oldest Ayurveda system as anticancer, antiulcer, anti-inflammatory, hypoglycemia, antiarthritic, and hepatoprotective potential (Srinivasan *et al.* 2008). This Ayurvedic plant is highly recommended in the COVID-19 outbreak, as it strengthens and rejuvenates the immune system to fight the global pandemic (Prasad *et al.* 2020). The herbal drugs of this plant exhibit potential immunomodulatory effects to overcome immunosuppression as well as anticancerous activities on human breast cancer and prostate cancer (Sachan *et al.* 2019; Deepa *et al.* 2019). In recent studies, the molecular basis of the anti-inflammatory and antioxidant properties of *T. cordifolia* has been validated (Reddi and Tetali 2019). Besides, this species is also used in many Ayurvedic pharmaceutical industries to produce a cure against chikungunya and dengue (Mittal and Sharma 2017). *Tinospora cordifolia* contains several alkaloids, including berberine, palmatine, tembetarine, magnoflorine, choline, tinosporin, isocolumbin, tetrahydropalmatine, etc. (Singh *et al*. 2003). Berberine is an important isoquinoline alkaloid found in a surplus amount in the stem of *T. cordifolia* and reported to have a wide range of pharmaceutical activities, including antimalarial, antipyretic, anti-inflammatory, antimicrobial, antidiabetic, antitumor, etc. (Srinivasan *et al.* 2008). Among different berberine-containing plants, *T. cordifolia* covers a wide region in India spreading from Kumaon Mountains to Kanyakumari and mostly found in the wild habitats. Because of the distribution and availability, this plant has become the major source of berberine in India (Panchabhai *et al*. 2008).

The significant market growth of medicinal plants and herbal drugs in the global context over the last few decades has conveyed the message of consumer's faith in natural drugs over synthetic ones (Ravi and Bharadvaja 2019). As stated by the National Medicinal Plant Board (NMPB), India, among 960 species of traded medicinal plants, the consumption of 178 species is estimated to be more than 100 metric tons annually (Ved and Goraya 2007). The enormous medicinal values of *T. cordifolia* in traditional medicine elicit the estimated annual demand from 2000 to 5000 metric tons with annual growth registered at 9.1% according to the NMPB, India, in 2012 (Abhijeet and Mokat 2018). Owing to its increased demand, *T. cordifolia* is placed on the priority list of the NMPB to cultivate in the agro-climatic zones of Rajasthan, Uttar Pradesh, and Madhya Pradesh in India (Mridula *et al*. 2017). To enhance the production growth, mass multiplication and commercial-level cultivation of this plant have been prioritized. Before practicing any commercial-level cultivation for mass production, identification, selection, and characterization of medicinal plants are indispensable (Nyarumbu *et al*. 2019). In this context, basic genetic information by chromosome analysis is essential for the elementary genetical characterization of species to introduce them into plant breeding and crop improvement programs (Arroyo Martinez *et al*. 2017). Other than conventional breeding, whole-genome duplication or artificial polyploidy induction of medicinal plants is now a flourishing approach to increase the secondary metabolite production of therapeutic value. To step forward in this modern biotechnological research and crop improvement program, accurate knowledge of the chromosomal profile is unconditional. Despite the enormous economic importance of medicinal plants, genotype information explored by karyotype analysis is still not sufficient, which may help breeders in identification, selection, and efficient crop management (Peruzzi and Eroğlu 2013).

Karyotype analysis and meiotic behavior provide a cytogenetic framework of a plant species, which is subsequently used in the study of genomics, taxonomy, evolution, and reproductive biology (She 2016; Kaur and Singhal 2019). However, classical karyotype analysis with only chromosomal measurement by orcein-based staining lacks significant markers for individual chromosome identification (She 2016). Therefore, to tackle this challenge, karyotype analysis through differential chromosome banding with Giemsa and fluorochrome dyes is beneficial (Levin 2002). Chromomycin A3 (CMA) and 4',6-diamidino-2-phenylindole (DAPI) show preferential binding to the GC- and AT-rich DNA sequences, respectively, and give us the access to identify different types of heterochromatin and also serve as a chromosomal marker for karyotype analysis in plants (Barros e Silva and Guerra 2010). In contrast, analysis of the meiotic chromosomes enlightens the genomic behavior during gamete formation and generates the idea about reproductive performance, which helps the breeders in productive manipulation of plants of economic interest (Souza *et al.* 2015, Guidini *et al.* 2017). Very restricted information is available considering the genetic identity through the analysis of chromosomal characteristics and karyotype of *T. cordifolia*. Almost the entire study was limited to the numerical value of the chromosomes, while details of structural features of the chromosomes and their measurement remain unresolved (Table 1). Therefore, karyomorphological analysis with accurate chromosomal landmarks is becoming essential for the prospect of this species.

