Journal of Cytology and Genetics 2025 VOL. **26** (NS): 79–87

RESEARCH ARTICLE

MOLECULAR CHARACTERIZATION IN SOME MONOCOTS USING RAPD MARKERS

RAMESH AHIRWAR [1]* AND R. C. VERMA[2]
[1]Department of Botany, Government College, Jatara 472 118, India
[2]School of Studies in Botany, Vikram University, Ujjain 456 010, India
*For correspondence. Email: rameshbotvu@gmail.com

(Received 10 October 2025, revised accepted 15 November 2025)

SUMMARY The RAPD markers were used to assess genetic diversity among some members of the monocot families viz., Amaryllidaceae, Asphodelaceae, Asparagaceae and Colchicaceae. The DNA was isolated from young leaves by CTAB methods with some modifications. Four primers were used to assess genetic diversity among the species. A total number of 381 bands were observed. Maximum number of 112 bands was observed with primer RPI08 and minimum 87 with RPI06. All the species showed 100% polymorphism. UPGMA based dendogram was constructed using PAST software and genetic diversity was analyzed. Dendogram showed 2 major clusters and only 0.07% similarity among the species.

Keywords: RAPD markers, genetic diversity, monocots.

INTRODUCTION

The development of molecular techniques for genetic analysis has led to a great increase in our knowledge of plants and our understanding of the structure and behaviour of plant genomes. These molecular techniques, in particular, the use of molecular markers, have been used to monitor DNA sequence variation among the species. Analysis of genetic variation can help us to understand the molecular basis of various biological phenomena in plants. DNA based marker techniques such as RFLP, RAPD, SSR, and AFLP are routinely being used in ecological, evolutionary, taxonomical and genetic studies of plant science (Agarwal & Shrivastava 2008).

Randomly Amplified Polymorphic DNA (RAPD) markers are excellent tools to study the genetic diversity, eliminate duplicates in germplasm, study genetic relationships, gene tagging and genome mapping. These markers measure diversity at the DNA level in various species which are seldom influenced by environmental conditions. RAPD analysis is technical simplicity speed and reliable and used for diversity analysis in plant species (Fu et al. 2003).

Peruzzi et al. (2009) summarized the present status of chromosome diversity and evolution in the family Liliaceae. Nayanakantha et al. (2010) studied the genetic diversity in *Aloe* species by RAPD. The phylogenetic relationships within *Allium* have been investigated using various molecular markers such as RAPDs, RFLPs, and AFLPs by Ricroch et al. (2005). The main objective in the present study was the molecular characterization of some of the monocotyledonous species using molecular marker RAPD.

MATERIALS AND METHODS

Materials for molecular study was collected during October to February in 2011 winter season and June to September in 2012 rainy season from different localities. Identification was done by consulting floras such as Flora of Madhya Pradesh, Flora of Madhya Pradesh (Western Part), website, https: //identify.planmet.org/k-world-flora/families and followed APG system IV.

Twentyeight samples of 20 species belonging to 13 genera under 4 families were collected from different localities (Tables 1, 2). The DNA was isolated from young leaves by CTAB method. The isolated DNA was subsequently analyzed for its purity by spectrophotometric analysis and qualitative analysis was confirmed by gel electrophoresis. Finally, RAPD amplification of all the samples were performed to screen the possible polymorphism among them. The major steps for RAPD analysis are mentioned below.

For extraction, purification and amplification of DNA, very little amount of plant material is required. Young leaves were collected from field grown plants. Fresh leaves were stored at –80º C and freeze-dried for grinding.

Young leaves (300 mg) were used for extracting genomic DNA using protocol of Doyle & Doyle (1987) with some modifications. Surface sterilized leaf tissue was crushed in pre-chilled mortar and pestle and immediately transferred to a centrifuge tube containing 5 ml modified CTAB extraction buffer (100 mM Tris HCl (pH 8.0), 25 mM EDTA, 1.5 M NaCl, 2.5% CTAB, 0.2% 2 Mercapto-ethanol (v/v), and 1% PVP (w/v), added immediately before use) and mixed well to form a slurry and incubated at 70° C for 30 min. The mixture was cooled at room temperature and an equal volume of the mixture of phenol: chloroform: isoamyl alcohol (25:24:1) was added prior to centrifugation at 5500 rpm for 20 min. To the aqueous phase, equal volumes of ice-cold isopropanol and NaCl was added and incubated at –20° C for 30 min followed by centrifugation at 5500 rpm for 20 min at 4° C. The supernatant was decanted carefully and pellet was dried at room temperature followed by dissolving in 1 ml of Tris-EDTA (TE) buffer and 5 ml RNase solution and incubated at 37° C. An equal volume of the mixture of phenol:chloroform:isoamyl alcohol (25:24:1) was added and centrifuged at 5500 rpm for 20 min at 4° C. To the supernatant, 600 μl of chilled absolute ethanol was added and incubated at –20° C for 30 min followed by centrifugation at 10000 rpm for 10 min at 4° C. The pellet was washed with 70% ethanol twice and dried at room temperature. Finally, the DNA pellet was dissolved in 50 ml of deionized (RNase, DNase free) water. The yield of DNA was measured using a UV-spectrophotometer (ND-1000) at 260 nm. The purity of DNA was

TABLE 1: List of species collected from different localities.

Sl. No.	Name	Location
1	*Allium cepa* L. Deshi variety	Vegetable market, Ujjain
2	*A. cepa* L. Nasik variety	Vegetable market, Ujjain
3	*A. cepa* L. White variety	Vegetable market, Ujjain, Sendhava, Distt,. Barwani
4	*A. cepa* L. Kashmiri variety	Melahura, Kashmir
5	*A. sativum* L. Deshi variety	Vegetable market, Ujjain
6	*A. sativum* L. Kashmiri variety	Melahura, Kashmir
7	*A. tuberosum* Rottl.	Vikram University, Ujjain
8	*Crinum asiaticum* L.	Vikram University, Ujjain
9	*C. latifolium* L.	Vikram University, Ujjain
10	*Haemanthus multiflorus* Martyn	Vikram University, Ujjain
11	*Aloe barbadensis* Mill	Malwa nursery, Ujjain
12	*A. barbadensis* Mill	Central Arid Zone Research Institute, Jodhpur
13	*Haworthia limifolia* Marloth	Malwa nursery, Ujjain
14	*Hemerocallis fulva* L.	Malwa nursery, Ujjain
15	*Asparagus racemosus* Willd.	Vikram University, Ujjain
16	*A. denciflorus* Meyersii.	Malwa nursery, Ujjain
17	*A. sprengeri* Regel	Vikram University, Ujjain
18	*Chlorophytum tuberosum* Roxb.	Bamanvarhi Distt, Dhar
19	*C. borivilianum* Santapau & R. R. Fern.	Bidwal, Distt, Dhar, Raibidpura Distt, Khargone
20	*C. laxum* R. Br.	Vikram University, Ujjain, Tinchha fall, Indore
21	*Drimiopsis kirkii* Baker.	Vikram University, Ujjain
22	*Ophiopogon japonicas* Kew-Gawl.	Vikram University, Ujjain
23	*Scilla hyacinthina* Roth.	Sanavadiya Distt, Indore
24	*Urginea indica* Roxb.	Bamanvarhi Distt, Dhar
25	*Gloriosa superba* L.	Sanavadiya Distt, Indore

TABLE 2: Sample details with botanical names, families and codes.

Sl. No.	Species	Family	Sample code
1	*Allium cepa* (Deshi variety)	Amaryllidaceae	Alcd
2	*A. cepa* (Nasik variety)	,,	Alcn
3	*A. cepa* (White variety)	,,	Alcw
4	*A. cepa* (Kashmiri variety)	,,	Alck
5	*A. sativum* (Deshi variety)	,,	Alsd
6	*A. sativum* (Kashmiri variety)	,,	Alsk
7	*A. tuberosum*	,,	Alt
8	*Crinum asiaticum*	,,	Cra
9	*C. latifolium* (Deptt. accession 1)	,,	Crl1
10	*C. latifolium* (Deptt. accession 2)	,,	Crl2
11	*Haemanthus multiflorus*	,,	Ham
12	*Aloe barbadensis* (Local accession)	Asphodelaceaee	Albl
13	*A. barbadensis* (Jodhpur accession)	,,	Albj
14	*Haworthia limifolia*	,,	Hal
15	*Hemerocallis fulva*	,,	Hef
16	*Asparagus racemosus*	Asparagaceae	Asr
17	*A. denciflorus*	,,	Asd
18	*A. sprengeri*	,,	Ass
19	*Chlorophytum tuberosum*	,,	Cht
20	*C. borivilianum*	,,	Chb
21	*C. laxum* (Deptt. accession)	,,	Chld
22	*C. laxum* (Tinchha accession 1)	,,	Chlt1
23	*C. laxum* (Tinchha accession 2)	,,	Chlt2
24	*Drimiopsis kirkii*	,,	Drk
25	*Ophiopogon japonicas*	,,	Opj
26	*Scilla hyacinthina*	,,	Sch
27	*Urginea indica*	,,	Uri
28	*Gloriosa superba*	Colchicaceae	Gls

determined by calculating the ratio of absorbance at 260 nm to that of 280 nm. DNA concentration and purity was also determined by running the samples on 0.8% agarose gel, based on the intensities of band when compared with the DNA marker (used to determine the concentration).

DNA amplification was performed in a Corbett Research gradient automatic thermal cycler using RAPD markers. Genomic DNA (50 ng) was amplified via the PCR reaction using 50 µl reaction volumes under the following conditions: 2x red dye PCR mix (25 µl), primer (2 µl) (Bangalore Genei), template DNA (1 µl) and sterile nano pure water (22 µl). After initial denaturation of the DNA at 94° C for 5 min, thermal cycling was performed with following temperature regimes: 08 cycles of 45 sec at 94° C, 1 min at 55° C and 1.5 min at 72° C; 30 cycles of 45 sec at 94° C, 1 min at 58° C and 1 min at 72° C and final extension at 72° C at 10 min and a hold temperature at 4° C. Control reactions without template DNA (negative control) and without enzyme (positive control) were also run in the experiments. All the experiments were repeated thrice to ensure reproducibility. Amplified DNA fragments were separated by electrophoresis at 60 V in 1 X TAE buffer for 3–4 h on 1.2% agarose gels stained with ethidium bromide and photographed by gel documentation system (Alpha innotech). Raw gel images were recorded through Alphaview software. All PCR experiments were done at least twice and the best gels of the replicates were used for band scoring.

TABLE 3: Primer accession numbers.

