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RESEARCH ARTICLE

MICROPROPAGATION OF *LAGENANDRA NAIRII*, AN ENDEMIC AROID

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SUMMARY An effective procedure for micropropagation by producing multiple shoots from the basal buds of *Lagenandra nairii*, a priceless ornamental aquatic plant, has been developed. The basal bud explants initiated shoot buds at the earliest on full strength Murashige and Skoog medium supplemented with 4.0 mg/l 6-benzyl aminopurine (BAP). The same medium was the best for in vitro shoots to multiply to their greatest extent also. Maximum length of the in vitro shoots was observed on MS medium containing 0.5 mg/l BAP. Among various media tried, medium containing half strength of the MS mineral salts and devoid of growth regulators (half MS medium) alone could produce root formation. Hardening and planting of the in vitro raised plantlets in the aquarium were also successful. The methodology can be used for industrial scale propagation as a strategy to both conserve and market this endemic ornamental plant.

Keywords: Micropropagation, *Lagenandra nairii*, ornamental, aquatic.

INTRODUCTION

Lagenandra nairii Ramam. & Rajan, belonging to the family Araceae, is an aquatic plant endemic to Thrissur district, Kerala, South India (Ansari & Jeeja 2006, Pooja et al. 2020, Rao et al. 2019). The plant is a rhizomatous herb with bright green ovate leaves which are glabrous on the upper surface and hairy on the lower surface. Whorls of staminate and pistillate flowers are borne on dark purple or yellow coloured spathes. The beautiful foliage pattern and the colourful floral characters add to the ornamental value of this plant. *L. nairii* is well suited to be grown in aquaria owing to its adaptability to the habitat with good oxygen transport system and ability to utilize bicarbonates present in the alkaline water as source of CO_2 for photosynthesis (Ansari & Jeeja 2006). In spite of its potentials to excel in the international aquarium industry, *L. nairii* faces the challenge of habitat destruction due to anthropogenic interferences. In order to protect this endemic plant from the danger of extinction, rapid multiplication of plantlets through tissue culture is of utmost importance. So far, there has been a single report on the micropropagation of a threatened species under *Lagenanadra* viz., *L. thwaitesii* (Ranasinghe et al. 2001). The present study deals with micropropagation of *L. nairii* which has not been studied so far.

MATERIAL AND METHODS

Mother plants were collected from Chalakkudy River, Thrissur district, Kerala and maintained in the aquaculture laboratory, Department of Marine Biology, Microbiology and Biochemistry, Cochin

University of Science and Technology, Kerala (Fig.1). After removing the leaves, the rhizomes were drenched in dilute detergent (5% Cleansol, India) for 10 min and cleansed well in running tap water to clear out superficial dirt. Rhizome segments of 5–6 cm length were immersed in a 10–30% solution of commercial bleach made by Robin liquid bleach by Reckitt Benckiser, India for 10–30 min before being cleansed thrice in sterile water. The rhizome buds excised out together with a few basal tissues were then dipped in 100% ethanol for 2 sec and rinsed thrice with sterile distilled water. Inoculation of the rhizome buds on to various culture initiation media were done inside the laminar air chamber.

The culture initiation media included plant growth regulator (PGR)-free basal MS medium (Murashige & Skoog 1962) and MS medium supplemented with 1.0–5.0 mg/l BAP alone or in combination with 0.1 mg/l indole-3-acetic acid (IAA) or 1.0–5.0 mg/l kinetin alone. Initiated cultures were subcultured on fresh media with the same hormonal composition for shoot multiplication and the concentration of BAP in the media was lowered from 2.5 mg/l up to 0.5 mg/l during the subsequent subcultures to get satisfactory elongation of the multiplied shoots. As root formation was not observed in any of the media (including PGR-free MS medium) used for shoot multiplication or elongation, different combinations of BAP and IAA in the MS medium (0.5–1.5 mg/l of BAP in combination with 0.5–1.5 mg/l IAA) and half strength basal MS media were tried for rooting. The pH of the media was adjusted to 5.8 ± 2 using 0.1 N HCl or 0.1 N NaOH, dispensed in 150 × 25 mm culture tubes (15 ml medium per tube) or 15 ml conical flask and autoclaved at 121° C for 15 min. Cultures were maintained in a culture room at 25° C under a 16 h photoperiod with light intensity of 35 µmol photons $m^{-2}s^{-1}$ from Philips cool white fluorescent tubes. To test the effect of PGRs and their concentration in the MS medium on culture initiation, shoot multiplication, shoot elongation and root formation; 4 separate experiments were set up in completely randomized design (CRD) each with 6 treatments and 10 replications for each treatment. Data were analyzed by Univariate Analysis of Variance using SPSS ver.20. Differences between the means were compared by Tukey HSD (Zar 1999).

OBSERVATIONS

Out of various concentrations of commercial bleach and various durations of treatment tried to obtain axenic explants, treatment with 20% bleach for 10 min followed by a quick dip in 100% ethanol produced 90% contamination-free cultures. When the treatment duration and bleach concentration decreased from the optimum level, rate of contamination was steadily increased; whereas scorching of the tissue was observed with increase in bleach concentration and treatment duration.

Shoot initials without any callus formation were clearly visible on all the basal bud explants within 2 wks which subsequently developed into normal shoots in about 3 to 4 wks of culture. Statistical analysis showed that, time taken for bud release, number of shoots formed per explant and the shoot length were significantly influenced by the cytokinins and its concentration in the MS medium. Time taken for sprouting of shoots in media containing 1.0–5.0 mg/l BAP in combination with 0.1 mg/l IAA was longer when compared to that of the media with 1.0–5.0 mg/l BAP or kinetin alone ($p < 0.05$) (Tables 1, 2). The best medium for culture initiation from the rhizome buds of *L. nairii* was MS medium supplemented with 4.0 mg/l BAP in which early

TABLE 1: Effect of various PGRs on culture initiation and shoot multiplication in *L. nairii.*

PGRs (mg/l)	BAP		KIN		BAP+ 0.1mg/l IAA	
	No. of days taken for bud release	No. of shoots/explant	No. of days taken for bud release	No. of shoots/explant	No. of days taken for bud release	No. of shoots/explant
0	4.5431 ± 0.178^{d}	1.0828 ± 0.174^{a}	4.5431 ± 0.178^{d}	1.0828 ± 0.174^{a}	4.5431 ± 0.178^{d}	1.0828 ± 0.174^{a}
1.0	4.6532 ± 0.130^{d}	1.8124 ± 0.129^{c}	4.4721 ± 1.048^{c}	1.5731 ± 0.167^{c}	4.6709 ± 0.140^{cde}	1.2485 ± 0.213^{a}
2.0	4.5453 ± 0.089^{d}	2.0944 ± 0.121^{d}	3.9157 ± 2.805^{b}	1.2899 ± 0.200^{ab}	5.2114 ± 0.094^{e}	1.2389 ± 0.269^{a}
3.0	3.9146 ± 0.105^{b}	2.3175 ± 0.180^{e}	4.5825 ± 1.169^{d}	1.3217 ± 0.242^{b}	4.9488 ± 0.105^{de}	1.4999 ± 0.236^{b}
4.0	3.6038 ± 0.124^{a}	2.5672 ± 0.101^{f}	3.6744 ± 1.048^{a}	1.7856 ± 0.112^{cd}	4.1736 ± 0.312^{abc}	1.0828 ± 0.174^{a}
5.0	4.1807 ± 0.162^{c}	1.5999 ± 0.211^{b}	3.9287 ± 1.048^{b}	1.8928 ± 0.138^{d}	3.9509 ± 0.261^{a}	1.7588 ± 0.847^{c}

Means ±SD of 10 observations. Treatment means with same superscripts in the same column belong to the same homogenous subgroup.