Meanwhile, with the identification of a particular genotype of medicinal plants by analyzing chromosomal

Table 1. Previous chromosome counts in *Tinospora cordifolia*.

Chromosomes in Sporophytic (2n) or Gametophytic (n)	Chromosome number	Karyotype*	Reference
n	12	-	Joshi (1934), Joshi and Rao (1935)
2*n*	24	-	Nanda (1962)
n	12	-	Sanjappa (1978)
n	13	-	Abraham (1942)
2*n*	26	-	Sharma and Bhattacharyya (1955)
2*n*	26	-	Sharma and Sharma (1957)
n	13	-	Sarkar et al. 1980
2*n*	22	asymmetric	Jain and Prasad (2014)
2*n*	26	-	Mathew (1958)

*Reports of the karyotype are based on the online available resources.

metrics corroborated with the differential banding characters, it is also necessary to assess their medicinal efficacy prior to any crop improvement and conservation program. Quantity and quality of the active principle in a medicinal plant species are a dynamic array, which can differ with the natural genetic variation of the species (Kroymann 2011). Therefore, assessment of the natural products present in the medicinal plants must link with the genetic characters. Hence, quantification of the berberine content in *T. cordifolia* is an essential part to evaluate their medicinal quality. High-performance liquid chromatography (HPLC) is considered a powerful analytical technique for the separation of natural products from a complex matrix to analyze and quantify them in a reliable and reproducible way. Although the berberine content has been quantified with HPLC in this species earlier, quality assessment linked with the particular genetic characteristic received less attention.

Therefore, in this study, for the first time, attempt has been made to analyze the precise chromosome characteristic to construct the karyotype by differential fluorochrome banding. The meiotic behavior of the reproductive cells of *T. cordifolia* has also been analyzed. Moreover, the berberine content of the genotype has been quantified to evaluate its medicinal efficiency. The outcome of this study may help in the selection and domestication of the species for future biotechnological and agricultural programs to improve from an economic perspective.

MATERIAL AND METHODS

Somatic chromosome preparation

The growing roots of *T. cordifolia* were collected from the medicinal plant garden. Chromosomes were prepared following Santra *et al.* (2020) with minor modifications. Roots were pretreated with 4 mM 8-hydroxyquinoline solution at 16 °C for 5 h and then fixed in acetic acid and methanol solution (1:3) overnight. Digestion of the cell wall was performed with an enzyme mixture containing 1% cellulase (Onozuka-RS, Sigma), 0.5% pectolyase (Sigma), and 0.75% macerozyme (Himedia) in a sodium citrate buffer (pH 4.6) at 37 °C for 60 min. After washing with the same buffer twice, the root tip was broken down into small pieces on a clean slide with the addition of freshly prepared fixative. The slide was air-dried for at least 24 h before staining.

Chromosome staining

Chromosomes were stained with 2% Giemsa solution in phosphate buffer solution with a ratio of 1:15 (pH 6.8) followed by rinsing with distilled water and analysis under microscope. Prior to fluorescent staining, slides were destained with 70% methanol for 15 min and air-dried. Slides were preincubated in McIlvaine buffer (pH 7.0) supplemented with 5 mM $MgCl_2$, followed by staining with 0.25 mg mL^{-1} CMA for 20 min in dark. After a short rinse in the same buffer, slides were mounted with 50% glycerol containing 5 mM $MgCl_2$ and kept in 4 °C for 48 h before further analysis. After preincubation in McIlvaine buffer (pH 7.0), chromosomes were stained with 0.5 µg mL^{-1} DAPI solution for 20 min in the dark followed by a rinse with the buffer and mounted with 50% glycerol. Chromosomes were analyzed under the fluorescent microscope Zeiss Axio Scope A1 equipped with CMA and DAPI specific filter cassette. Photomicrographs were taken with an AxioCam ICc 5 and ZEN application suite. Individual chromosomes were measured with AxioVision 4.9.1 and categorized based on the arm ratio following Levan *et al.* (1964).