Sl. No.	Primer	Accn No.
1	RPI-6	AM773771
2	RPI-7	AM773312
3	RPI-8	AM773773
4	RPI-9	AM773315

The PCR protocol, as adopted in the study, resulted in reproducible pattern of amplicons using specific combination of accession and primer. Only the primers which displayed reproducible, scoreble and clear bands were considered for analysis. The image profiles of banding patterns were recorded and molecular weight of each band was determined by Alphaview software. The banding pattern was scored based on the presence or absence of clearly visible and reproducible bands. The results were analyzed based on the principle that a band is considered to be 'polymorphic' if it is present in some individuals and absent in others, and 'monomorphic' if present in all the individuals or accessions. Similarity index of bands which were common between 2 samples were estimated. Using dice coefficients, similarity matrix among the samples were generated with NTSYS-pc Ver. 2.02 and PAST. Dendogram was constructed using the unweighted pair group method with arithmetical averages (UPGMA).

OBSERVATIONS

Molecular investigations have been made in 20 species, their varieties and accessions of some monocots. Twentyeight samples were collected in 20 species from different locations in which 6 species belong to Amaryllidaceae, 3 species belong to Asphodelaceae, 10 species belong to Asparagaceae and 1 species belongs to Colchicaceae and were analyzed for RAPD profiles and an attempt has been made to find out the diversity at molecular level. Amplicon profiles obtained at different runs by polymerase chain reaction of DNA extracted independently. RAPD of the 28 samples of 20 species were carried out using 4 primers. Four primers used for restriction were, RPI-06 (5'-ATTGGC-3'), RPI-07 (5'-GATCTG3'), RPI-08 (5'TCAAGT-3') and RPI-09 (5'-CTGATC-3'). The restricon produced by these RPI markers were analyzed for analysis of genetic diversity among the species and their varieties and accessions. The amplification products produced by these primers are presented in Figures 1 and 2.

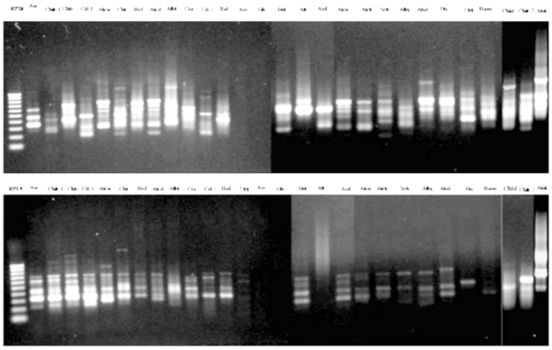


Fig. 1: RAPD profile of 28 samples of 20 species and their varieties and accession of the monocots with primers RPI-06 (upper) and RPI-7 (lower).

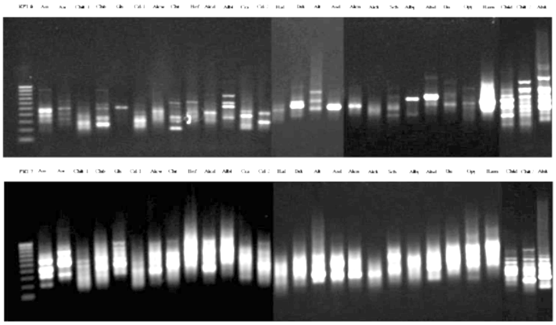


Fig. 2: RAPD profile of 28 samples of 20 species and their varieties and accession of the monocots with primers RPI-08 (upper) and RPI-9 (lower).

The DNA fingerprints obtained with those species were scored for present (1) and (0) of the restricted bands of various molecular weights obtained though PCR amplification reaction. The data thus obtained in the form of binary matrix was analyzed to obtain Jaccards coefficient among different samples. The un-weight pair group method with an arithmetic average (UPGMA) algorithm and software PAST (Version-1.91) were used for the generation of the dendogram. The RAPD profiles of different accessions of those species were used to generate distances between accessions. The amplicons of each primer differed in number and intensity. A total number of 343 bands were observed in RAPD profile in which 78 in RPI06, 82 in RPI07, 106 in RPI08 and 87 in RPI09. Maximum number 106 bands were observed with primer RPI08 minimum 78 in RPI06. All the species showed 100% polymorphism.

UPGMA based dendogram was constructed (using PAST software) and analyzed the genetic diversity. Dendogram showed 2 major clusters. Among the presently investigated species samples, the analysis revealed that there are 2 distinct main groups showing only 0.07% similarities. This indicates that these groups are genetically different from each other. Group I has 2 species namely, Gls and Ass showing 0.076%.

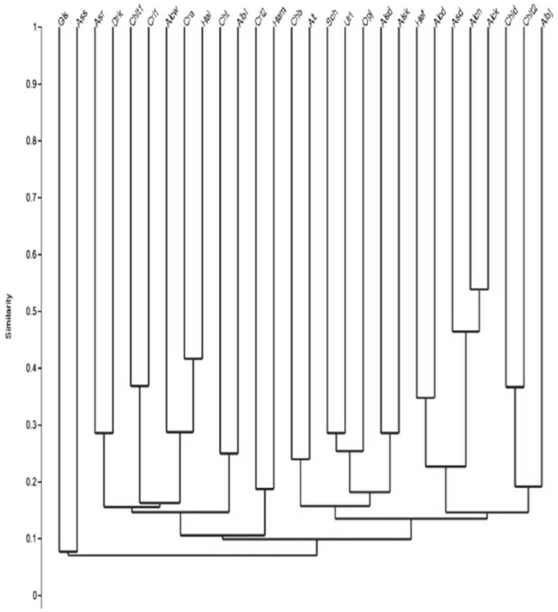


Fig. 3: UPGMA cluster dendogram (Jaccard coefficient) showing the genetic relations among the some monocots species on 4 RAPD markers.

Group II has 26 samples under 18 species and their varieties and accessions namely, Asr, Drk, Chlt1, Crl1, Alcw, Cra, Hal, Cht, Albl, Crl2, Ham, Chb, Alt, Sch, Uri, Opj, Alsd, Alsk, Hef, Alcd, Asd, Alcn, Alck, Chld, Chlt2 and Albj. This group consists of 3 major subgroups. In subgroup I, 9 samples namely, Asr, Drk, Chlt1, Crl-1, Alcw, Cra, Hal, Cht and Albd are present. In this subgroup, Cht and Albd showed minimum 0.25% and Cra and Hal showed maximum 0.41% similarity. Subgroup II has 2 species namely, Crl2 and Ham showing 0.18% similarity. Subgroup III has 15 samples namely, Chb, Alt, Sch, Uri, Opj, Alsd, Alsk, Hef, Alcd, Asd, Alcn, Alck, Chld, Chlt2 and Albj, This group shows minimum of 0.13% in between Chlt2 and Albj and maximum of 0.53% between Alcn and Alck similarity. Overall, very less similarity (0.07%) among the species, minimum 0.076% similarity between *A. sprengeri* and *G. superba* and maximum 0.53% between 2 varieties (Nasik and Kashmir) of *A. cepa* was found (Fig. 3).

DISCUSSION

Molecular methods have become an essential part of most of the recent studies on genetic diversity and distribution and in the analyses of breeding system, bottlenecks and other key features affecting genetic diversity patterns (Rao & Hodgkin 2002). RAPD markers are excellent tools to study the genetic diversity, eliminate duplicates in germplasm, study genetic relationships, gene tagging, genome mapping. These markers measure diversity at DNA level in various species which are seldom influenced by environmental conditions. Several workers have done RAPD study in *A. cepa* (Maniruzzaman et al. 2010, Mukherjee et al. 2013), *Aloe* (Nayanakantha et al. 2010), *A. racemosus* (Vijay et al. 2009, Sing et al. 2013), *C. borivilianum* (Dwivedi & Sharma 2011, Samantaray et al. 2011) and *G. superba* (Rajgopal & Kandhasamy 2013).

In the present study, genetic diversity was assessed among the species using RAPD markers to provide genetic data for protection of the germplasm of important species. Hence, an attempt has been made to investigate variation among 20 species by using RAPD markers. A total number of 343 bands were observed in RAPD profile in which maximum number of 106 bands were observed with primer RPI08 and minimum of 82 in RPI06. All the species showed 100% polymorphism. Very less similarity of

0.07 to 0.53% were observed among the species. Lowest similarity (0.076%) was observed between *A. sprengeri* and *G. superba* and highest similarity (0.53 %) was observed between the Nasik and Kashmiri varieties of *A. cepa*.

Genetic diversity is usually thought of as the amount of genetic variability among individuals of a variety, or population of a species. It results from many genetic differences between individuals and may be manifested in differences in DNA sequence (Brown 1983). Rao & Hodgkin (2002) suggested that the variation that underpins genetic diversity arises from mutation and recombination which determinate natural selection, genetic drift and gene flow act on the alleles in different species to cause variation in the diversity among the species. The present investigation found high genetic/molecular diversity among the species. The reason for this is the morphological and cytological differences among of them. Another reason may be abiotic and biotic factors which are also responsible for creation of genetic diversity among the species. RAPD method is convenient for a better understanding of genetic diversity among the species. Molecular characterization of selected species would help to identify different variants at the molecular level and to use this information in genomics.

Declaration

The authors declare that this paper is based on the original research work of RA having no conflict of interest.