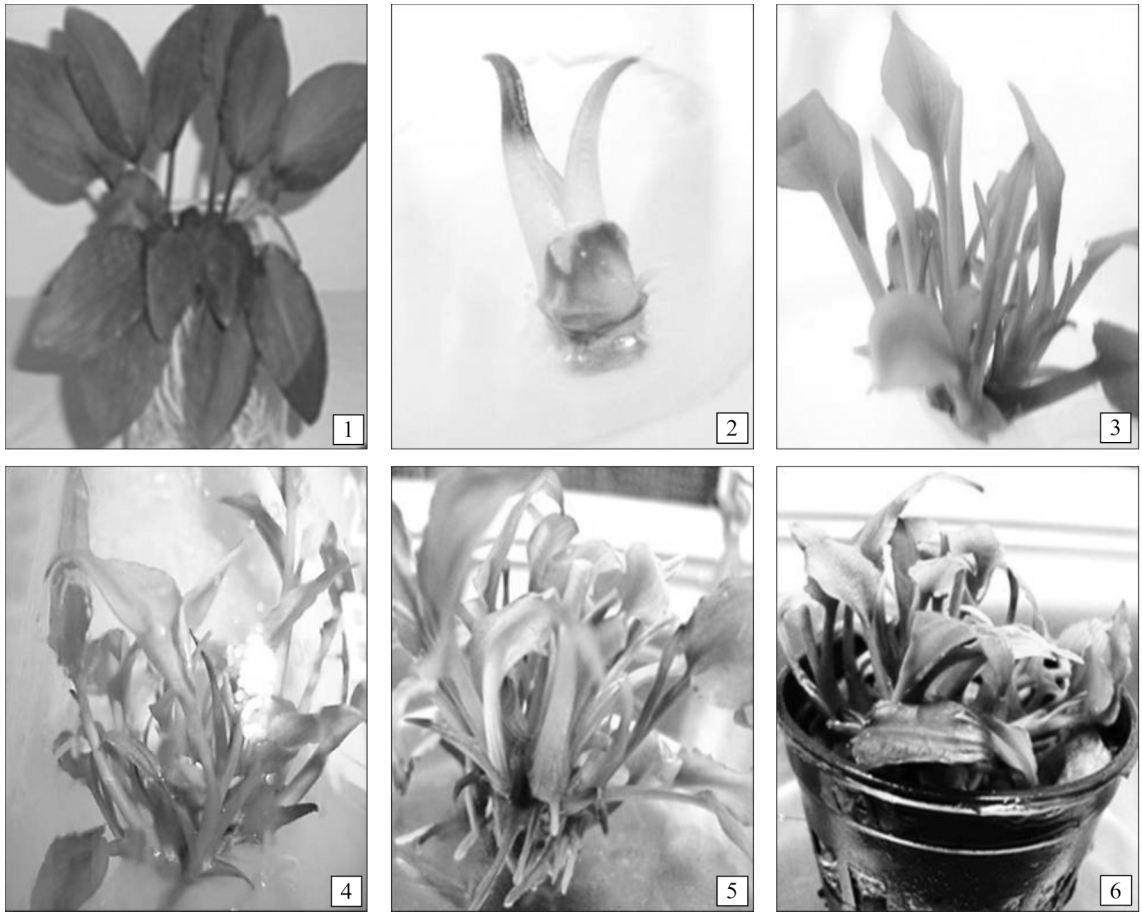
TABLE 2: Effect of PGRs on shoot elongation in *L. nairii.*

PGRs (mg/l)	Length of the shoot (cm)		
	BAP	KIN	BAP + 0.1 mg/l IAA
0	2.4100 ± 0.04434^{d}	2.4100 ± 0.04434^{e}	2.4100 ± 0.04434^{e}
0.5	2.8702 ± 0.04650^{f}	2.2753 ± 0.05600^{d}	1.8381 ± 0.15414^{cd}
1.0	2.6939 ± 0.05454^{e}	2.0703 ± 0.06679^{c}	1.5386 ± 0.11944^{b}
1.5	1.9397 ± 0.17458^{c}	2.0072 ± 0.03332^{c}	1.8799 ± 0.13308^{d}
2.0	1.8034 ± 0.09215^{b}	1.9129 ± 0.03069^{b}	1.8334 ± 0.17822^{cd}
2.5	1.6122 ± 0.10988^{a}	1.7794 ± 0.06551^{a}	1.2174 ± 0.09367^{a}

Means ±SD of 10 observations. Treatment means with same superscripts in the same column belong to the same homogenous subgroup.

release of adventitious shoots occurred on an average of 6.33 d following inoculation (Fig. 2). In the present study, BAP was found to be more effective than kinetin on shoot multiplication too ($p < 0.05$) and maximum shoot multiplication was obtained on MS medium supplemented with 4.0 mg/l BAP with an average of 2.56 ($p < 0.05$) shoots per explant (Fig. 3).

Multiple shoots produced in MS media containing higher concentrations of BAP or kinetin did not elongate considerably in the same media and stunted mass of multiple shoots were observed with 3.0–5.0 mg/l BAP or kinetin. Reducing the concentration of BAP or kinetin from 2.5–0.5 mg/l favoured shoot elongation (Table 2). Of the various media tried for shoot



Figs 1–6: *L. nairii.* 1. Mother plant. 2. Culture initiation. 3. Multiple shoot formation in MS medium supplemented with 4.0 mg/l BAP. 4. Shoot elongation in MS medium supplemented with 0.5 mg/l BAP. 5. In vitro root formation in half MS basal medium. 6. Plantlets transferred to pot.

elongation, maximum elongation of the in vitro shoots (2.87 cm, $p < 0.05$) was obtained with 0.5 mg/l BAP while average length of the shoots in MS basal medium was 2.41 cm (Fig. 4, Table 2). Addition of 0.1 mg/l IAA to the BAP-containing medium (0.5–2.5 mg/l BAP) did not show any superior effect on shoot elongation than MS media supplemented with 0.5–2.5 mg/l BAP or kinetin alone (Table 2).

Roots were not developed in the shoots cultured on MS basal media or MS media supplemented with 0.5–5.0 mg/l BAP or kinetin alone or in combination with 0.1 mg/l IAA. Of the various media tried, root development was achieved only on half MS basal medium with an average of 2.31 roots per shoot suggesting that, lowering the MS salt concentration to one half is essential for in vitro root formation in *L. nairii* (Fig. 5).

Hardening was done by transferring the rooted plantlets from culture vessels into plastic cups containing sterilized Neopete, which were

kept inside the culture room with controlled conditions of 25° C and 16 h/day illumination with cool fluorescent light for 2 wks. The humidity was adjusted at 80% for the first wk and at 60% for the next wk. The acclimatized plantlets were then transferred to garden pools and 92% of them were established successfully (Fig. 6).

DISCUSSION

In the present study, 100% of the explants surface sterilized with commercial bleach produced contamination-free healthy green shoots. Ram & Kakkar (1983) have reviewed inherent difficulties in obtaining aseptic cultures from aquatic plants, which dissuade investigators from using hydrophytes as experimental material for in vitro propagation. Jenks et al. (2000) also have reported difficulties in establishing axenic cultures in an aquatic plant, *Nymphoides indica,* with a success rate of only 3%. Dissanayake et al. (2007) have reported 60% and 65% contami-nation free cultures from the rhizome explants of *Echinodorus argentinensis* and *Cryptocoryne wendtii* respectively when 0.1% mercuric chloride was used as the surface sterilant. Use of mercuric chloride for explant sterilization has also been reported by Sonia & Das (2002) in *Piper longum,* Smitha et al. (2005) in *Myriophyllum aquaticum* and Sharma et al. (2010) in *Bacopa monnieri*. Present study points out that, use of harmful mercuric chloride can be substituted with commercial bleach in tissue culture studies; and our results support the earlier reports on explant sterilization using commercial bleach in *Ludwigia repens* (Ozturk et al. 2004), *Phyla nodiflora* (Ahmed et al. 2005) and *Rosa damascena* (Nikbakth et al. 2005).

MS medium supplemented with 4.0 mg/l BAP was the best for culture induction in the present study. Culture induction on MS medium containing BAP alone has also been reported in *B. monnieri* by Tiwari et al. (1998) and Sharma et al. (2010). Smitha et al. (2005) reported earlier shoot regeneration from the nodal explants of *M. aquaticum* on MS medium containing BAP alone at a concentration of 1.0 mg/l whereas BAP in combination with kinetin was reported in *Avicenia* (Abdulaziz & Al-Khayri 2003), *P. longum* (Sonia & Das 2002) and *Dioscorea rotundata* (Adeniyi et al. 2008). Even though auxin-cytokinin combinations are reported as more efficient for culture establish-ment in *Acorus calamus* (Anu et al. 2001), *L. repens* (Ozturk et al. 2004), *P. nodiflora* (Ahmed et al. 2005), *R. damascena* (Nikbakth et al. 2005), *E. cordifolius* (Dissanayake et al. 2007) and *D. alata* (Adeniyi et al. 2008), in the present study, no superior effect of IAA was seen on early induction of culture development when 0.1 mg/l IAA was added to 1.0–5.0 mg/l BAP. Therefore, the present investigation supports the opinion of Dissanayake et al. (2007) that, PGR requirement of plants are species-specific and specified hormonal treatments should be determined for in vitro propagation of aquatic plants.