Study of meiotic behavior and pollen viability

Tinospora cordifolia is a dioecious creeper with unisexual flowers. Male buds of the appropriate size were taken and fixed in the Carnoy's fixative (acetic acid: ethanol: 1:3 v/v) at 4 °C until use. Anthers were then removed and squashed with 2% acetocarmine stain. Pollen viability assessment through acetocarmine staining was performed according to Haque and Ghosh (2017). The pollen viability was estimated after an analysis of more than 1000 pollens. To stain with DAPI, anther was squashed with 45% acetic acid, and then, cover glass was removed by freezing the slides at –80 °C temperature for 10 min. Slides were then stained with 0.5 μg mL^{-1} DAPI solution and mounted with 50% glycerol in McIlvaine's buffer (pH 7.0). Photomicrographs were taken with an AxioCam ICc 5 and ZEN application suite equipped with bright field and DAPI-specific filter cassette.

Quantification of berberine content

Preparation of standard

A stock solution (1.0 mg mL^{-1}) of berberine (Sigma-Aldrich) was prepared with HPLC-grade methanol freshly.

Preparation of sample

The stem was dried at room temperature, and fine powder was prepared in a mechanical grinder. HPLC-grade methanol was dissolved with 20 mg of powder and was sonicated for 45 min at 40 °C. After centrifugation at 5000 RPM for 5 min, samples were filtered through 0.22 mm Teflon-coated membrane and aliquoted. The analysis was performed with three replicas.

HPLC conditions

The analytical HPLC experiments were performed with the Waters 1525 binary HPLC pump and the Waters 2489 UV–Vis. detector. The separation was carried out with reverse-phase C-18 column (5 μm particle size, 4.6×250 mm) using potassium dihydrogen phosphate buffer (Solvent A) with pH 3.2 adjusted by orthophosphoric acid and acetonitrile (Solvent B) with different solvent scales (0 min 90:10 v/v, 18 min 5:95 v/v, and 20 min 90:10 v/v); the flow rate was 1.0 mL min^{-1} under gradient condition. Injected sample volume was 20 μL with 20 min run times. Berberine was detected in a UV detector at 266 nm.

RESULTS

Karyomorphological studies

In this analysis with *T. cordifolia,* it has been found that they possess $2n$=26 chromosomes in somatic cells (Figure 1A-C). Chromosomes are small in length and range between 1.8 and 2.4 μm. Individual chromosome size has been mentioned in Table 2. Detailed karyotype analysis revealed that 11 pairs of chromosomes have median to nearly median primary constriction, whereas two pairs have submedian primary constriction. Hence, the karyotype formula is 22m+4sm (Figure 1I). Two pairs of submetacentric chromosomes are also associated with secondary constrictions in the long arm (Figure 1A, I). The position of all secondary constrictions is intercalary. The karyotype is symmetric and falls into 1A category of Stebbins's (1971) classification. Staining with CMA reveals that two pairs of chromosomes show bright CMA positive banding in their secondary constrictions (Figure 1B). Whereas DAPI stained the metaphase chromosomes uniformly, no DAPI positive band has been found (Figure 1E). Instead, DAPI negative bands have been detected in the secondary constrictions colocalized with the CMA positive bands (Figure 1F–H). However, when stained with DAPI at the prometaphase stage, some of the less condensed chromosomes reveal DAPI positive signals in their centromeric and pericentromeric regions (Figure 1D).