REFERENCES

AGARWAL M & SRIVASTAVA N 2008 Advance in molecular marker techniques and their applications in plant science *Plant C Rep* **27** 617–631

BROWN W L 1983 Genetic diversity and genetic vulnerability – an appraisal *Econ. Bot* **37** 4–12

DOYLE J J & DOYLE J L 1987 A rapid DNA isolation procedure for small quantities of fresh leaf tissue *Phytochem Bull* **19** 11–15

DWIVEDI R & SHARMA D K 2011 Assessment of genetic diversity among collected genotypes of *Chlorophytum borivilianum* using random amplified polymorphic DNA (RAPD) markers *Afr J Biotechnol* **10** 5268–5272

FU C, QIU Y & KONG H 2003 RAPD analysis for genetic diversity in *Chagium smyrnioides* (Apiaceae) an endangered plant *Bot Bull Acad Sin* **44** 13–18

MANIRUZZAMAN M E, HAQUE M M, SAYEM M A & AMIN M 2010 Molecular characterization on onion (*Allium cepa*) using RAPD makers *Bangla J Agri Res* **35** 313–322

MUKHERJEE A, SIKDAR B, GHOSH B, BANERJEE A, GHOSH E, BHATTACHARYA M & ROY S C 2013 RAPD and ISSR analysis of some economically important species varieties and cultivars of the genus *Allium* (Alliaceae) *Turk J Bot* **37** 605–618

NAYANAKANTHA N M C, SINGH B R & GUPTA A K 2010 Assessment of genetic diversity in *Aloe* germplasm accessions from India using RAPD and morphological markers *Cey J Sci* **39** 1–9

PERUZZI L, LEITH I J & CAPARELLI K F 2009 Chromosome diversity and evolution in Liliaceae *Ann Bot* **103** 459–475

RAJAGOPAL C & KANDHASAMY R 2013 Assessment of genetic diversity of *Gloriosa superba* L accessions detected by random amplified polymorphic DNA analysis *J Medic Plan Res* **7** 2122–2127

RAO V R & HODGKIN T 2002 Genetic diversity and conservation and utilization of plant genetic resources *Plant Cell Tiss Org Cult* **68** 1–19

RICROCH A, YOCKTENG R, BROWN S C & NADOT S 2005 Evolution of genome size across some cultivated *Allium* species *Genome* **48** 511–520

SAMANTARAY S, PATEL T K, GEETHA K A & MAITI S 2011 Identification and assessment of genetic relationships in three *Chlorophytum* species and two high yielding genotypes of *C borivilianum* through RAPD makers *Biologia* **66** 244–250

SINGH A, RAI V P, SINGH M, SINGH A K & SINHA B 2013 Molecular diversity analysis of *Asparagus racemosus* and its adulterants using random amplified polymorphic DNA (RAPD) *J Medic Plant Res* **7** 1050–1056

VIJAY N, SAIRKAR P, SILAWAT N, GARG R K & MEHROTRA N N 2009 Genetic variability in *Asparagus racemosus* (Willd) from Madhya Pradesh India by random amplified polymorphic DNA *Afr J Biotech* **8** 3135–3140

Journal of Cytology and Genetics 2025 VOL. **26** (NS): 89–94

RESEARCH ARTICLE

DISCOVERY OF HAPLOID *PIPER NIGRUM* L. 'PAJ': A GAME CHANGER FOR BLACK PEPPER GENETICS AND BREEDING

P. J. MATHEW[1]*, THOMSON DAVIS[1], I. MANJU BABY[1], M. ABDUL JABBAR[1] AND P. M. MATHEW[2†]
[1]Plant Genetic Resource Division, Kerala State Council for Science, Technology and Environment - Jawaharlal Nehru Tropical Botanic Garden and Research Institute (KSCSTE-JNTBGRI), Palode, Thiruvananthapuram 695 562, India
[2]Perakathusseril, Muttada P.O., Thiruvananthapuram 695 025, India
*For correspondence. Email: matjol2004@yahoo.co.uk

(Received 30 October 2025, accepted 10 November 2025)

SUMMARY Black pepper is the most widely used spice of the world. A hybridization effort was made between a wild genotype of black pepper, 'PMM' (female parent), and a high-yielding cv. 'Karimunda' (male parent), to transfer the high-piperine content-trait of fruits of 'PMM' to cv. 'Karimunda'. Out of the 9 fruits obtained as a result of the attempted hybridization, 5 germinated, of which one odd seedling exhibited retarded growth and the 4 others showed normal growth. The seedling which showed initial retarded growth, established eventually. The fully grown plant of the odd seedling showed some morphological traits of the female parent, 'PMM' and exhibited no morphological features of the male parent, cv. 'Karimunda', whereas the 4 seedlings which showed normal growth were true to type to the female parent, 'PMM'. Mitotic studies on the plants propagated using stem cuttings of the 2 morphotypes revealed that the plants obtained from the normal seedlings were 2n = 52, whereas the plants propagated from the seedling which showed retarded growth initially, were 2n = 26, and this confirmed the haploid genome constitution of the latter. Hence, we inferred that the plants with 2n = 26 genome constitution are dihaploids, originated via parthenogenesis from an egg cell of 'PMM', which is having allotetraploid genome constitution. This only reported haploid black pepper genotype has been named 'PAJ', and its discovery is highly significant as regards to the genetics and breeding of black pepper.

Keywords: *Piper nigrum*, dihaploid, DH technology, hybrid vigour.

INTRODUCTION

Haploids possess only half the number of chromosomes of the species in their somatic cells, and they are unusual among the angiosperms. Mathew et al. (2016) discovered the first haploid genotype of *P. nigrum*, among the progenies of the lemon scented genotype of black pepper (*P. nigrum* 'PMM'), which was produced in an attempted

---

Deceased †

hybridization between the 2 genotypes of the species, 'PMM' (female) and the popular cv. 'Karimunda' (male).

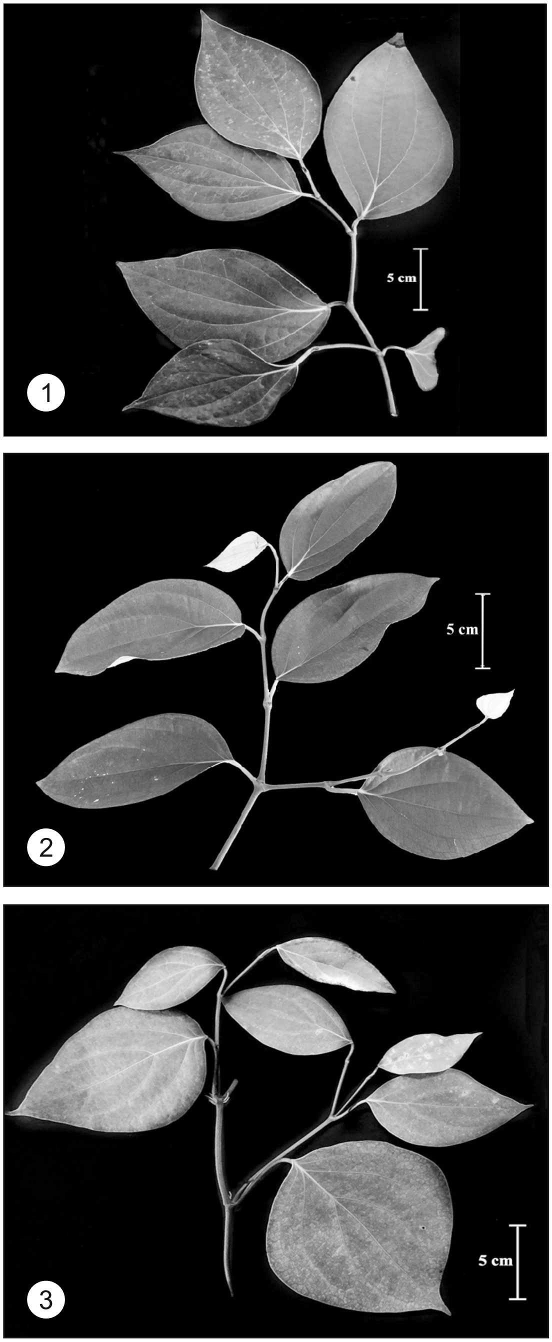
In the realm of crop improvement of black pepper, achievements have been limited to the release of a few high yielding lines, and there was only very limited success in the development and release of disease resistant cultivars (Krishnamurthy et al. 2024). Black pepper, which is blessed with the twin advantage of viable sexual reproduction and vegetative propagation, offers much scope for exploitation of hybrid vigour. But, the advantage of heterosis is hampered in the crop due to lack of homozygous lines (Ravindran et al. 2000). Development of many generations of inbred lines is needed for attaining homozygosity in a highly heterozygous crop like black pepper. The problem is more acute, since *P. nigrum* is perennial in habit and its seedlings take at least 6–7 y for attaining reproductive maturity. However, if doubled haploids (DH) are produced, completely homozygous lines can be obtained in one or two generations (Nei 1963), which can lead to exploitation of hybrid vigour as well as doubled haploid technology (DH) for crop improvement in black pepper.

MATERIALS AND METHODS

One 3 y old, well-established plant of each of the 2 genotypes of black pepper - lemon scented genotype 'PMM' (Fig.1) (Rameshkumar et al. 2008) and the cv. 'Karimunda' (Fig.2) maintained in the Field Gene Bank of Medicinal and Aromatic plants at JNTBGRI were selected as the female and male parents respectively for the hybridization programme.

Ten young spikes of each of the parent plants were selected based on random sampling and the spikes were bagged using transparent polythene covers. Given the majority of flowers on the selected spikes of the cv. 'Karimunda' getting opened, the bags covering the selected spikes were removed and washed it with drops of distilled water, using a camel hair brush. The wash, a suspension of pollen grains of cv. 'Karimunda', was collected and applied on the flowers of the selected spikes of the female parent using the brush. Ten randomly selected spikes of the female parent, 'PMM' were applied with pollen suspension using the brush and they were bagged and labelled showing date and time of the experimental pollination.

Seven months after the pollination, the bags were removed and 9 ripened fruits developed in total, in all the pollinated spikes collected. In the laboratory, the pericarp of the 9 fruits were removed and the seeds were washed using distilled water and shade dried for 2 subsequent days. After shade drying, on the next day, the 9 seeds were sowed in 2 clay pots, which were potted with river sand as the potting medium. The pots containing the seeds were kept in nursery and watered as and when needed. Out of the 9 seeds, 5 seeds germinated, of which one showed retarded growth, whereas the other 4 seedlings showed normal growth. All the 5 seedlings were transplanted to pots containing 1:1:1 mixture



Figs 1–3: Twigs of *P. nigrum* genotypes. 1. 'PMM'. 2. cv. 'Karimunda'. 3. 'PAJ'.

of top soil, cow dung and river sand. The plants, on establishment, were transplanted to the conservatory house of the Field Gene Bank at JNTBGRI. The seedlings, on attaining maturity, were propagated using stem cuttings.

OBSERVATIONS

Morphological observations on the plants propagated using the stem cuttings, which were obtained from the fully grown odd seedling as well as the plants propagated using stem cuttings of the fully grown plants of the 4 healthy seedlings revealed that the former showed considerable morphological similarity as well as differences with its female progenitor, 'PMM'. They exhibited no morphological similarity with the male parent, cv. 'Karimunda'. While the plants obtained from the normal seedlings were true to type to the female parent, 'PMM'. Mitotic studies using root tips of the stem cuttings of the plants propagated from the odd seedling revealed that they possess only 26 chromosomes in their somatic cells, whereas plants produced using the stem cuttings of the plants raised from the normal seedlings showed 2n = 52. This haploid black pepper genotype having only 26 chromosomes in their somatic cells has been named 'PAJ' (Fig. 3) in honour of the late Sri. P. A. Jacob, Karyakulathu Paravanparampil, Pathamuttom, Kottayam District, Kerala State, India, who was the source of inspiration and guidance to the senior author, throughout his research career in plant science.