Among all media tried, MS medium supplemented with 4.0 mg/l BAP gave the best results for shoot multiplication. Presence of BAP in the medium showed better multiplication of shoots than that with kinetin in any of the concentrations tried. Similar observations were made by Anu et al. (2001) in *A. calamus*, Smitha et al. (2005) in *M. aquaticum* and Royani et al. (2021) in *Indigofera zollingeriana.* However, in the present study, when the concentration of BAP in the medium exceeded the optimum level, there was a decrease in the rate of shoot multiplication. Inhibition of shoot multiplication by cytokinins beyond the optimum level has also been reported by Espinosa et al. (2006) in *Prunus serotina* and Pandeya et al. (2010) in *Clitoria ternatea.*

Observations on the elongation of in vitro shoots in the present study while supporting the opinions of Pijut et al. (1991) and George et al. (2014) that high cytokinin concentration in the medium has a suppressive effect on axillary shoot elongation, contradict their opinion that addition of low concentrations of auxins nullifies the suppressive effect of cytokinins. The present investigation is at variance with the opinion that the presence of growth regulators is not essential for the growth of the in vitro developed shoots (Ozturk et al. 2004), as the shoots on MS media containing 0.5 mg/l BAP were the longest. However, the results of the present study are in line with the findings of Geng et al. (2016) who reported in apple that, optimum concentrations of BAP in the tissue culture medium had stimulatory effect on shoot multiplication as well as shoot elongation.

None of the media tried except half MS basal medium produced in vitro roots in the present study. Similar reports are also available in *Cryptocoryne wendtii* and *Echinodorus cordifolius* where root development occurred when the shoots were transferred from full MS to half MS basal medium (Dissanayake et al. 2007). The methodology followed here will be helpful for both ex situ sustentation of this endemic ornamental aquatic plant and large scale propagation to suit market demands.

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RESEARCH ARTICLE

CHROMOSOMAL DIVERSITY IN INDIAN WILD MULBERRY COLLECTIONS OF *MORUS LAEVIGATA* AND *M. SERRATA* THROUGH EMA BASED FLUOROCHROME STAINING AND rDNA ANALYSIS OF *M. INDICA* VAR. KANVA-2

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SUMMARY Wild mulberries in India are represented by two native species viz., *Morus laevigata* and *M. serrata*. The former is distributed throughout India, including the Andaman Islands. But the latter is mainly confined to the foot hills of the Himalayan ranges. These wild resources are potential sources of rich genetic diversity and harbour genes for resistance to pest and diseases, as well as tolerance to frost damage, salinity, etc. Chromosomal investigations on these species are poorly documented due to extremely small size, holocentric nature and polypoidy. In this context, we analysed chromosome complement of 10 collections of these 2 species by enzymatic maceration and air-drying method (EMA). EMA method, followed by DAPI counterstaining, yielded a large number of cytoplasm-free metaphase plates with distinct chromosomes. The mean chromosome length among the species varied from 0.44 to 2.32 µm and mean total chromosome length ranged from 22.35 to 78.27 µm. No localized centromere was observed in chromosomes. We assigned ideogram formula based on the comparative mean size of the chromosomes. Detailed analysis has revealed karyotype diversity in terms of chromosome length, size and DAPI^{+ve} staining. The distinction of small chromosomes of wild species was useful in identification of chromosome complements in a diverse cytogenetic scenario. Bicolour FISH, using 25S rDNA and 5S rDNA probes showed discrete signals at respective loci on chromosome 5 and 11 of the popular mulberry cultivar, Kanva-2. The study addresses the fundamental needs towards utilization of wild mulberries in crop genetic enhancement.

Key words: Wild mulberries, fluorochrome, rDNA analysis, FISH, holocentric, karyotype.

INTRODUCTION

Mulberry (*Morus* spp.), is an economically important tree species and a prominent taxon of the family Moraceae. It is cultivated for its foliage in silk production as a small or medium bush or small tree in over 40 countries worldwide (Venkatesh & Munirajappa 2013). The National Active Germplasm Site (NAGS) for mulberry at the Central Sericultural Germplasm Resources Centre (CSGRC), Hosur, India has an assembly of 1317 diverse accessions (1032 indigenous and 285 exotic) from 29 countries in an *ex-situ* field gene bank (http://www.csgrc.res.in/dbmul). High variability of morpho-economic traits and high genetic diversity have been recorded in mulberry germplasm, specifically in wild mulberry

collections (Krishnan 2014, Pinto et al. 2018). India is one of the centres of diversity of mulberry owing to the existence of a natural wild population and the domestication process that started long ago, coupled with discrete agro-nomical practices. *M. laevigata* and *M. serrata* are the 2 well known native wild mulberry species that have been documented to occur in India. *M. laevigata* has been found growing scattered throughout India, including the remote Andaman Islands, but *M. serrata* is confined to the Himalayan region (Naik et al. 2015). Even though, wild mulberries are quite distinct and highly diverse, the taxonomic identity and categorization of cultivated species are debatable (Zeng et al. 2015). Therefore, comparative insight into the cytogenetical aspects, such as chromosome number, ploidy and karyological observations of wild species, may be supportive in establishing systematic and evolutionary links with the cultivated types, resolving taxonomic difficulties and indicating a better understanding of how they diverged from one another (Mehravi et al. 2022). In commercially important crops, chromosomal analysis has aided in the conservation of plant genetic resources for crop enhancement programmes (Jha 2019).

Several studies on morphological, biochemical, and molecular aspects of mulberry were undertaken, but the plant has been confronted with extreme limitations in the field of cytogenetical studies. The mulberry species are not easily amenable to cytogenetic analysis due to small size of chromosomes, dense cytoplasmic background and holocentric nature of chromosomes with near similar morphology (Xuan et al. 2017). Further, intra- and interspecific ploidy variation with chromosome numbers (2n) ranging from 14 to 308 also complicated the cytological investigations. Till date, the chromo-somal evolutionary process in mulberry species remains vague.

Two basic chromosome numbers exist in mulberry, x = 7 (He et al. 2013) and x = 14 (Xuan et al. 2022). The first to initiate cytological investigation in the genus *Morus* was Tahara (1909). Chromosome analysis was attempted in a number of studies and reported varying chromosome counts. Osawa (1920) reported 2n = 28 in *M. alba*, a diploid, while Janaki Ammal (1948) was the first to report the existence of a dodecosoploid (2n = 308) in *M. nigra*. Datta (1953) reported a tetraploid collection of *M. laevigata* (2n = 56). Seki (1952) and Basavaiah et al. (1990) observed tetraploidy in *M. tiliaefolia* and *M. serrata* respectively. All these authors have utilized the traditional staining methods to assess the chromosome numbers, and concluded that the mulberry chromosomes were extremely small, packed in dense cytoplasm and devoid of satellites, secondary constrictions or other structures (Datta 1953). Due to its small size and similar chromosomal appearance, it is difficult to identify individual chromosomes using conventional methods. Because of this fact, the cytological investigation of mulberry was found to be cumbersome. Hence, it appears that cytologically the mulberry did not receive the attention it deserved (Xuan et al. 2022). Holocentric chromosomes are a unique trait in mulberry and several other plants where centromeric position is diffused and the entire chromosome (small and dot like) exhibits the kinetochores along the entire length. Holokinetic conditions favour karyotype differentiation for agmatoploidy (fission), symploidy (fusion) and polyploidy (Luceño & Guerra 1996, Bureš & Zedek 2014).

In *M. notabilis*, a wild species endemic to China, the enzymatic maceration and air-drying method (EMA) followed by Giemsa staining was utilized for improvisation of karyomorphological analysis and resolution of structural

details of chromosomes. This method, overcame the technical limitations of the conventional aceto-orcein technique (He et al. 2013). EMA method-based chromosomal preparations enable easy applications of the fluorochrome banding technique as a suitable upgradation of karyotyping in the era of molecular cytogenetics (Ghosh et al. 2018). The use of base specific fluorochromes like the adenine-thymine (AT)-specific 4',6-diamidino-2-phenylindole (DAPI) facilitates the distinction of morphology-alike chromosomes, which in a broad sense contribute to karyotype refinements and the characterization of individual genotypes or varieties in particular (Bhowmick & Jha 2015). The advent of molecular cytogenetics enabled techniques such as fluorescence *in situ* hybridisation (FISH), which is more powerful than the classical methods and has been used in the plants with small chromosomes. FISH with probes for specific DNA sequences can be employed for evolutionary studies (Anamthawat-Jónsson 2003) and it allows chromosomes to be accurately identified by specific DNA sequences based on signal patterns. The rDNA probes are the most exploited probes in cytogenetical studies.

In the present study, we report EMA based karyotyping in 2 important wild mulberry species, viz., *M. laevigata* and *M. serrata*. Kanva-2, a popular cultivar belonging to *M. indica,* has been used for validation of rDNA sites on mulberry chromosomes. The study was also aimed at localizing the 5S and 25S rDNA sites on chromosomal complement and using the data together with DAPI banding patterns to facilitate linear chromosomal differentiation in mulberry.