Meiosis studies

Meiosis in the male flowers of *T. cordifolia* revealed 13 bivalents in metaphase-I confirming n=13 chromosomes in the studied material. Chiasma formation in diplotene and diakinesis stages has been found to be normal, and the frequency is 10.83±1.16 per pollen mother cell (Figure 2A). Metaphase-I in some pollen mother cell, when stained with acetocarmine and DAPI, revealed 13 perfect bivalents (Figure 2B, C). In addition to the usual bivalents, secondary association between the chromosomes causes the formation of trivalent and tetravalent and multivalent configurations frequently (Figure 2D, E). In addition to the secondary associations, several other meiotic abnormalities such as chromosome stickiness, laggard chromosome and precocious movement have been recorded. Chromosome stickiness has been observed in 21.66% and 17.86 % of total metaphase-I and metaphase II respectively (Figure 2F, G, L). In anaphase-I, separation of the chromosomes in most of the plates was regular; however, chromosomes showed stickiness between themselves in each pole (Figure 2H-J). In addition, 2.83% of total anaphase-I stage having laggard

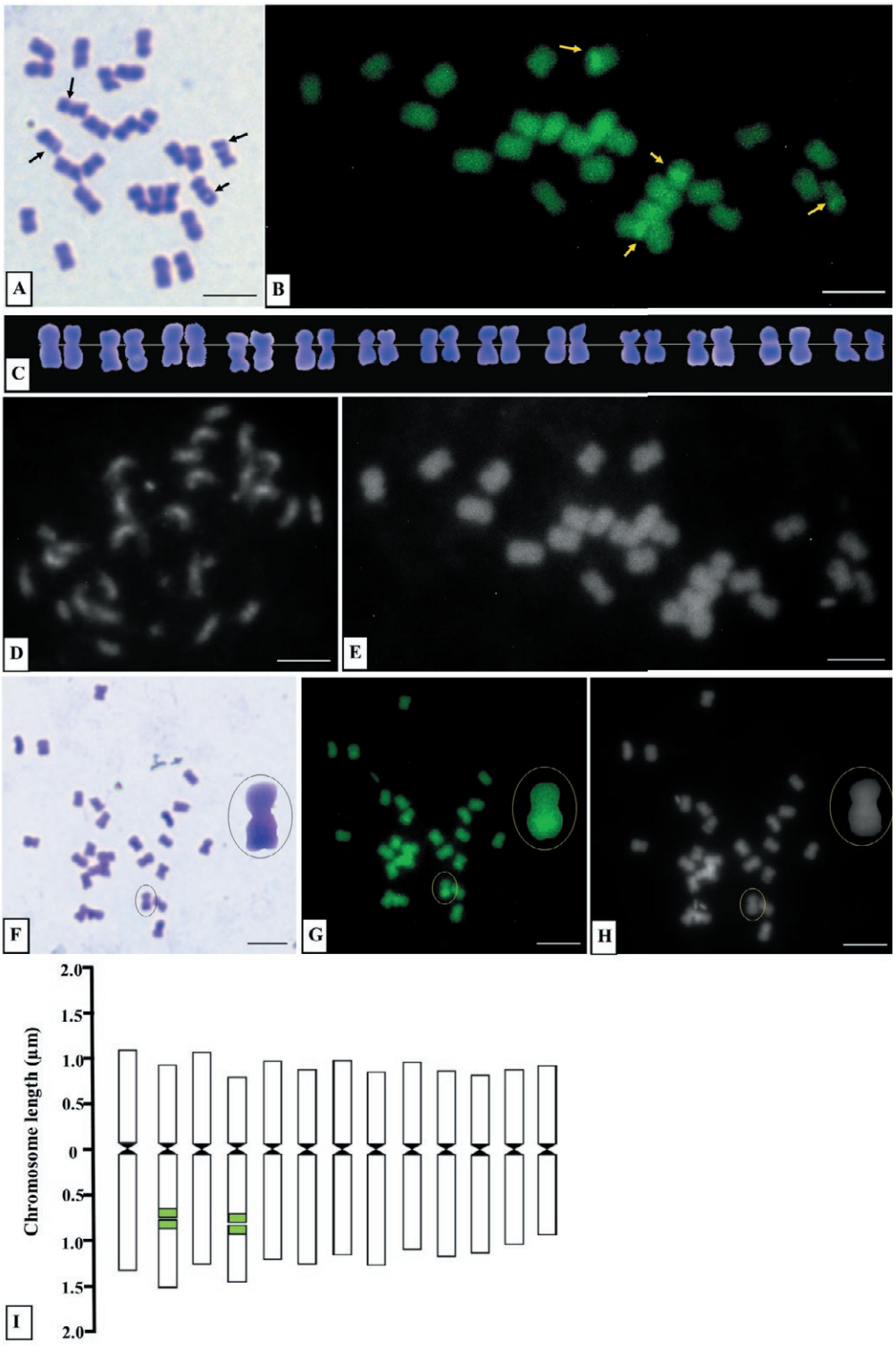

Figure 1. Somatic metaphase chromosomes of *Tinospora cordifolia* showing 2n=26 chromosomes; (A) Enzymatic maceration of the cell wall and Giemsa staining of chromosomes (arrows indicate secondary constrictions); (B) Metaphase plate stained with CMA, showing 4 positive bands at the secondary constriction region (marked with arrows); (C) Karyogram representation of Giemsa stained chromosomes; (D) Pro-metaphase stained with DAPI; (E) Metaphase plate stained with DAPI; (F-H) Same metaphase plate stained with Giemsa, CMA and DAPI respectively. The magnified chromosomes having a secondary constriction showing CMA positive and DAPI negative bands; (I) Ideogram representation of the chromosomes in metaphase. Scale bars=5µm.