DISCUSSION

Following the pioneering studies on cytogenetics of the genus *Piper* by Mathew (1958) and the first report on the correct chromosome number of *P. nigrum*, many workers had reported 2n = 52 in the wild genotypes and cultivars of the species. The 2 genotypes selected for crossing - the lemon scented wild genotype, 'PMM” (Davis et al. 2013), cv. 'Karimunda' (Mathew 1997) and the plants raised from the progenies which showed normal growth are having the somatic chromosome constitution of 2n = 52. Mitotic studies on the plants propagated from the odd seedling ('PAJ') showed 2n = 26 (Mathew et al. 2016, Parthasarathy et al. 2024). Mathew (1958) reported allotetraploid (2n = 4x = 52) genome constitution (AABB) of *P. nigrum*, based on the basic chromosome number of the genus *Piper* (x = 13), and subsequently, many workers have confirmed his finding (Mathew & Mathew 1982, Jose & Sharma 1984). 'PAJ' having 2n = 26, constitutes a dihaploid (2n = 2x = 26) genotype (with genome constitution AB) of *P. nigrum*, which is an allotetraploid with genome constitution AABB. The ploidy status of 'PMM' and 'PAJ' were confirmed using flow cytometric analysis by the senior author and co-workers (unpubl.).

Hybridization, both intraspecific and interspecific, involved in producing haploids has been known to occur in many crop species. As a consequence of hybridization, parthenogenesis happens and haploids were generated from diploids, and diploids from tetraploids. Parthenogenesis occurs either spontaneously or can be induced by modified pollination methods in vivo or in vitro culture of immature male or female gametophyte (Dunwell 2010). Induction of haploidy has also been attained through delayed pollination (Rotarenco & Mihailov 2007). Stimulus of pollination may be the causative factor of parthenogenetic origin of the haploid black pepper genotype 'PAJ' (Fig. 3) from 'PMM' (Fig. 1). Usually parthenogenesis occurs at low frequency, and such rare haploid seedlings among the many diploids are difficult to detect and distinguish (Kendall 1934). The considerable degree of morphological similarity as well as differences between 'PAJ' and its female parent, 'PMM', and its total dissimilarity in morphological features with cv.'Karimunda' (Fig. 2) along with the haploid count in this genotype supports the parthenogenetic origin of the haploid black pepper 'PAJ' from the female parent 'PMM'. The plants propagated vegetatively from all the 4 sister seedlings (2n = 52) of 'PAJ' were true to type to 'PMM' in their morphological traits, and this indicates their apomictic origin from sporophytic cells of their maternal progenitor, 'PMM'. Apomictic development of fruits was reported earlier in cultivars of *P. nigrum* (Gentry 1955, Ravindran et al. 2000).

In depth studies on genetics of black pepper have not been initiated hitherto, especially investigations on the genetics of resistance against the devastating pathogens like *Phytophthora* and *Fusarium* (Ravindran et al. 2000). Non-availability of genes characterised for desirable traits such as yield and quality, disease, pest and drought resistance is a limiting factor for pyramiding the genes in a single cultivar of the crop (Parthasarathy et al. 2008). Haploids have been exploited in a wide range of theoretical and applied aspects of plant biology such as in cytogenetic analysis, development of linkage maps, genome sequencing, mutant isolation and transformation, horticulture and plant breeding (Dunwell 2010). Hence, the discovery of haploid black pepper is a revolutionary step for exploring the genetics of the crop.

In the realm of crop improvement of black pepper, achievements have been limited to the release of a very few high yielding lines (Ravindran et al. 2000). Black pepper, which is blessed with the twin advantage of viable sexual reproduction and vegetative propagation, offers much scope for exploitation of hybrid vigour. However, it is hampered in the crop due to lack of homozygous lines (Ravindran et al. 2000). Development of many generations of inbred lines are needed for attaining homozygosity in a highly heterozygous crop like black pepper, which is imperative for exploiting hybrid vigour. This problem of heterozygosity of black pepper is more acute, since the F_1 seedlings will take a minimum of 5–6 y for attaining reproductive maturity. However, if homozygous diploids or doubled haploids (DH) are produced, completely homozygous lines of the crop can be produced in one or two generations (Nei 1963). Production of polyploid lines from diploids has already been achieved in black pepper, employing colchicine treatment (Nair & Ravindran 1992). Given the highly heterozygous genetic constitution of *P. nigrum* as a result of allopolyploid origin of the species, haploidy breeding may be the most desired method of crop improvement in black pepper, since it is potential for the exploitation of hybrid vigour as well as doubled haploid technology in the crop. Hence, the discovery of *P. nigrum* 'PAJ' will be a game changer in the realms of genetics as well as breeding of black pepper.

ACKNOWLEDGEMENTS

We are thankful to the Director, KSCSTE-JNTBGRI, Palode, Thiruvananthapuram for facilities. We are also thankful to Dr. K. C. Koshy, Scientist-F (Retd), for critical feedback on the manuscript.

Declaration

The authors have no conflict of interest.

REFERENCES

DAVIS THOMSON, MANJU BABY I, MATHEW P J & MATHEW P M 2013 Cytology of *Piper nigrum* L 'PMM' – A lemon-scented genotype of 'Black pepper' *J Cytol Genet* **14** (NS) 97–100

DUNWELL J M 2010 Haploids in flowering plants *Plant Biotechnol* J **8** 1–48

GENTRY H S 1955 Apomixis in black pepper and Jojoba *J Hered* **46** 8–13

JOSE J & SHARMA A K 1984 Chromosome studies in the genus *Piper* L *J Indian Bot Soc* **63** 313–319

KENDALL J 1934 A parthenogenetic aberrant tobacco plant *J Hered* **21** 363–366

KRISHNAMURTHY K S, SHARON A, MEENA R S, JANAKIRAM T & NIRMAL BABU K 2024 Improved varieties of spice crops *In* Ravindran P N Sivaraman K Devasahayam S & Babu K N (eds) *Handbook of spices in India: 75 years of research and development* Springer Singapore pp 284–394

MATHEW P J 1997 *Morphological cytological and palynological investigations on the family Piperaceae with special reference to the genus Piper* Ph D Thesis University of Kerala

MATHEW P J, DAVIS, T, MANJU BABY I, ABDUL JABBAR M & MATHEW P M 2016 Discovery of haploid black pepper (*Piper nigrum* L 'PAJ') 2016 *In* Archak S Agarwal A Rana J C Karihaloo J L & Tyagi R K (eds) Abstract book of the *1st International Agrobiodiversity Congress* Indian Society of Plant Genetic Resources and Bioversity International New Delhi p 180

MATHEW P M 1958 Studies on Piperaceae *J Indian Bot Soc* **37** 155–171

MATHEW P M & MATHEW P J 1982 Cytology of hybrid pepper variety Panniyur-I *Curr Sci* **51** 530 –531

NAIR R R & RAVINDRAN P N 1992 Induced polyploidy in black pepper (*Piper nigrum*) *JOSAC* **1** 151–153

NEI M 1963 The efficiency of haploid breeding of plant breeding *J Hered* **18** 95–100

PARTHASARATHY V A, SRINIVASAN V & MADAN M S 2008 Black pepper research for future *In* Krishnamurthy K S (ed) *Piperaceae crops – Technologies and future perspectives* Indian Institute of Spices Calicut pp 1–9

PARTHASARATHY V A, SRINIVASAN V, MATHEW P J, NEEMA V P, KRISHNAMURTHY K S, SHIVAKUMAR M S, JAYASHREE E & RAVINDRAN P N 2024 Black pepper *In* Ravindran P N Sivaraman K Devasahayam S & Nirmal Babu K (eds) *Handbook of spices in India: 75 Years of research and development* Springer Singapore pp 1393–1576

RAMESHKUMAR K B, MATHEW P J & GEORGE V 2008 Discovery of a potential genotype of 'Black pepper' (*Piper nigrum* L 'PMM”) from the Western Ghats Yesodharan E P (ed) *Proceedings of the 20th Kerala Science Congress* Kerala State Council for Science Technology and Environment Thiruvananthapuram pp 503–505

RAVINDRAN P N, NIRMAL BABU K, SASIKUMAR B & KRISHNAMURTHY K S 2000 Botany and crop improvement of black pepper *In* Ravindran P N (ed) *Black pepper – Piper nigrum* Harwood Academic Publishers Amsterdam pp 2–142

ROTARENCO V A & MIHAILOV M E 2007 The influence of ear age on the frequency of maternal haploids produced by a haploid-inducing line *Maize Genet Newsl* **81** 9–10

Journal of Cytology and Genetics 2025 VOL. **26** (NS): 95−114

REVIEW ARTICLE

ABIOTIC AND BIOTIC STRESS-RESPONSIVE EXPRESSION AND REGULATORY FEATURES OF *ARABIDOPSIS* GAMETOPHYTE-SPECIFIC GENES**

DANA BASHEER AND JASMINE M. SHAH*
Department of Plant Science, Central University of Kerala, Periye, Kasaragod 671 325, India
*For correspondence. Email. jasmine@cukerala.ac.in

(Received 23 October 2025, revised accepted 10 November 2025)

SUMMARY A comprehensive bioinformatic analysis was carried out to investigate the regulation of gametophyte-specific (GS) genes in *Arabidopsis thaliana*. A curated list of 20 GS genes was made, and their transcription factor binding site (TFBS) data were collected for analysis. The frequencies and distribution of these TFBSs were assessed. Expression data of these genes under various biotic, abiotic and developmental conditions were studied to check the relationship between the putative TFBSs and the gene activity. This study revealed an overall inverse correlation between the number of putative TFBSs and expression levels of GS genes. This observation was opposite to that of stress-responsive genes. The study provides insights into the transcriptional regulation of GS genes of *A. thaliana*.

Keywords: *Arabidopsis thaliana*, gametophyte-specific gene, gene expression, transcription factor, transcription factor binding site.

INTRODUCTION

Transcription is the first stage of gene expression in which mRNA is synthesized from the DNA, on which post-transcriptional processes like RNA splicing are carried out and later translated into proteins. Not all genes are expressed in all the tissues. Transcription factors (TFs) are proteins that regulate gene expression by binding to the promoter region of a gene and thereby promoting or inhibiting transcription. Transcriptional regulation by TFs can be considered as the primary level of regulation of genes (Wray et al. 2003). The specific DNA sequences where the TFs bind in a sequence-specific manner, as revealed by structural studies of protein-DNA complexes, are known as transcription factor binding sites (TFBS) (Paillard & Lavery 2004, Rohs et al. 2010). They are short, conserved sequences within gene regulatory regions, usually spanning a few to around 20 base pairs, and a single TF often recognizes similar sequences across different target genes. Several computational methods are followed to predict the binding sites for TFs on the DNA

** Paper presented at the XV All India Conference on Cytology and Genetics held on 11th and 12th September 2025.

sequence (Wang et al. 2021).