MATERIALS AND METHODS

Plant material

Actively growing shoot tips of *M. laevigata* and *M. serrata* collections, along with variety Kanva-2 of *M. indica* were obtained from the experimental plot under Augmented Randomized Block Design (ARBD) at the Central Sericultural Research and Training Institute (CSRTI), Mysuru (12˚ 15' 38.6" N, 76˚ 37' 30.6" E) and the *ex situ* gene bank at Central Sericultural Germplasm Resources Centre (CSGRC), Hosur (12˚ 45" N, 77˚ 51" E) and utilized in somatic chromosome analysis (Table 1).

Somatic metaphase chromosome preparation

Shoot tips were pre-treated with 2 mM 8-hydroxyquinoline solution at 4˚ C for 4 h, washed thoroughly with water and fixed in freshly prepared Carnoy's solution (ethyl alcohol:acetic acid 3:1) overnight and stored at −20˚ C until further use. For metaphase spreads, enzymatic maceration and the air-drying method (EMA) was followed with slight modification (Anamthawat-Jónsson 2003). Pre-treated shoot tips were digested in a mix of 2% cellulase (Onozuka RS, Japan), 2.5% pectinase (Sigma P-9179, USA) and 1 mM EDTA (pH 4.2) for 4 h. Digested shoot tips were macerated and filtered using a nylon mesh to obtain protoplast suspension. The protoplast suspension was incubated at 4˚ C for 2 h. To the suspension, 75 mM KCl solution was added and mixed well. Later, the protoplast suspension was centrifuged at 5000 rpm for 5 min, the supernatant was discarded and the pellet was resuspended in 1.5 ml of freshly prepared Carnoy's solution and incubated for 5 min. Again, the protoplast was spun at 5000 rpm for 5 min and the supernatant was discarded. Fixative treatment was repeated twice to obtain protoplast pellet. The protoplast pallet was resuspended in 1 ml of ice-cold Carnoy's solution. The protoplast pellet was dropped onto the ice-cold slide and air-dried. The slides were stored at 4˚ C until further use.

Fluorochrome staining

The air-dried slides with protoplasts were stained

TABLE 1: Details of ten indigenous wild mulberry collections along with chromosome number and ploidy status.

Species	Name/place of collection	Accession number	State/territory of collection	Chromosome number (2n)	Estimated ploidy
M. laevigata	Doomar Nali	MI-0365	Andaman Island	28	2x
	Chidia Tapu – 2	MI-0606	Andaman Island	28	2x
	Birds Foot	MI-0079	Karnataka	42	3x
	Unlobed	MI-0164	West Bengal	42	3x
	New Delhi	MI-0540	New Delhi	56	4x
	Karanjotli-1	MI-0633	Jharkhand	56	4x
	Veterinary College	MI-0787	Maharashtra	56	4x
	RSCSGRC, Kalipong	MI-0805	West Bengal	56	4x
M. serrata	Bhowali Farm	MI-0569	Uttar Pradesh	84	6x
	Kathpuria	MI-0571	Uttar Pradesh	84	6x

TABLE 2: Characterization of chromosomal complements of eight *M. laevigata* and two *M. serrata* collections with number of DAPI^{+ve} signals detected.

Accession number	Chromosome number (2n)	Mean length of shortest chromosome (µm) ± SD	Mean length of longest chromosome (µm) ± SD	Mean total chromosome length (µm) ± SD	No of DAPI^{+ve} signals
MI-0365	28	0.54 ± 0.22	1.75 ± 0.21	22.35 ± 0.42	22
MI-0606	28	0.80 ± 0.13	1.98 ± 0.26	25.43 ± 0.29	08
MI-0079	42	0.64 ± 0.12	2.19 ± 0.19	48.3 ± 0.36	12
MI-0164	42	0.65 ± 0.14	1.79 ± 0.15	46.32 ± 0.29	12
MI-0540	56	0.68 ± 0.21	1.83 ± 0.26	66.36 ±0.21	12
MI-0633	56	0.47 ± 0.14	1.60 ± 0.23	61.65 ± 0.19	08
MI-0787	56	0.53 ± 0.21	1.92 ± 0.18	64.21 ± 0.35	20
MI-0805	56	0.53 ± 0.30	2.32 ± 0.26	59.45 ± 0.56	10
MI-0569	84	0.44 ± 0.24	0.96 ± 0.24	76.8 ± 0.28	16
MI-0571	84	0.52 ± 0.25	1.10 ± 0.19	78.27 ± 0.41	14

using fluorochrome DAPI as per Bhowmick & Jha (2015) with a few modifications. The slides were stained with 0.3 µg/mL DAPI with actinomycin D as a counterstain for 15 min and observed under a fluorescence microscope (Carl Zeiss, Germany) with a DAPI filter cassette and the metaphase chromosome images were captured in Axiocam 208 colour and DAPI signals were detected.

The chromosomes were counted among 10 cells per sample. Chromosome measurements were made using the freeware computer application KaryoMeasure (University of Kurdistan, Iran). A total of 10 good metaphase spreads per sample with similar condensation were measured. The mean length of the karyotype and the length of the shortest and longest chromosomes of the complement were determined for categorization. As chromosomes lack centromeric constrictions, they were categorized into long (> 1.5 µm), medium (1.0 to 1.5 µm) and short (< 1.0 µm) types and abbreviated as L, M and S respectively for the purpose of ideogram formula (Nijalingappa & Bai 1990).

Probe preparation

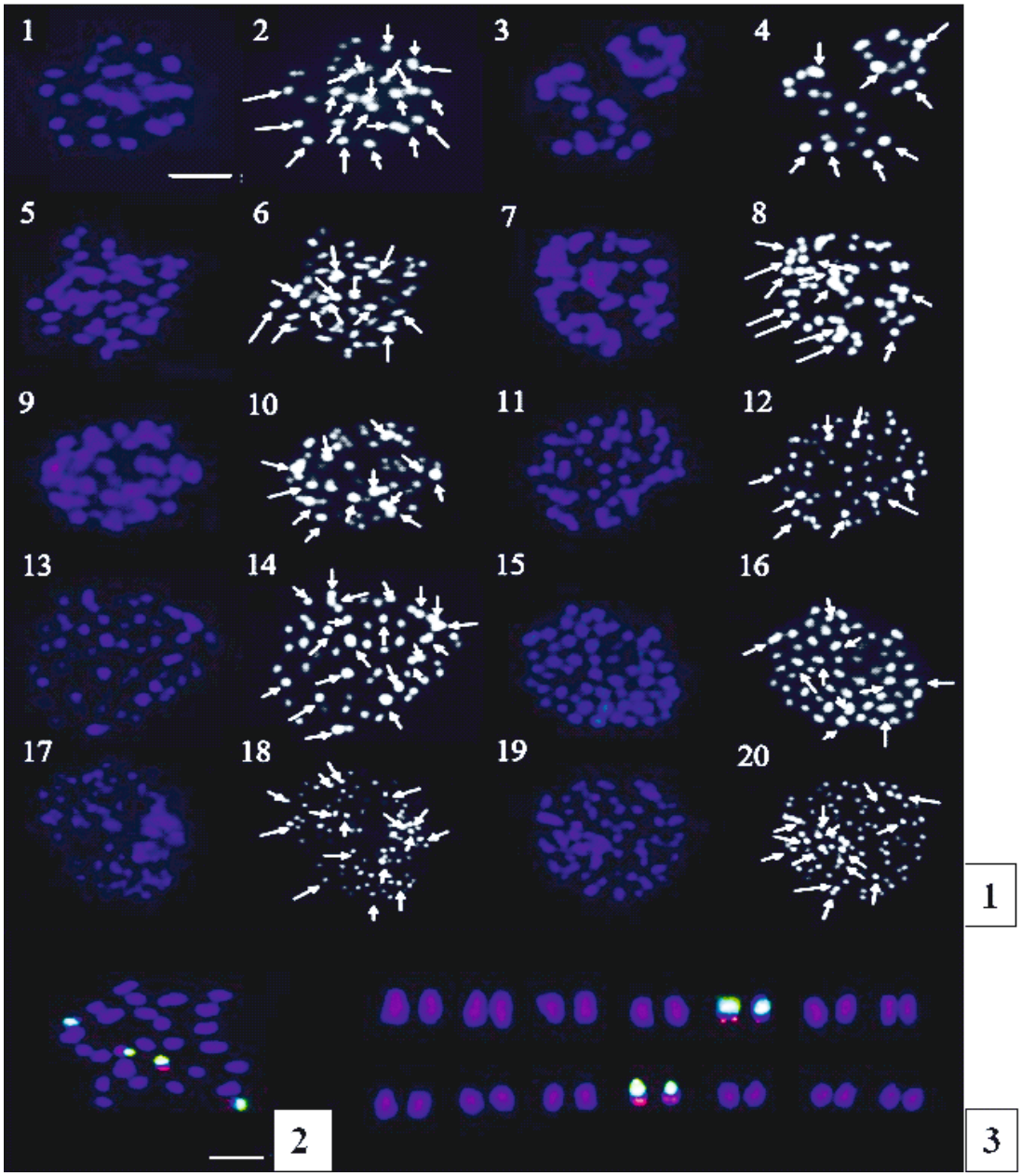
The primers suggested by Xuan et al. (2017) for 5S and 25S rDNAs were synthesized (Juniper Lifesciences, India) and utilized in the study. The 5S and 25S rDNA sequences were cloned into a pMD19-T vector and then labelled with biotin-16-dUTP and digoxigenin-11-dUTP, respectively, using a PCR DIG Probe Synthesis Kit (Roche, Switzerland) with labelling conditions based on the product manual. The PCR cycles consisted of an initial denaturation of 95˚ C for 5 min; followed by 32 cycles of 95˚ C for 30 sec, 58˚ C for 30 sec, 72˚ C for 1 min; with a 7 min of final extension.