Table 2. Chromosome parameters in *Tinospora cordifolia.*

Chromosome number	S (μm)	L (μm)	Total (μm)	Arm Ratio	Chromosome type*	DAPI/CMA bands
1	1.07±0.06	1.34±0.01	2.42±0.06	1.25	m	-
2	0.9±0.13	1.51±0.11	2.41±0.01	1.67	sm	DAPI negative/CMA positive
3	1.05±0.07	1.26±0.07	2.31±0	1.20	m	-
4	0.76±0.04	1.44±0.07	2.21±0.04	1.89	sm	DAPI negative/CMA positive
5	0.95±0.07	1.21±0.09	2.15±0.02	1.27	m	-
6	0.85±0.04	1.26±0.14	2.11±0.05	1.48	m	-
7	0.96±0.03	1.15±0.02	2.11±0.01	1.19	m	-
8	0.83±0.09	1.27±0.09	2.1±0	1.53	m	-
9	0.94±0.09	1.09±0.08	2.03±0.01	1.15	m	-
10	0.84±0.01	1.17±0.01	2.02±0.01	1.39	m	-
11	0.8±0.08	1.13±0.06	1.93±0.03	1.41	m	-
12	0.85±0.03	1.03±0.01	1.88±0.02	1.21	m	-
13	0.9±0	0.92±0.02	1.82±0.02	1.02	m	-

*m = metacentric, sm= submetacentric.

chromosomes during the separation (Figure 2I). Precocious movement found in 7.56% of total metaphase-I resulted in early separation of some bivalents (Figure 2K). Staining of pollens with acetocarmine shows bold red colors for the viable pollens and weak or colorless for the nonviable pollens. The estimated pollen viability was 47.17% (Figure 2M).

Estimation of berberine through HPLC

In HPLC, chromatogram of standard berberine peak was obtained at 10.706 min (Figure 3A), and a peak at 10.773 min was obtained from the methanolic extract of *T. cordifolia* (Figure 3B). In the case of our plant, the berberine content was found to be 0.424±0.02% on dry weight (DW) basis.