Genes that are expressed more highly in a tissue than in other tissues are known as tissue-specific genes (Whitehead & Crawford 2005), and those predominantly expressed in male and/or female gametophytes are known as gametophyte-specific (GS) genes. Analysis of the promoter of a tissue-specific gene will provide insights into the factors that provide specificity to that gene. Study of GS genes in plants and their regulatory factor has its importance in the field of plant genetic engineering and crop improvement. The study brings computational approaches to explore the regulation of GS genes in *Arabidopsis thaliana*. Bioinformatics tools were employed to explore TFBS and expression profiles of GS genes.

METHODS

Promoter sequences (spanning from-2000 bp upstream to +500 bp downstream) of the 20 *Arabidopsis* GS genes were retrieved from the Eukaryotic Promoter Database (EPD). Putative TFBSs within the retrieved promoter sequences were predicted using the PlantRegMap tool in the Plant TFDB database for *A. thaliana* (http://planttfdb.cbi.pku.edu.cn/). Binding site data was processed in Microsoft Excel, where sorting and filtering tools were used to organize the entries. The number of TFBSs for each gene was grouped by TF families, and parameters such as count (total number of genes showing the sites), frequency (total number of sites divided by total number of genes), and diversity (total number of TF family types) were calculated.

Expression profiling of the selected genes was carried out using the Electronic Fluorescent Pictograph (eFP) Browser (http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi) for *A. thaliana* by submitting gene IDs individually to examine expression patterns across various tissues, developmental stages, and under different biotic and abiotic stress conditions. Promoter sequences of GS genes and stress-responsive genes retrieved were analysed for stress-responsive TFBSs. Expression values under various biotic and abiotic stresses were compiled for each gene. Genes were ranked by the sum of expression level values, and high and low expressing groups were defined to compare TFBS frequencies. The frequency of 29 stress-responsive TF families was calculated for each group, and fold-change values between high and low expressing groups were calculated.

OBSERVATIONS

Twenty GS genes (Table 1) were selected for the study. The putative binding sites for 43 TF families (Table 2) were counted on the promoters of these genes (Fig. 1). Counting of the total number of genes with respective sites, and their frequencies showed that not all gene promoters had all 43 TFBSs (Fig. 2). Putative binding sites for 15 TFs such as ERF, NAC, MYB_related, MYB, MIKC_MADS, LBD, GRAS, GATA, G2-like, ERF, Dof, C2H2, bZIP, bHLH, B3, and AP2 were present on all promoters as their total count (20) was highest (Fig. 2). As the number of sites on each promoter varied, their frequencies were calculated (Fig. 2). While ERF had highest frequency of 264.15, a lowest of 0.25 was

TABLE 1: Gametophyte-specific genes in *Arabidopsis thaliana*.

SI. No.	Gene ID	Accession No.	Function	Reference
1	AGL11	AT4G09960	Development of ovule, determination of ovule identity.	Nain et al. 2008
2	AGP6	AT5G14380	Pollen development, pollen tube growth and stamen function, pollen viability, nexine formation.	Coimbra et al. 2010, Jia et al. 2015, Levitin et al. 2008, Moreira et al. 2022
3	AGP11	At3g01700	Pollen development, pollen tube growth and stamen function, pollen viability.	Coimbra et al. 2010, Levitin et al. 2008, Moreira et al. 2022
4	AGP23	AT3G57690	Pollen development.	Moreira et al. 2022, Nguema-Ona et al. 2012
5	AGP40	AT3G20865	Pollen development.	Moreira et al. 2022, Nguema-Ona et al. 2012
6	ATAGP24	AT5G40730	Pollen-pistil interaction.	Moreira et al. 2022
7	AtFLA3	At2g24450	Microspore development and affecting cellulose deposition.	Li et al. 2010
8	AtPTEN1	AT5G39400	Pollen development and maturation.	Gupta et al. 2002
9	AtSTP6	AT3G05960	H+/monosaccharide transporter during the late stages of pollen development.	Scholz-Starke et al. 2003
10	AtVEX1	At5g62580	Mediates pollen galactose sensitivity via GALK that is required for efficient galactose uptake in pollen during pollen germination.	Engel et al. 2005, Wang et al. 2022
11	BCP1	AT1G24520	Pollen development.	Xu et al. 1993, Xu et al. 1995
12	DUO3	AT1G64570	Cell cycle progression and sperm cell specification.	Brownfield et al. 2009
13	HAP2	AT4G11720	Pollen tube guidance and fertilization.	von Besser et al. 2006
14	MSP1	At4g20090	Controls early sporogenic development.	Honys et al. 2006, Nonomura et al. 2003
15	MSP2	At5g46795	Role in pollen development during the early bicellular stage.	Honys et al. 2006
16	PIRL6	AT2G19330	Male and female gametogenesis.	Forsthoefel et al. 2018, Hossain et al. 2022
17	RPG1	At5g40260	Exine pattern formation and cell integrity of microspores.	Guan et al. 2008, Kim et al. 2013
18	TAG1	AT2G19450	Proper development of stamens and carpels and the prevention of indeterminate growth of the floral meristem.	Galli et al. 2003, Pnueli et al. 1994
19	TAPNAC	At1g61110	Tapetal development.	Alvarado et al.2011
20	TUA1	AT1G64740	Pollen development and pollen tube growth.	Carpenter et al. 1992

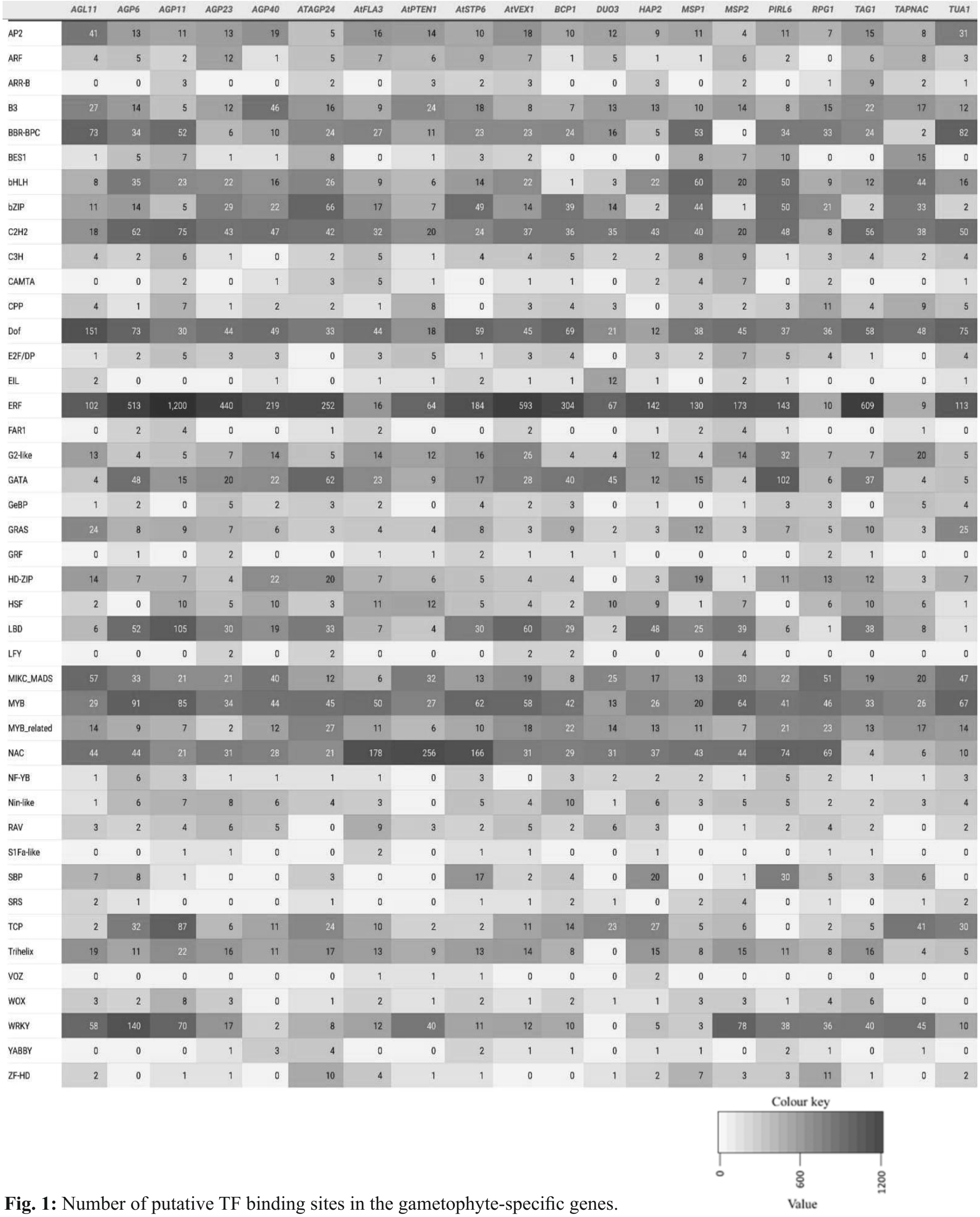
TABLE 2: Functions of TF families.