Fluorescence *in situ* hybridisation

Fluorescence *in situ* hybridization (FISH) was carried out in variety, Kanva-2. The 25S rDNA and the 5S rDNA probes were directly labelled by nick translation with the fluorochromes-fluorescein (green) and Cy3.5 (red), respectively. The labelled probes were resuspended with 50 times the excess of sheared salmon sperm DNA in a hybridization mixture comprised of 50% formamide, 2x SSC and 5% dextran sulphate. Chromosomal DNA was stabilized by washing slides for 10 min in a solution of 1x PBS/50 mM $MgCl_2$/1% formaldehyde which was followed by degradation in a graded ethanol series at –20˚ C. The probe solution of 10 µl was added to each slide and simultaneously denatured on a hotplate for 5 min at 80˚ C and the slides were left for overnight hybridization in a humid chamber at 37˚ C. The hybridized slides were then washed for 5 min at 42˚ C in 2x SSC, followed by 10 min in 0.2x SSC (Snowdon et al. 2000). Chromosomes were later counterstained with DAPI-antifade and fluorescence was visualized using a fluorescence microscope (Carl Zeiss, Germany) with a DAPI filter cassette and chromosome images were captured in Axiocam 208 colour.

OBSERVATIONS

Chromosomal analysis of 10 wild mulberry collections belonging to 2 indigenous wild mulberry species, *M. laevigata* and *M. serrata* was carried out (Table 1). Enzymatic digestion and DAPI staining period played an important role in the removal of the cell wall and rich cytoplasmic contents and staining of small chromosomes. This is essential for accurate determination of chromosome number and estimation of the chromosome size in the somatic metaphase stage. Out of 10 collections analysed, 2 diploids, 2 triploids, 4 tetraploids and 2



Figs 1–3: 1. Mitotic metaphase spreads of wild mulberry collections with DAPI^{+ve} signals shown by arrow marks. (1–2) MI-0365. (3–4) MI-0606. (5–6) MI-0079. (7–8) MI-0164. (9–10) MI-0540. (11–12) MI-0633. (13–14) MI-0787. (15–16) MI-0805. (17–18) MI-0569. (19–20) MI-0571. 2. DAPI stained mitotic metaphase chromosomal preparation of var. Kanva-2 (*M. indica*, MI-0014) and FISH mapping of single copy probe indicates 25S rDNA (green) and 5S rDNA (red) sites. 3. Karyotype of var. Kanva-2 (*M. indica*, MI-0014) with 25S rDNA (green) and 5S rDNA (red) sites. Scale bar – 5 µm.

hexaploids were recorded. The chromosome number ranged from 2n = 28 to 84 (Fig. 1). *M. laevigata* was represented by diploid (2n = 28), triploid (2n = 42) and tetraploid (2n = 56) collections, whereas both the *M. serrata* collections were represented by hexaploid (2n = 84) genome. All the chromosomes were extremely small and dot like, holokinetic and look alike in morphology. The details of the chromosome number and measurements (mean values), along with the ploidy status of individual wild mulberry collections are presented in Table 2. The shortest chromosome (0.44 µm) was observed in a hexaploid collection, *M. serrata* (Bhowali Farm), whereas longest chromosome (2.32 µm) was measured in a tetraploid, *M. laevigata* (RSCSGRC, Kalimpong). Further, in terms of total chromosome length, the smallest (22.35 µm) was recorded in a diploid *M. laevigata* collection, Doomar Nali but the longest (78.27 µm) was observed in the hexaploid genome of the *M. serrata* collection namely, Kathpuria.

The mitotic chromosome counts of Doomar Nali (MI-365) and Chidia Tapu-2 (MI-0606) collections of *M. laevigata* were 2n = 28 (diploid). These two collections were sourced from the Andaman Islands. The diploid complement was constituted by 8 large, 16 medium and 4 small chromosomes in Doomar Nali (ideogram formula, 8L + 16M + 4S), the length of chromosomes varied from 0.54 to 1.75 µm and the total length of the chromosomes in the diploid complement was 22.35 µm. The other diploid collection of *M. laevigata* (Chidia Tapu-2) was represented by the ideogram formula 10L + 14M + 4S, with the length of chromosomes varying from 0.8 to 1.98 µm and the total length of chromosomes in the diploid complement was 25.43 µm. The ideogram formulae of 2 *M. laevigata* triploid (2n = 42) collections, Birds Foot and Unlobed, were 18L + 16M + 8S and 14L + 24M + 4S respectively. The corresponding chromosome length of these 2 collections ranged from 0.64 to 2.19 µm and 0.65 to 1.79 µm respectively. The remaining 4 collections of *M. laevigata* from New Delhi, Karanjotli-1, Veterinary College-1 and RSCSGRC, Kalimpong were found to be tetraploids. The ideogram formulae of these collections were 12L + 36M + 8S, 6L + 44M + 6S, 8L + 28M + 20S and 16L + 20M + 20S respectively. Among these, the collection from Kalimpong (MI-0805) recorded the longest chromosome length of 2.32 µm. Detailed collection-wise chromosomal measurement statistics are provided in Table 2. Two hexploid (2n = 84) collections namely, Bowali Farm and Kathpuria belonging to *M. serrata* represented by the smallest chromosomes among all. The collection from Bowali Farm showed only small-sized chromosomes with the length varying from 0.44 to 0.96 µm and an ideogram formula of 84S, without any representation of long and medium chromo-somes. The ideogram formula of Kathpuria was 50M + 34S, with the chromosome size varying from 0.52 to 1.1 µm and also lacked long chromosomes. The highest number of 22 $DAPI^{+ve}$ signals was found in Doomar Nali which depicts the presence of a higher AT base rich complex. The least number of $DAPI^{+ve}$ signals were observed in collections, Chidia Tapu-2 and Karanjotli-1.

FISH resulted in the detection of 4 25S rDNA loci located proximally and 8 5S rDNA loci present in the telomeric chromosome regions of var. Kanva-2 (Fig. 2). Chromosome number 5 and 11 showed the presence of both 25S rDNA and 5S rDNA probe binding sites (Fig. 3). Chromosome number 11 had a stronger and larger signal of 25S rDNA compared to chromosome number 5. Chromosome number 5 had a low intensity signal

of 5S rDNA probe. Two signals of each 5S rDNA were present, whereas only single probe signal of 25S rDNA was detected on each chromosome pair.

DISCUSSION

Crop wild relatives (CWRs) are key factors for effective and sustainable plant genetic enhancement in breeding schemes (Renzi et al. 2022). The negative implications of uniformity in modern day breeding can be countered by using CWRs to increase crop heterogeneity as well as genetic diversity in new varieties. These genetically enhanced varieties can adapt to climatic changes and challenges rendered by abiotic and biotic stresses. The wild *M. laevigata* has a wide distribution with greater variability and adapted to varied ecological niche ranging from the coastal belts of the Andaman Islands to the plains of South and Central India and extending to sub-Himalayan regions (Parkinson 1923, Ravindran et al. 1997). The wide adaptability of this species, along with the high regenerating ability of specifically the Andaman collections, render them desirable in breeding. Andaman collections of the species are tolerant to saline soils and termites, with superior timber-grain quality (Naik et al. 2015) and can be used as resources for crop diversification. The Doomar Nali collection of Andaman Islands has recorded the largest leaves in mulberry. Therefore, it is likely to impact positively on the economics of silk production, as mulberry foliage is the feed used in silkworm rearing. Sericulture in temperate regions are constrained by frost damage of mulberry plantations during the winter and can be addressed by breeding tolerant variety using *M. serrata*, a species known to survive in extreme low temperatures. The mechanism of tolerance to frost is similar to that of drought and can be used simultaneously to develop improved genotypes for varied situations. Besides, this species is known for its resistance to pests and diseases that infect mulberry. *M. serrata* has a higher moisture retention capacity, an important economic trait for the development of elite lines.