DISCUSSION

Chromosomes of the Menispermaceae family are small and have basic chromosome number, x=12 and 13. Only a very few genera of the Menispermaceae family have been considered for cytological assessment, and the same is also true for the genus *Tinospora*. Mitotic cell division and mitotic index have been recorded earlier in *T. cordifolia* that revealed different growth responses of the plant, based on the changing eco-climate (Shervani and Mishra 2020). Previous chromosome investigations revealed that the species have three cytotypes, 2n=22, 24, and 26 (Table 1). In the present report, chromosome analysis performed on *T. cordifolia* having diploid somatic chromosome number 2n=26 (Figure 1A) based on basic number x=13 agrees with the study by Sharma and Bhattacharyya (1955), Sharma and Sharma (1957), and Mathew (1958). As per the online available resources, analysis of chromosomal characters and their measurements have not been done before in this cytotype (2n=26). Karyological measurement has been reported for the cytotype having 2n=22 chromosomes (Jain and Prasad 2014). Mathew (1958) stated the range of chromosome size as small (2–3 μm). Individual chromosome measurement has not been mentioned. Further centromeric position of chromosomes could not be revealed. Nonetheless, these parameters are very essential for chromosome characterization. In plants with small chromosomes, it remains a challenge to prepare high-quality chromosomes spread by a conventional squashing method, where the details of chromosomal attributes can be resolved (Yamamoto *et al.* 2019). This may be a reason for the limited studies on cytogenetic of the family Menispermaceae despite being very important and economic. Therefore, enzymatic maceration of the cell wall becomes a reliable technique that removes the wall and helps spread the chromosomes on a cytoplasm-free background, so chromosomal details can be studied more rigorously. The chromosomes of *T. cordifolia* are small and categorized into metacentric and submetacentric (Figure 1A, C). In the cytotype with 2n=22, the presence of a subtelocentric pair has been reported, which is absent in the present investigation (Jain and Prasad 2014). The two pairs of submetacentric chromosomes are also associated with the secondary constriction, which is intercalary in