SI. No.	TF family	Function	Reference
1	ERF	Growth and development, response to various biotic and abiotic stresses.	Nakano et al. 2006
2	NAC	Regulating senescence, cell division, and wood formation, mediates auxin signalling to promote lateral root development, response to biotic and abiotic stresses.	Fang et al. 2008
3	Dof	The regulation of gene expression in processes like seed storage, protein synthesis in developing endosperm, light regulation of genes involved in carbohydrate metabolism, plant defence mechanisms, seed germination, gibberellin response in post germinating aleurone, auxin response and stomata guard cell-specific gene regulation.	Lijavetzky et al. 2003
4	MYB	Development, metabolism, response to biotic and abiotic stress.	Dubos et al. 2010
5	C2H2	Growth and development, response to environmental stresses.	Han et al. 2020
6	LBD	Plant growth and development, respond to environmental stress.	Rong et al. 2024
7	GATA	Light-responsive transcription.	Teakle et al. 2002
8	MIKC_MADS	Formation of floral organs and reproductive development in plants, biotic and abiotic stress response.	Dai et al. 2023
9	bZIP	Growth and development, abiotic and biotic stress response.	Wang et al. 2018
10	bHLH	Secondary metabolite synthesis, growth and development.	Wang & Liu 2020
11	B3	Growth and development.	Xia et al. 2019
12	AP2	Regulation of developmental processes.	Nakano et al. 2006
13	MYB_related	Plant development and responses to various environmental stressors.	Yang et al. 2021
14	G2-like	Regulate chloroplast development.	Fitter et al. 2002
15	GRAS	Plant development, including root development, axillary shoot development and maintenance of the shoot apical meristem and phytochrome signalling.	Hirsch et al. 2009
16	WRKY	Plant growth and development and biotic and abiotic stress response.	Chen et al. 2017
17	BBR-BPC	Homeotic gene regulation and developmental processes, hormone signaling, stress, circadian oscillation, and sex determination processes.	Sahu et al. 2023
18	TCP	Plant development.	Martín-Trillo & Cubas 2010
19	Trihelix	Light and abiotic stress response.	Xu et al. 2018
20	HD-ZIP	Meristem maintenance or organ and vascular develoment.	Ariel et al. 2007
21	ARF	Regulate auxin mediated transcriptional activation/repression.	Okushima et al. 2005
22	Nin-like	Regulate nitrate-response genes, plant root development.	Hsin et al. 2021
23	C3H	Embryogenesis, apical domain of the embryo.	Li & Thomas 1998

(Contd)

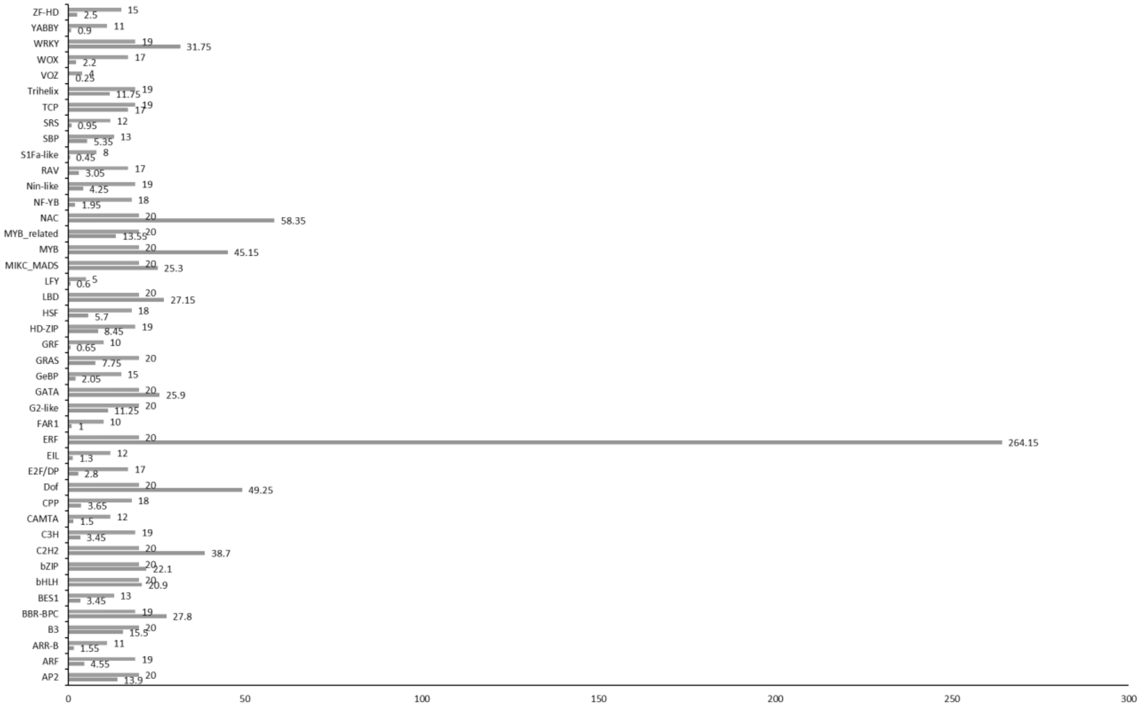
TABLE 2: (Concluded).

SI. No.	TF family	Function	Reference
24	HSF	Plant heat stress (hs) response.	Kotak et al. 2004
25	CPP	Development of reproductive tissue and control of cell division in plants.	Yang et al. 2008
26	NF-YB	Plant growth and development and biotic and abiotic stress response.	Bhattacharjee & Hallan 2023
27	RAV	Ethylene response and brassinosteroid response.	Nakano et al. 2006
28	E2F/DP	Regulation of cell cycle and cell proliferation.	Van Den Heuvel & Dyson 2008
29	WOX	Key developmental processes in plants, such as embryonic patterning, stem-cell maintenance and organ formation. These functions can be related to either promotion of cell division activity and/or prevention of premature cell differentiation.	van der Graaff et al. 2009
30	ZF-HD	Establishment of the characteristic expression pattern of the C4 PEPCase gene.	Windhovel et al. 2001
31	GeBP	Cytokinin hormone pathway regulation.	Chevalier et al. 2008
32	SBP	Flower development.	Guo et al. 2008
33	BES1	Plant growth and development and BR-regulated gene expression.	Yin et al. 2005
34	CAMTA	Regulating CBF2 expression.	Finkler et al. 2007
35	EIL	Regulate expression of the GCC-box-binding transcription factors, ethylene-response factors.	Yamasaki et al. 2005
36	SRS	Regulators of plant organ growth and development, abiotic stress response.	Lu et al. 2023
37	ARR-B	Nutrient sensing, root and shoot development, circadian clock oscillation maintenance, hormonal transport and signalling.	Safi et al. 2017
38	YABBY	Specify abaxial identity in lateral organs produced by the apical and flower meristems in *Arabidopsis.*	Siegfried et al. 1999
39	FAR1	Light signalling.	Lin et al. 2007
40	GRF	Cell expansion in leaf and cotyledon tissues.	Kim et al. 2003
41	S1Fa-like	Represses the rps1 promoter preferentially in roots and in etiolated plants.	Zhou et al. 1995
42	LFY	Switch from vegetative to reproductive development in dicotyledonous plant species.	William et al. 2004
43	VOZ	Participate in flowering and biotic and abiotic stress signalling pathways.	Uluisik et al. 2023