One of the main impediments is the lack cytogenetical studies on wild resources in order to scientifically integrate them into the present day mulberry breeding programme. Stray attempts have been made to utilize wild resources in mulberry breeding (Tikader & Dandin 2007, Tikader & Kamble 2008), but these efforts are arbitrary and not directional without any consideration of the chromosome complements and ploidy of the breeding materials. But mulberry resources show ample variability in chromosome number such as 2n = 14, 28, 35, 42, 49, 56, 84, 112, 126 and 308 and ploidy (Zheng et al. 2015, Xuan et al. 2019). Unlike the majority of agricultural crops, cytogenetical investigations in mulberry, specifically of wild species, remained unattractive and tedious due to the extreme small size of the chromosomes coupled with a lack of localized centromere and polyploidization. Conventional staining techniques are not suitable to resolve the mulberry chromosomal characteristics. EMA based chromosome preparation, a basic molecular cytogenetic technique, has enormously benefited plant chromosome research. He et al. (2013) have initiated the EMA method in mulberry to overcome these cytogenetical challenges. EMA-based pachytene chromosome analysis in mulberry was also reported by Xuan et al. (2017) and Xuan et al. (2022). In our study, the EMA method not only provided cytoplasm-free chromosome preparations but also, allowed us to reuse the same slide for other molecular cytogenetic techniques. In the present study, the EMA method followed by DAPI staining was able to unambiguously

resolve the chromosome numbers of the wild collections and categorise them into diploids, triploids, tetraploids and hexploids.

The somatic chromosomes in mulberry examined here have no centromeric constrictions and the same was also reported by Datta (1954). There are a number of other reports available on cultivated mulberry karyotype analysis using traditional methods of staining (Susheelamma et al. 1990, Venkatesh & Munirajappa 2013) but, none have documented holokinetic nature of chromosomes in their studies. Due to the small chromosome size and being holokinetic, typical karyotypic classification cannot be derived in mulberry based on the position of the constrictions. But they can be distinguished as long, medium and short chromosomes based on their chromosome size (Nijalingappa & Bai 1990). Our study also categorised the chromosomes into small, medium and long in all the wild collections of mulberry. However, among 2 *M. serrata* collections, both were hexploids (2n = 84) in which the Bowali Farm collection was represented by only small chromosomes (84S) and the Kathpuria collection by medium and small-sized chromosomes (50M + 34S) only. In higher ploidy levels of mulberry, long chromosomes were totally absent and only medium and small chromosomes were observed. A similar situation has been reported in *Carex* (Nijalingappa & Bai 1990). Chromosomal divergence in terms of size and number in both the wild species is interesting and Xuan et al. (2022) suggested that long terminal repeat retrotransposons (LTR) are the vital factors for genome divergence and evolution in mulberry species. The basic chromosome number of these indigenous wild mulberry species is suggested to be 14 as is the case with *M. alba*. Phylogenetic analysis based on molecular markers suggests a closer relationship of *M. alba* with the rest of the indigenous species (Pinto et al. 2018). The comparative genomics of *M. alba* and *M. notabilis* suggest a high chromosomal synteny between 14 chromosomes of the former and 6 chromosomes of the latter species. *M. notabilis* is an ancient wild mulberry species endemic to China and diverged from *M. alba* about 10 million years ago (Zeng et al. 2015, Jio et al. 2020). Xuan et al. (2022) conclusively proved that the basic chromosome number of *M. notabilis* is 7 using FISH based comparative chromosome analysis and showed that the mitotic chromosome 7 fused with 5 to form the chromosome 5 in diakinesis stage. It is proposed that a large scale fusion and fission events have contributed to speciation in mulberry and its evolution.

Fluorescent banding with DAPI targets the AT-rich constitutive heterochromatin region on chromosomes and generates positive and negative signals, aiding in advanced cytogenetic research in many plant species (Guerra 2008, Schwarzacher 2016). Moreover, DAPI staining is preferred over other staining methods as it can provide a stronger signal (Van Laere et al. 2008). Prior to this study, the application of base specific fluorochrome banding to indigenous mulberry species was not undertaken as researchers relied on conventional staining methods. However, as previously stated the resolution achieved by these conventional staining methods were not good enough to karyotype the mulberry chromosomes. Using the DAPI staining, extremely small and medium sized chromosomes of *M. serrata* could also be measured and characterized. The DAPI staining procedure was optimized with 0.3 µg/mL for 15 min of incubation for better staining and detecting positive signals. It is reported that plants

with small chromosomes face difficulties with fluorochrome banding due to the insufficient resolving power of a light microscope and relatively low copy number of repetitive sequences (Jha 2019, Meharavi et al. 2022). However, mulberry chromosomes even though very small, could reveal positive signals with DAPI staining.

The application of bicolour FISH technique using 2 different rDNA single copy probes (5S rDNA and 25S rDNAs) was carried out for the first time in indigenous mulberry variety, Kanva-2. The variety was chosen for FISH analysis basically for 2 reasons: i) Kanva-2 genome sequencing was already deciphered (Jain et al. 2022) and comparative analysis for localization of rDNA loci can be done, ii) the chromosomes of this variety were slightly longer and signals of 25S and 5S rDNAs can be clearly visualized or discriminated. As expected, we observed that 4 25S rDNAs were proximal and 8 of the 5S rDNAs were detected at telomeric regions of the chromosome 5 and 11. This finding is also in agreement with the previous report (Xuan et al. 2022) on variety of *M alba*. Chen & Hu (1999) reported 4 pairs of signals for 25S rDNA in Golden mushroom (genus *Cantharellus*). The variability in rDNA loci was also reported in the genus *Oryza* (Fukui et al. 1994). Similarly, the 5S rDNA signal pattern also showed polymorphism - 2 pairs in *Helianthus annus* (Schrader et al. 1997) and one pair in *Capsicum* species (Park et al. 2000). In mulberry, molecular markers-based genetic maps have been constructed (Venkateswaralu et al. 2006, Naik et al. 2014), but a physical map is still elusive. Chromosome identification and karyotype analysis by the FISH technique are likely to aid in the integration of genetic and physical mapping of the mulberry genome. Besides, the FISH technique can be utilized to assay chromosome variation and characterize addition or substitution lines in mulberry breeding.

The study has provided much awaited cytogenetical information on 2 important indigenous wild mulberry species with unique and novel characteristics. We hope that the findings of this study will help the breeders in the utilization of wild resources in mulberry genetic improvement programmes. The chromosome number and ploidy status of all the collections have been diligently assessed using contemporary EMA-based fluorochrome staining and ideogram formulae have been suggested for the distinction of wild genomes. The use of rDNA-based fluorescence probes for characterization/distinction of chromosomes and karyotypic analysis has been demonstrated in an indigenous mulberry variety. However, there should be a concerted effort to refine and address challenging issues in mulberry cytogenetics by integrating advancements in the fields of genomics, molecular cytogenetics, cell biology and bioinformatics. The identification of the entire chromosome complement and compilation of karyotype diversity of mulberry resources will be useful in understanding the genetic base and development of a chromosomal database for mulberry to aid future breeding programmes.

AUTHORS' CONTRIBUTION

VGN and HSK conceived the study. HSK designed the experiments and generated data and drafted the manuscript. VGN revised the manuscript. Both the authors read and approved the final manuscript.

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RESEARCH ARTICLE

STUDY ON DROUGHT TOLERANCE IN SHORT DAY ONION GENOTYPES AND ITS INHERITANCE

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SUMMARY Drought is a significant abiotic stress that negatively impacts crop growth and yield, including onion crop globally. This study was aimed to evaluate and characterize various onion genotypes for their tolerance to drought using PEG-8000 under controlled environmental conditions and to investigate the inheritance pattern of drought tolerance. The results revealed a decrease in germination, root length and shoot length with increasing concentrations of PEG and at 9% PEG concentration, the germination dropped to 50%. So, the concentration of 9% PEG was identified as the LD-50 dose for screening onion genotypes for drought tolerance. Yellow genotypes were found to be more tolerant to drought followed by white and least by red genotypes. High genetic coefficient of variation (GCV), heritability and genetic advance values were observed for germination, root and shoot length, suggesting the presence of additive gene action.

Keywords: Onion, drought, PEG, inheritance.

INTRODUCTION

Onion (*Allium cepa* L., 2n = 16) is a highly important vegetable crop grown extensively around the world and production of onion ranks second among the vegetables. In India, it is one of the important exports earning vegetable crops and the second most widely grown vegetable during the 2020-2021 period. Onions possess numerous health promoting properties such as lipid lowering, antidiabetic, antihypertensive and antimicrobial attributes (Karavelioglu & Hoca 2022). Its nutritional value, coupled with its distinct flavour, makes it a common ingredient in different culinary traditions and is regarded as the queen of the kitchen (Selvaraj 1976).