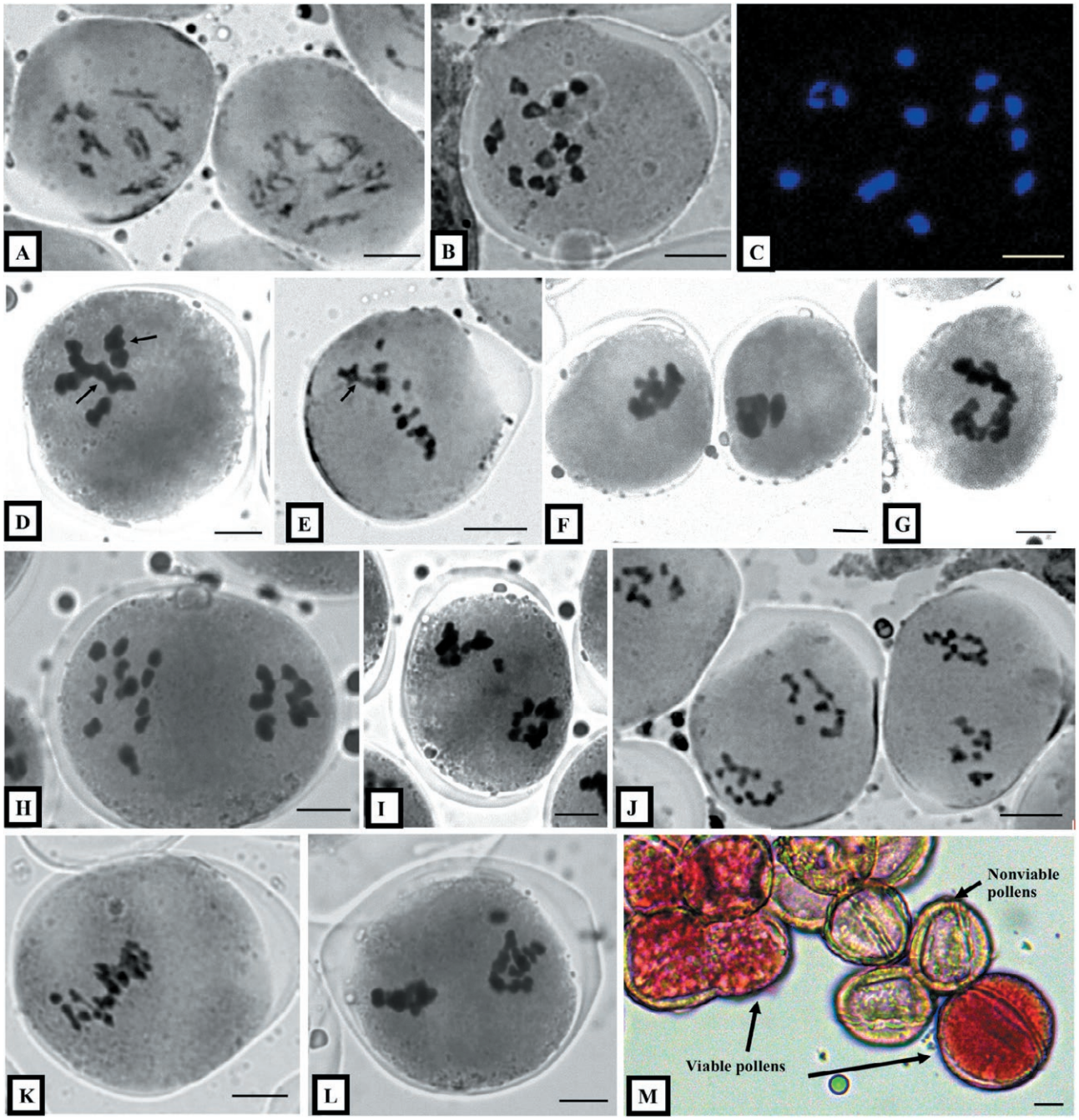

Figure 2. Meiosis and pollen viability of *Tinospora* cordifolia; (A) Diplotene stage with chiasma; (B-C) Metaphase-I, showing n=13 bivalents (stained with acetocarmine and DAPI respectively); (D-E) Secondary associations between chromosomes in metaphase-I showing bivalents trivalents, tetravalents and multivalents configuration (arrows indicate secondary association); (F-G) Chromosome stickiness in metaphase-I; (H) Separation of chromosomes in anaphase-I; (I) Laggard in anaphase-I; (J) Stickiness between the chromosomes after anaphasic separation; (K) Precocious movement in metaphase-I; (L) Chromosome stickiness in metaphase-II; (M) Viable pollens and non-viable pollen stained with acetocarmine. Scale bars=10μm.

position. Intercalary secondary constriction originated through chromosome breakage and inversion events that hold several evolutionary implications in species formation, studied in different plant genera such as *Richardia* and *Melilotus* (Schlarbaum *et al.* 1984; Siljak-Yakovlev *et al.* 2017).

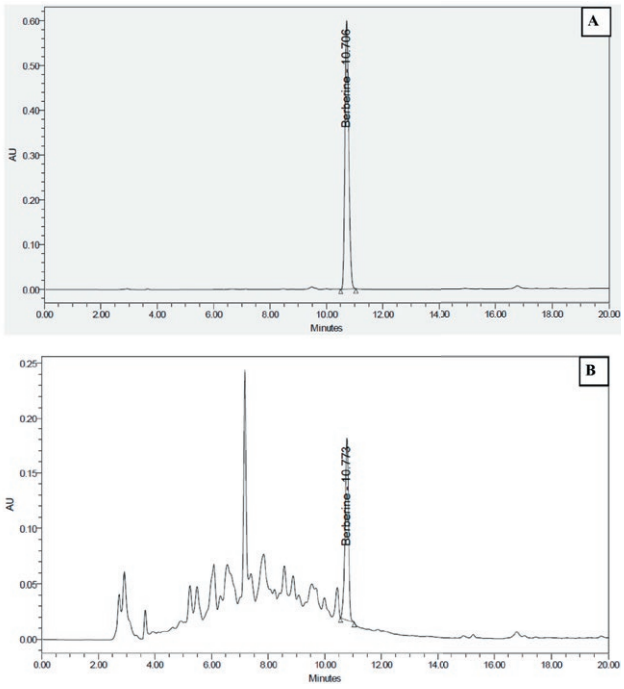

Figure 3. HPLC chromatogram of berberine. (A) Standard. (B) Stem of *Tinospora cordifolia.*