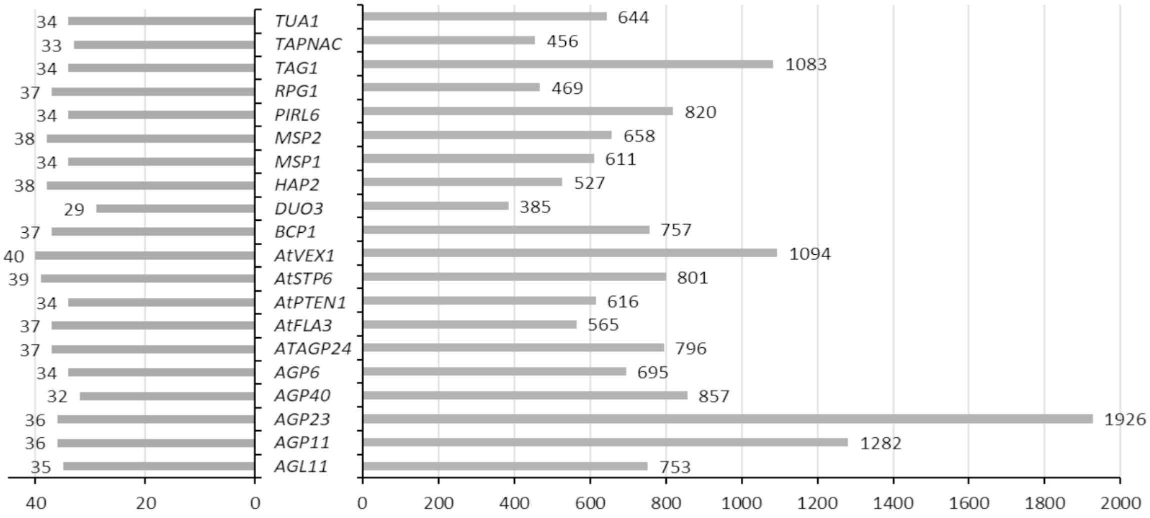


	AGL11	AGP6	AGP11	AGP23	AGP40	ATAGP24	AtFLA3	AtPTEN1	AtSTP6	AtVEX1	BCP1	DUO3	HAP2	MSP1	MSP2	PIRL6	RPG1	TAG1	TAPNAC	TUA1
AP2	41	13	11	13	19	5	16	14	10	18	10	12	9	11	4	11	7	15	8	31
ARF	4	5	2	12	1	5	7	6	9	7	1	5	1	1	6	2	0	6	8	3
ARR-B	0	0	3	0	0	2	0	3	2	3	0	0	3	0	2	0	1	9	2	1
B3	27	14	5	12	46	16	9	24	18	8	7	13	13	10	14	8	15	22	17	12
BBR-BPC	73	34	52	6	10	24	27	11	23	23	24	16	5	53	0	34	33	24	2	82
BES1	1	5	7	1	1	8	0	1	3	2	0	0	0	8	7	10	0	0	15	0
bHLH	8	35	23	22	16	26	9	6	14	22	1	3	22	60	20	50	9	12	44	16
bZIP	11	14	5	29	22	66	17	7	49	14	39	14	2	44	1	50	21	2	33	2
C2H2	18	62	75	43	47	42	32	20	24	37	36	35	43	40	20	48	8	56	38	50
C3H	4	2	6	1	0	2	5	1	4	4	5	2	2	8	9	1	3	4	2	4
CAMTA	0	0	2	0	1	3	5	1	0	1	1	0	2	4	7	0	2	0	0	1
CPP	4	1	7	1	2	2	1	8	0	3	4	3	0	3	2	3	11	4	9	5
Dof	151	73	30	44	49	33	44	18	59	45	69	21	12	38	45	37	36	58	48	75
E2F/DP	1	2	5	3	3	0	3	5	1	3	4	0	3	2	7	5	4	1	0	4
EIL	2	0	0	0	1	0	1	1	2	1	1	12	1	0	2	1	0	0	0	1
ERF	102	513	1,200	440	219	252	16	64	184	593	304	67	142	130	173	143	10	609	9	113
FAR1	0	2	4	0	0	1	2	0	0	2	0	0	1	2	4	1	0	0	1	0
G2-like	13	4	5	7	14	5	14	12	16	26	4	4	12	4	14	32	7	7	20	5
GATA	4	48	15	20	22	62	23	9	17	28	40	45	12	15	4	102	6	37	4	5
GeBP	1	2	0	5	2	3	2	0	4	2	3	0	1	0	1	3	3	0	5	4
GRAS	24	8	9	7	6	3	4	4	8	3	9	2	3	12	3	7	5	10	3	25
GRF	0	1	0	2	0	0	1	1	2	1	1	1	0	0	0	0	2	1	0	0
HD-ZIP	14	7	7	4	22	20	7	6	5	4	4	0	3	19	1	11	13	12	3	7
HSF	2	0	10	5	10	3	11	12	5	4	2	10	9	1	7	0	6	10	6	1
LBD	6	52	105	30	19	33	7	4	30	60	29	2	48	25	39	6	1	38	8	1
LFY	0	0	0	2	0	2	0	0	0	2	2	0	0	0	4	0	0	0	0	0
MIKC_MADS	57	33	21	21	40	12	6	32	13	19	8	25	17	13	30	22	51	19	20	47
MYB	29	91	85	34	44	45	50	27	62	58	42	13	26	20	64	41	46	33	26	67
MYB_related	14	9	7	2	12	27	11	6	10	18	22	14	13	11	7	21	23	13	17	14
NAC	44	44	21	31	28	21	178	256	166	31	29	31	37	43	44	74	69	4	6	10
NF-YB	1	6	3	1	1	1	1	0	3	0	3	2	2	2	1	5	2	1	1	3
Nin-like	1	6	7	8	6	4	3	0	5	4	10	1	6	3	5	5	2	2	3	4
RAV	3	2	4	6	5	0	9	3	2	5	2	6	3	0	1	2	4	2	0	2
S1Fa-like	0	0	1	1	0	0	2	0	1	1	0	0	1	0	0	0	1	1	0	0
SBP	7	8	1	0	0	3	0	0	17	2	4	0	20	0	1	30	5	3	6	0
SRS	2	1	0	0	0	1	0	0	1	1	2	1	0	2	4	0	1	0	1	2
TCP	2	32	87	6	11	24	10	2	2	11	14	23	27	5	6	0	2	5	41	30
Trihelix	19	11	22	16	11	17	13	9	13	14	8	0	15	8	15	11	8	16	4	5
VOZ	0	0	0	0	0	0	1	1	1	0	0	0	2	0	0	0	0	0	0	0
WOX	3	2	8	3	0	1	2	1	2	1	2	1	1	3	3	1	4	6	0	0
WRKY	58	140	70	17	2	8	12	40	11	12	10	0	5	3	78	38	36	40	45	10
YABBY	0	0	0	1	3	4	0	0	2	1	1	0	1	1	0	2	1	0	1	0
ZF-HD	2	0	1	1	0	10	4	1	1	0	0	1	2	7	3	3	11	1	0	2

Fig. 1: Number of putative TF binding sites in the gametophyte-specific genes.

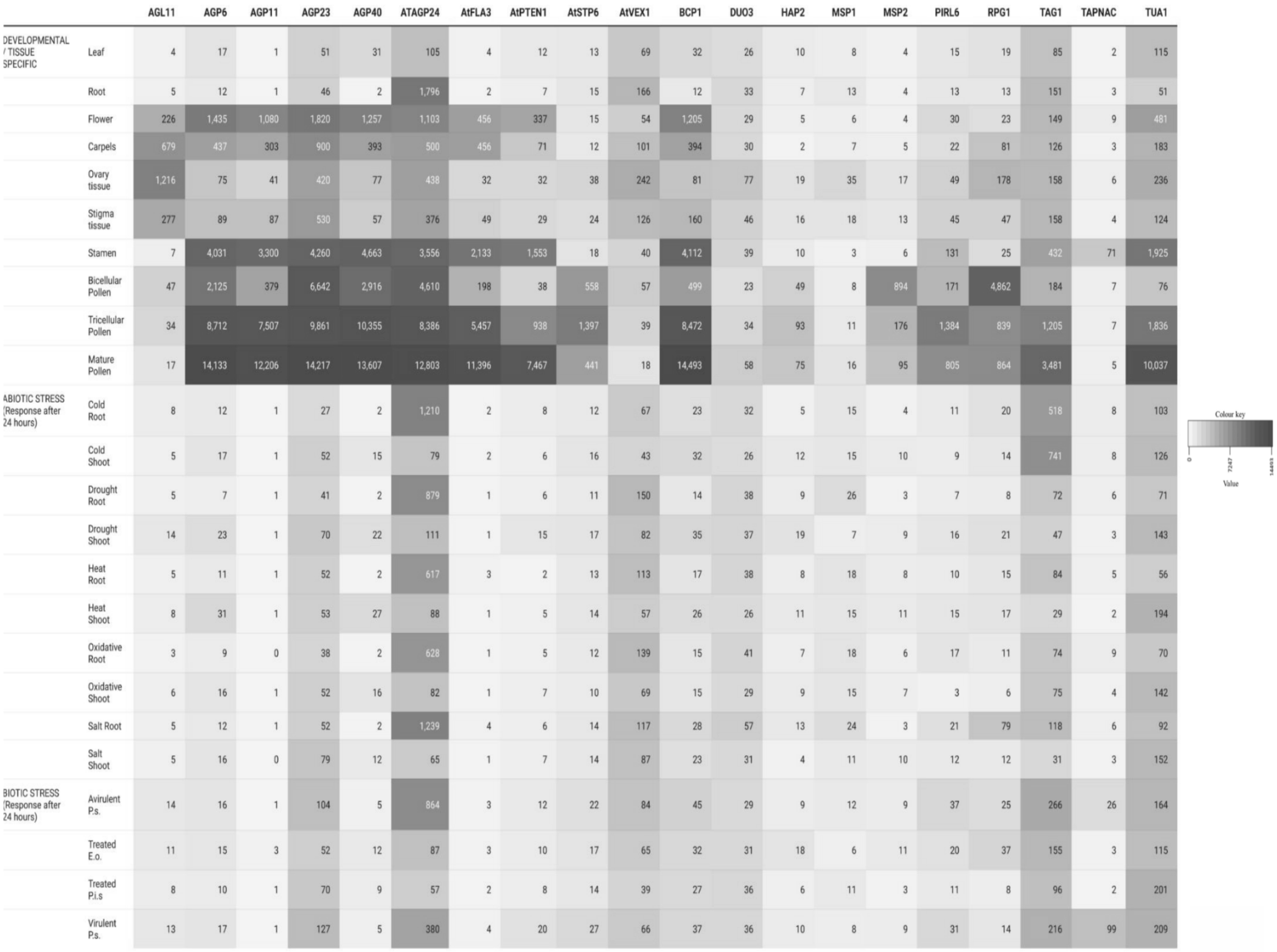


2



3

Figs 2 & 3: 2. Frequency and count of TF binding sites in *Arabidopsis* gametophyte-specific genes. 3. Graphs comparing the diversity (blue) and count (red) of putative binding sites of TF families among the gametophyte-specific genes.



		AGL11	AGP6	AGP11	AGP23	AGP40	ATAGP24	AtFLA3	AtPTEN1	AtSTP6	AtVEX1	BCP1	DUO3	HAP2	MSP1	MSP2	PIRL6	RPG1	TAG1	TAPNAC	TUA1
DEVELOPMENTAL / TISSUE SPECIFIC	Leaf	4	17	1	51	31	105	4	12	13	69	32	26	10	8	4	15	19	85	2	115
	Root	5	12	1	46	2	1,796	2	7	15	166	12	33	7	13	4	13	13	151	3	51
	Flower	226	1,435	1,080	1,820	1,257	1,103	456	337	15	54	1,205	29	5	6	4	30	23	149	9	481
	Carpels	679	437	303	900	393	500	456	71	12	101	394	30	2	7	5	22	81	126	3	183
	Ovary tissue	1,216	75	41	420	77	438	32	32	38	242	81	77	19	35	17	49	178	158	6	236
	Stigma tissue	277	89	87	530	57	376	49	29	24	126	160	46	16	18	13	45	47	158	4	124
	Stamen	7	4,031	3,300	4,260	4,663	3,556	2,133	1,553	18	40	4,112	39	10	3	6	131	25	432	71	1,925
	Bicellular Pollen	47	2,125	379	6,642	2,916	4,610	198	38	558	57	499	23	49	8	894	171	4,862	184	7	76
	Tricellular Pollen	34	8,712	7,507	9,861	10,355	8,386	5,457	938	1,397	39	8,472	34	93	11	176	1,384	839	1,205	7	1,836
	Mature Pollen	17	14,133	12,206	14,217	13,607	12,803	11,396	7,467	441	18	14,493	58	75	16	95	805	864	3,481	5	10,037
ABIOTIC STRESS (Response after 24 hours)	Cold Root	8	12	1	27	2	1,210	2	8	12	67	23	32	5	15	4	11	20	518	8	103
	Cold Shoot	5	17	1	52	15	79	2	6	16	43	32	26	12	15	10	9	14	741	8	126
	Drought Root	5	7	1	41	2	879	1	6	11	150	14	38	9	26	3	7	8	72	6	71
	Drought Shoot	14	23	1	70	22	111	1	15	17	82	35	37	19	7	9	16	21	47	3	143
	Heat Root	5	11	1	52	2	617	3	2	13	113	17	38	8	18	8	10	15	84	5	56
	Heat Shoot	8	31	1	53	27	88	1	5	14	57	26	26	11	15	11	15	17	29	2	194
	Oxidative Root	3	9	0	38	2	628	1	5	12	139	15	41	7	18	6	17	11	74	9	70
	Oxidative Shoot	6	16	1	52	16	82	1	7	10	69	15	29	9	15	7	3	6	75	4	142
	Salt Root	5	12	1	52	2	1,239	4	6	14	117	28	57	13	24	3	21	79	118	6	92
	Salt Shoot	5	16	0	79	12	65	1	7	14	87	23	31	4	11	10	12	12	31	3	152
BIOTIC STRESS (Response after 24 hours)	Avirulent P.s.	14	16	1	104	5	864	3	12	22	84	45	29	9	12	9	37	25	266	26	164
	Treated E.o.	11	15	3	52	12	87	3	10	17	65	32	31	18	6	11	20	37	155	3	115
	Treated P.i.s	8	10	1	70	9	57	2	8	14	39	27	36	6	11	3	11	8	96	2	201
	Virulent P.s.	13	17	1	127	5	380	4	20	27	66	37	36	10	8	9	31	14	216	99	209