Global warming has become a worldwide problem within the past decade and as a consequence, several dramatic changes in climatic conditions have occurred in recent years, the major being abiotic stresses affecting crop growth in a dangerous way. Among these abiotic stresses, drought poses a critical environmental constraint that severely limits global crop production and horticultural productivity (Raza et al. 2019). Drought stress disrupts various morphological, physiological and metabolic processes in plants and ultimately leading to reduced plant growth, impaired photosynthesis and substantial yield losses (Sairam & Saxena 2000).

Among the crops affected by drought, onions are particularly vulnerable due to their shallow root system and high water requirement, approximately 45 lakh liters per hectare. A significant proportion of onion cultivation relies on monsoon rainfall to meet its water demand. The occurrence of frequent drought episodes associated with climate change has led to an approximately 30% decrease in global bulb production (Gedam et al.

2021). The extent of damage to bulb yield depends on the genotype and phenological stage at which drought stress occurs (Ghodke et al. 2018). Therefore, developing drought tolerant onion varieties with stable performance is crucial for enhancing its productivity in water scare regions.

Drought tolerance is a complex phenomenon, controlled by many genes and highly influenced by uncontrollable environmental factors. So, development of drought stress tolerant cultivars and hybrids is very difficult (Wang et al. 2021). One of the important sustainable strategies for development of drought tolerant cultivars is through characterization, selection followed by hybridization. However, controlled and uniformly repeated simulation of drought in the field cannot be easily achieved (Zeigler & Puckridge 1995). Using natural field conditions is difficult because rainfall can eliminate water deficits. However, in vitro drought screening methods are facilitating progress in our understanding of drought tolerance and in selection of drought tolerant genotypes. So, study of the influence of the drought using osmotic solutions is one of the methods in the evaluation of tolerance during the germination phase. Exposure to polyethylene glycol (PEG) solutions has been effectively used to mimic drought stress with limited metabolic interference as those associated to the use of low molecular weight osmolytes that can be taken up by the plant. PEG based in vitro screening for drought tolerance has been proven to be a suitable method to effectively screen large sets of germplasm with good accuracy (Kulkarni & Deshpande 2007).

In addition to germplasm characterization, it is very important to know the inheritance pattern involved in the expression of drought tolerance as this knowledge is useful in deciding the plant breeding procedure for genetic improvement. Therefore, this study was aimed to screen onion genotypes for drought tolerance and investigate the inheritance pattern associated with drought tolerance.

MATERIALS AND METHODS

A series of laboratory experiments were carried out at I and B seeds Pvt Ltd. during 2022-2023 to characterize onion genotypes for drought tolerance using PEG-8000 as per the method suggested by Panchalingam (1983). In the preliminary experiments, best performing red ONH-12 and white WH-8 genotypes along with standard check N2-4-1 which was previously characterized as drought tolerant by Singh & Gopal (2019) were screened under different PEG concentrations ranging from 0% to 20% to find out LD-50 dose. The second experiment with fine tuned concentrations at 0%, 3%, 6%, 9% and 12% was laid out to determine the exact dose of LD-50 and it was found to be 9%. Then, 8 red, 8 yellow and 7 white genotypes that were maintained in the germplasm bank of I and B seeds were screened at 9% PEG. In order to study the inheritance of drought tolerance, red hybrid RH-5, white hybrid WH-8 and their parents were screened at 9% PEG.

All the 4 experiments were conducted in completely randomized design (CRD) with 2 replications. The seeds were soaked in distilled water for 4 h to eliminate nonviable seeds and 25 uniform seeds for each of the genotypes per replication were placed on moistened filter paper with 5 ml of 9% PEG in each petridish separately. The petridishes were kept in seed germinator at 25

± 1° C for 8 d and observations were recorded on number of seeds germinated, root length and shoot length. The data were analysed following standard statistical methods.

OBSERVATIONS

The results of the preliminary experiment are presented in Fig. 1. All the 3 genotypes almost stopped germination beyond 10% PEG and LD-50 was between the range of 5 and 10% PEG. In a modified experiment, it was demonstrated that the exact concentration for LD-50 was 9% beyond which germination, shoot and root length were decreased (Table 1). Table 2 describes performance of red, yellow and white genotypes to induced drought at 9% PEG. Yellow genotypes were more drought tolerant followed by white with the higher values of germination count, root and shoot growth. All the red genotypes except 16/25 Gr3 OOH Mass were susceptible to drought which is a prime concern as vast onion area is under red onions. While white onions are cultivated on small area for processing and for culinary purpose in India, yellows are not in vogue.

Genotypes 16/25 Gr 3 OOH Mass (red), 16/7Y Gr3, 16/7Y G1T3 Gen, OM 10 Long storer (yellow) and 16/16 F4 W TSS17, 16/21 TSS16 WSP and 16/47 TSS18 SPS (white) could be characterized as drought tolerants. These genotypes recorded good germination, root and shoot length under 9% PEG with less reduction as compared to 0% PEG. Analysis of variance revealed significant differences among the parents and hybrids for germination, root length and shoot length. The male parent of RH-5 (Synth 4 LD 1:1) and WH-8 (16/21 TSS16 WSP) exhibited superior performance for germination

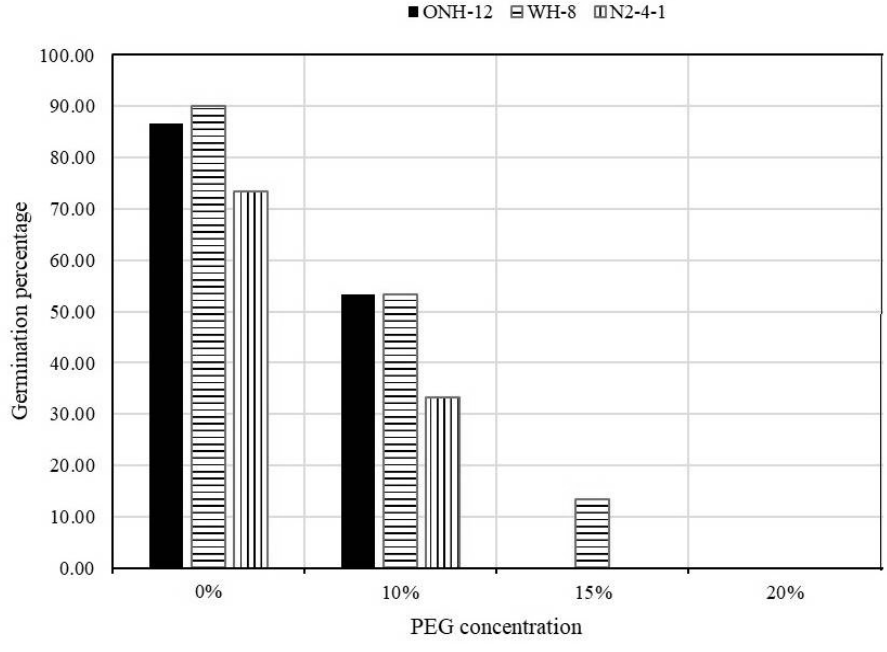

Fig. 1: Effect of different PEG concentrations on germination of onion genotypes.

TABLE 1: Effect of different PEG concentrations on germination, root and shoot growth of onion genotypes.

Concentration	Germination (%)				Root length (cm)				Shoot length (cm)			
	ONH-12	WH-8	N2-4-1	Mean	ONH-12	WH-8	N2-4-1	Mean	ONH-12	WH-8	N2-4-1	Mean
0% PEG	86.67	100.00	90.00	92.22	3.10	1.51	2.29	2.30	2.59	5.09	4.95	4.21
3% PEG	73.33	83.33	56.67	71.11	3.31	3.18	2.26	2.92	3.90	5.25	3.82	4.32
6% PEG	53.33	80.00	76.67	70.00	2.40	2.68	2.72	2.60	4.31	4.50	4.12	4.31
9% PEG	33.33	50.00	66.67	50.00	1.53	1.67	2.46	1.89	2.53	3.43	3.20	3.06
12% PEG	36.67	60.00	50.00	48.89	0.91	1.67	1.75	1.44	0.83	1.97	1.83	1.55
Mean	56.67	74.67	68.00		2.25	2.14	2.30		2.83	4.05	3.59	
	SE.m ±	C.D.			SE.m ±	C.D.			SE.m ±	C.D.		
Varieties (V)	0.46	1.33			0.21	NS			0.25	0.72		
PEG concn. (P)	0.59	1.71			0.27	0.78			0.32	0.93		
V × P	1.02	NS			0.46	NS			0.56	NS		

(75% and 97%, respectively), root length (3.03 cm and 2.88 cm, respectively) and shoot length (5.42 cm and 5.20 cm, respectively) as compared to their hybrids and female parents (Table 3). Mid parent heterosis of RH-5 shown positive heterosis for germination (7.44%), root length (7.41%) and negative heterosis for shoot length (-2.82%). Similarly, in WH-8, positive mid parent heterosis was observed for root (23.61%) and shoot length (20.68%) whereas negative heterosis for germination (1.39%).