The CMA positive bands colocalized with the nucleolar organizer region (NOR) associated heterochromatin part can readily be observed in different plant species (Guerra 2000; Guidini *et al.* 2017). In the present study, the four intense CMA positive bands signifying the presence of GC-rich content in the secondary constriction (Figure 1B). In contrast, the uniformity of DAPI stain all over the chromosomes and the absence of any particular DAPI positive bands in metaphase stage indicate a lack of sufficient AT repeats (Figure 1C). The AT stretches of the DNA are required to generate distinct fluorescent signals, as DAPI is predominantly specific to the AT-rich region (Bhowmick *et al.* 2016). However, DAPI negative band associated with CMA positive bands signifies GC-rich DNA contents. Noticeably, DAPI positive signals can be visible in the less condensed chromosome of prometaphase, where any higher condensation in metaphase failed to generate the DAPI positive signal (Figure 1D). Perhaps, the loose condensation of the prometaphase provides a better resolution for DAPI than over condensed metaphase chromosomes. The karyological data along with their fluorescent banding is found to be reproducible for this genotype.

The meiosis studies show the presence of 13 bivalents in metaphase I which is in agreement with the studies by Abraham (1942) and Mathew (1958). Similar to somatic chromosomes, meiosis studies on this genus are also very limited despite the significance of meiotic behavior in reproductive events. Failure in successful meiosis during gamete formation can lead to pollen sterility and reduction in reproductive performance (Shin *et al.* 2021). Along with the regular meiotic behavior, various abnormalities in pollen mother cells have been observed in the present study and are mainly categorized into two classes. The first one is chromosome stickiness that may be a consequence of secondary association in metaphases-I. Secondary association is a result of the residual attraction between distantly related chromosomes owing to their structural rearrangements such as duplication, interchanges, or stickiness and has been reported across different plant genera (Bala and Gupta 2011). Data from different plant families indicate that the presence of the secondary association between bivalents evidences the occurrence of polyploidy or interspecific hybridization events as an extent of the genome evolution of plant species (Heilborn 1936; Bala and Gupta 2011). Polyploidy and intergeneric hybridization have appeared in different genera, namely *Cocculus*, *Menispermum*, and even in *Tinospora* of the family Menispermaceae (Wang *et al.* 2004; Lian *et al.* 2019). However, due to the less attention to the meiotic study of this family, the naturally occurred secondary association between chromosomes was never mentioned before. In the artificial colchitetraploid species of *T. cordifolia,* the occurrence of different chromosomal association such as quadrivalents, trivalents, bivalents, and univalents in the metaphase-I of meiosis is similar to that mentioned in the present study, which also explains the link between the appearance of secondary association and polyploidization (Thakur *et al.* 2020). Chromosome stickiness is a common phenomenon in plants where the secondary association is involved between the chromosomes (Bala and Gupta 2011). In addition to these, other common meiotic irregularities such as laggard chromosome and precocious separation result from abnormal spindle activity (Kumar and Singh 2003). In the present study, the percentage of the sterile pollens is higher than that of the viable pollens, suggesting that the meiotic abnormalities significantly affect the microsporogenesis process, which later on decides the fate of sexual reproduction. Together, cytogenetic assessment aided with the fluorescence banding and analysis of pollen infertility is utilized as a potent tool in the identification of stable genotypes that are further used in the breeding programs (Samatadze *et al.* 2020).

In the previous studies, berberine content has been detected through chromatographic separation in the extract of *T. cordifolia* (Srinivasan *et al.* 2008; Satija *et al.* 2020). Srinivasan *et al.* (2008) also reported the

quantitative variation of the berberine content in different samples of *T. cordifolia* studied through HPLC technique. Therefore genetic characterization together with quantification of active compounds is essential that relates a genotype with the medicinal efficacy. Best of our knowledge, genetic assessment along with the measurement of berberine content in *T. cordifolia* is remaining very poor. Hence, in the present study, the amount of berberine has been measured after details cytogenetical characterization of the plant. The stem of *T. cordifolia* contains 0.424±0.02% (DW basis) of berberine that is more or less resembles the report of Srinivasan *et al.* (2008). Moreover, the above study would be beneficial for the correct assessment of a genotype and reproductive performance linked with their medicinal quality which again is significant for any further quality improvement programs.

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