The estimates of phenotypic coefficient of variation (30.37%, 29.34% and 15.83%, respectively) were higher than genotypic coefficient of variation (26.95%, 24.90% and 13.63%, respectively) for germination, root length and shoot length. To assess the heritable variation present in these traits, heritability was calculated. The heritability values were high for germination (78.84%), root length (72.42%) and shoot length (73.85%) and genetic advances as a percentage of the mean (GAM) values were high for these characters.

DISCUSSION

Drought is a major abiotic stress in recent years because of several dramatic changes in climatic conditions. Among the crops affected by drought, onions are more vulnerable to drought. Seed germination requires optimum soil moisture and establishment of crop and the final yield. The decrease in germination, root and shoot length with increasing PEG concentrations can be attributed to the gradient water potential to absorb moisture to germinate and further growth (Kulkarni & Deshpande 2007). The sequence of events leading to seed germination and root emergence is governed by water uptake from the external medium. Water availability plays a significant role in enzymatic reactions, solubilization and transportation of metabolites and also as a reagent in the hydrolytic breakdown of proteins, lipids and carbohydrates in the storage tissues of germinating seeds. Amylase enzyme plays an important role during seed germination, hydrolyzing the endosperm starch into metabolizable sugars, which provide the energy for the growth of roots and shoots. The

TABLE 2: Genetic variation in per se performance of red, yellow and white onion genotypes at 9% PEG concentration.

	Genotype	Germination (%)		Root length (cm)		Shoot length (cm)		% change over 0% PEG		
		0% PEG	9% PEG	0% PEG	9% PEG	0% PEG	9% PEG	Germination	Root length	Shoot length
A.	**Red accessions**									
1	16/25 Gr3	88.00	28.00	4.50	1.37	4.93	2.47	-68.18	-69.63	-50.00
2	16/11 Gr1	96.00	56.00	0.57	0.37	3.07	1.83	-41.67	-35.29	-40.22
3	16/77 Gr2 OOH-1	76.00	32.00	5.60	2.40	7.77	3.17	-57.89	-57.14	-59.23
4	16/25Gr3 OOH Mass	88.00	72.00	3.10	2.40	6.23	4.60	-18.18	-22.58	-26.20
5	16/26-1-1C OOH Gen	76.00	56.00	4.50	2.43	8.97	4.23	-26.32	-45.93	-52.79
6	16/11Gr1 Homo65 SSD	84.00	44.00	3.27	2.17	5.37	3.80	-47.62	-33.67	-29.19
7	Synth 2 HP 1 long storer	80.00	48.00	4.70	3.50	7.93	4.90	-40.00	-25.53	-38.24
8	16/26 Gr3	92.00	48.00	4.27	2.10	6.40	3.33	-47.83	-50.78	-47.92
	Mean	85.00	48.00	3.81	1.97	6.33	3.54			
	Standard deviation	9.80	13.18	1.44	0.97	1.88	1.44			
	T-value	25.31**	10.82**	8.00**	6.55**	10.46**	8.10**			
B.	**Yellow accessions**									
1	16/7Y G1T5 GEN	96.00	68.00	2.93	1.83	7.57	4.50	-29.17	-37.50	-40.53
2	16/7Y G1T2 Gp2	92.00	80.00	3.10	2.87	5.23	4.43	-13.04	-7.53	-15.29
3	16/7Y Gr3	96.00	92.00	3.00	2.10	5.93	5.07	-4.17	-30.00	-14.61
4	16/7Y G1T1 Mass	72.00	64.00	2.97	1.70	5.67	3.97	-11.11	-42.70	-30.00
5	16/7Y G1T4 GP2	80.00	44.00	1.47	1.07	5.07	3.87	-45.00	-27.27	-23.68
6	16/7Y G1T3 Gen	80.00	72.00	3.10	2.33	6.47	4.83	-10.00	-24.73	-25.26
7	16/7Y Gr3 OOH	80.00	56.00	1.27	1.33	3.90	3.27	-30.00	5.26	-16.24
8	OM 10 Long storer	88.00	72.00	5.20	4.37	6.93	5.67	-18.18	-16.03	-18.27
	Mean	85.50	68.50	2.88	2.20	5.85	4.45			
	Standard deviation	8.80	14.57	1.20	1.04	1.16	0.75			
	T-value	27.48**	13.30**	6.79**	5.97**	14.31**	16.70**			
C.	**White accessions**									
1	16/47 TSS 18 SPS	92.00	72.00	3.63	2.57	5.80	5.03	-21.74	-29.36	-13.22
2	17/R41-1W	76.00	52.00	6.80	3.47	7.50	5.33	-31.58	-49.02	-28.89
3	16/17 R-06 W self	100.00	68.00	4.67	2.93	8.70	6.23	-32.00	-37.14	-28.35
4	16/16 F4W TSS 17	84.00	80.00	2.30	3.27	5.50	4.87	-4.76	42.03	-11.52
5	16/21 TSS 16 WSP	80.00	72.00	1.43	2.07	3.63	3.43	-10.00	44.19	-5.50
6	16/21 Gr3	80.00	72.00	3.60	2.13	7.13	4.87	-10.00	-40.74	-31.78
7	16/2A x 16/21 TSS16	92.00	64.00	3.13	2.90	5.77	5.07	-30.43	-7.45	-12.14
	Mean	86.29	60.05	3.65	2.76	6.29	4.98			
	Standard deviation	31.53	25.57	2.06	1.10	2.69	1.92			
	T-value	6.77**	6.64**	4.39**	6.24**	5.78**	6.42**			

* and ** indicate significance of values at p = 0.05 and p = 0.01, respectively.

TABLE 3: Estimates of genetic variability parameters, mid parent heterosis and per se performance of onion parents and hybrids at 9% PEG concentration.

Parents/Hybrids	Germination (%)	Root length (cm)	Shoot length (cm)
16/17 DBT A Pink (Parent-1)	46.00	1.83	4.50
Synth 4 LD 1:1 (Parent-2)	75.00	3.03	5.42
Mean of parents / mid parent	60.50	2.43	4.96
RH-5 (Hybrid)	65.00	2.61	4.82
Mid parent heterosis (%)	7.44	7.41	-2.82
16/2A (Parent-1)	49.00	1.44	3.60
16/21 TSS16 (Parent-2)	97.00	2.88	5.20
Mean of parents / mid parent	73.00	2.16	4.40
WH-8 (Hybrid)	72.00	2.67	5.31
Mid parent heterosis (%)	-1.39	23.61	20.68
Mean	67.33	2.41	4.81
SE.m ±	4.71	0.19	0.20
CD	14.12	0.56	0.58
CV %	14.00	15.47	8.11
GCV %	26.95	24.90	13.63
PCV %	30.37	29.34	15.83
$H^2_{(B.S)}$%	78.74	72.42	73.85
Genetic advance	34.78	1.11	1.22
GAM %	51.66	46.06	25.36

activity of such enzymes is reduced by water stress with negative effects on carbohydrate metabolism (Bialecka & Kepczynski 2010).

Yellow genotypes were more drought tolerant followed by white with the higher values of germination count, root and shoot growth. Red genotypes were found to be the least tolerant. This variation in drought tolerance among the different genotypes suggest the presence of genetic factors involved in conferring drought tolerance. Plants respond and adapt to these adverse environmental stresses through a number of physiological and developmental changes (Philippe et al. 2010). These changes can be attributed to genetic mechanisms that enable plants to adapt and survive under water limited conditions. The genetic basis of these adaptive responses to drought involves the expression of numerous genes. Many of these genes have been shown to interact strongly with the environment, suggesting that their expression is influenced by genetic-environmental interactions (Wang et al. 2021). In the background of this, a breeder can think of using drought tolerant genotypes like 16/7Y Gr3, 16/7Y G1T3 Gen, OM 10 Long storer, 16/16 F4 W TSS17, 16/21 TSS16 WSP and 16/47 TSS18 SPS in yellow and white genotypes to transfer into red onions using appropriate breeding methods like back cross breeding and recurrent selection methods using the advantage of dominant genes with additive effects as indicated from the values in Table 3.

The study successfully identified onion genotypes that exhibit enhanced performance under drought conditions during the early seedling stage. However, further investigations are needed to validate the drought tolerance of these genotypes at subsequent growth stages, including vegetative and reproductive stages. The presence of dominant genes and additive gene action for drought tolerance suggest selecting plants based on germination count, root length and shoot length traits shows promising prospects for enhancing drought tolerance in onion crop through breeding. The identified genotypes can be effectively incorporated into breeding programmes for development of onion drought tolerant varieties and hybrids.

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