Fig. 4: Expression levels of gametophyte-specific genes in developmental, tissue-specific, biotic and abiotic stress conditions. *P. s.* indicates *Pseudomonas syringae* and *E. o.* denotes *Erysiphe orontii*

TABLE 3: Fold change in frequencies of putative binding sites of stress-responsive TFs on GS gene promoters

TF family	Frequency of TFBSs on least expressed genes (A)	Frequency of TFBSs on most expressed genes (B)	Fold change (A/B)
AP2	19.0	17.3	1.10
ARF	4.5	5.3	0.86
bHLH	17.3	19.0	0.91
bZIP	10.8	21.0	0.52
C2H2	42.5	46.3	0.92
CAMTA	3.0	1.3	2.40
CPP	4.0	3.5	1.14
Dof	58.2	52.8	1.10
ERF	319.3	391.8	0.82
GATA	14.8	33.0	0.45
GRAS	8.8	10.3	0.86
HD-ZIP	10.0	10.8	0.93
HSF	10.2	4.5	2.26
LBD	38.0	33.0	1.15
MIKC_MADS	33.8	24.3	1.40
MYB	54.2	50.8	1.07
MYB_related	11.7	18.0	0.65
NAC	101.3	16.5	6.14
NF-YB	1.5	1.3	1.20
Nin-like	4.7	3.5	1.33
RAV	4.7	2.3	2.07
SRS	1.0	1.0	1.00
TCP	24.2	17.5	1.38
Trihelix	17.3	13.0	1.33
VOZ	0.7	0.0	4.00
WOX	3.0	2.0	1.50
WRKY	44.2	17.5	2.52
YABBY	0.7	1.3	0.53
ZF-HD	2.2	3.3	0.67

Shaded boxes indicate <1 fold change.

TABLE 4: Stress-responsive genes in plants.

SI. No.	Gene ID	Accession No.	Function	Reference
1	*GRPL1*	AT4G18280	Defence against drought, salinity, and heat stresses.	Tawab et al. 2020
2	*STP4*	AT3G19930	Resistance to environmental stress.	Truernit et al. 1996
3	*ProDH2*	AT5G38710	Proline homeostasis.	Rizzi et al. 2017
4	*AtMGL*	AT1G64660	Methionine catabolism as stress responses and seed development.	Akinson et al. 2013
5	*GEA6*	AT2G40170	Stress response and late embryogenesis.	Manfre et al. 2006
6	*PRX33*	AT3G49110	Defense mechanism against pathogens.	Daudi et al. 2012
7	*RAB18*	AT5G66400	Cold acclimatization.	Lång & Palva 1992
8	*RD29A*	AT5G52310	Abiotic stress response.	Msanne et al. 2011
9	*RD29B*	AT5G52300	Abiotic stress response.	Msanne et al. 2011
10	*AtCOR47*	AT1G20440	Cold tolerance.	Ganguly et al. 2025
11	*ICS1*	AT1G74710	SA synthesis, immune defense.	Gawroński et al. 2013
12	*EDS5*	AT4G39030	Defense against viral pathogens.	Ishihara et al. 2008
13	*PAL1*	AT2G37040	Defense against pathogens.	Kavil et al. 2021
14	*NPRI*	AT1G64280	Controls systemic acquired resistance (SAR).	Cao et al. 1997
15	*NIMIN1*	AT1G02450	Modulate the plant's defense response.	Weigel et al. 2005
16	*DIR1*	AT5G48485	Long-distance signaling of systemic acquired resistance.	Champigny et al. 2013
17	*PR1*	AT2G14610	Provide resistance against biotic and abiotic stresses.	Han et al. 2023
18	*PR2*	AT3G57260	Plant Defense Signaling.	Oide et al. 2013
19	*OPR1*	AT1G76680	Jasmonic Acid (JA) biosynthesis.	You et al. 2025
20	*AOC1*	AT3G25760	Ethylene biosynthesis.	Poulaki et al 2020

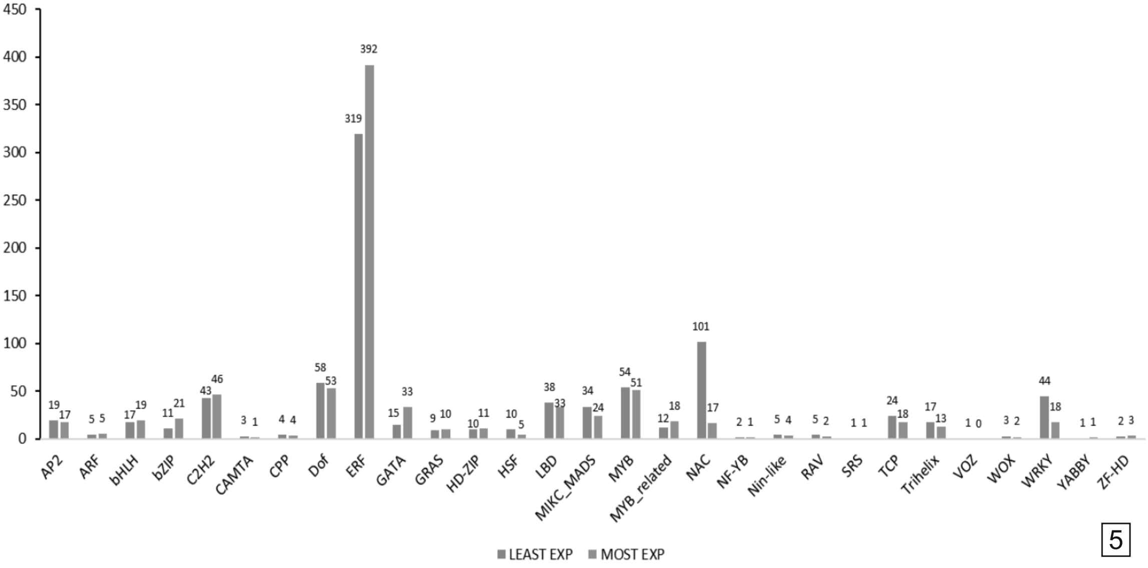
for VOZ. Putative binding sites for ERF were 4.5 times more than NAC, the second most frequent family. Among the 20 *Arabidopsis* GS genes, while promoter of *AGP11* had the highest number of 1926 putative binding sites, *AtVEX1* had highest diversity of 40 TF families (Fig. 3). The diversity of former being 36, and number of latter being 1094. Thus, highest number was not a representation of high diversity of sites.

Developmental/tissue-specific expression levels of the 20 GS genes were compared (Fig. 4). As majority of 29 out of 43 TF families had stress-

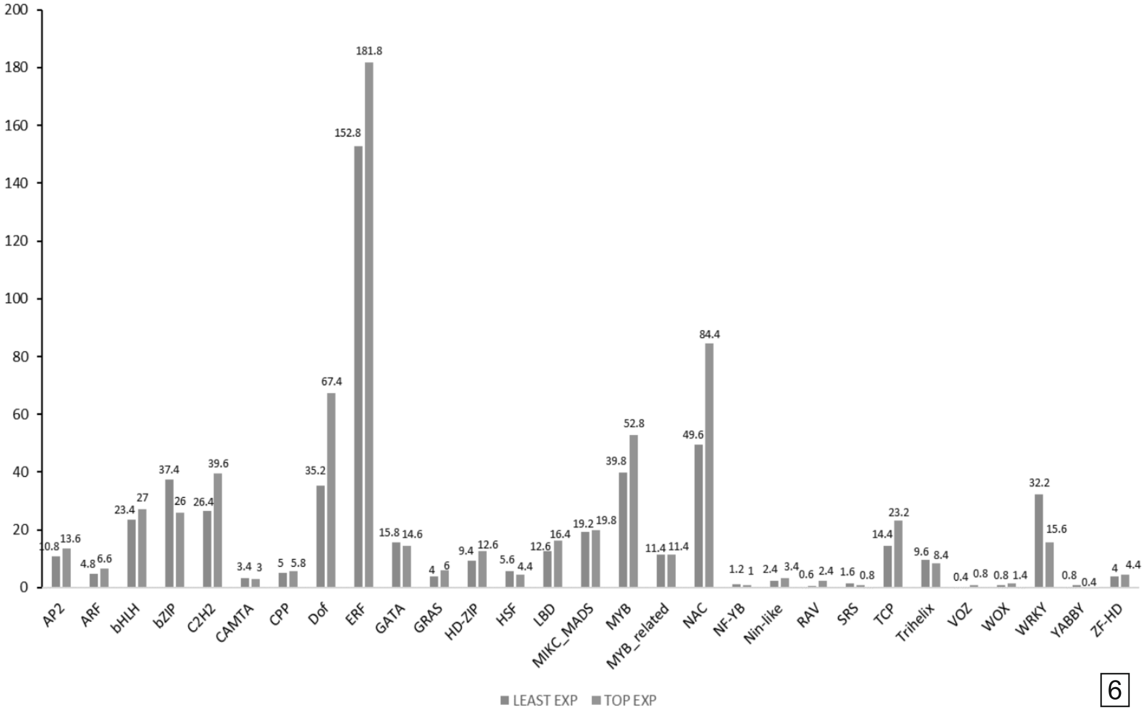
TABLE 5: Fold change in frequencies of putative binding sites of stress-responsive TFs on stress-responsive genes

TF family	Frequency of TFBSs on least expressed genes (A)	Frequency of TFBSs on most expressed genes (B)	Fold change (A/B)
AP2	10.8	13.6	0.79
ARF	4.8	6.6	0.73
bHLH	23.4	27	0.87
bZIP	37.4	26	1.44
C2H2	26.4	39.6	0.67
CAMTA	3.4	3	1.13
CPP	5	5.8	0.86
Dof	35.2	67.4	0.52
ERF	152.8	181.8	0.84
GATA	15.8	14.6	1.08
GRAS	4	6	0.67
HD-ZIP	9.4	12.6	0.75
HSF	5.6	4.4	1.27
LBD	12.6	16.4	0.77
	19.2	19.8	0.97
MYB	39.8	52.8	0.75
MYB_related	11.4	11.4	1.00
NAC	49.6	84.4	0.59
NF-YB	1.2	1	1.20
Nin-like	2.4	3.4	0.71
RAV	0.6	2.4	0.25
SRS	1.6	0.8	2.00
TCP	14.4	23.2	0.62
Trihelix	9.6	8.4	1.14
VOZ	0.4	0.8	0.50
WOX	0.8	1.4	0.57
WRKY	32.2	15.6	2.06
YABBY	0.8	0.4	2.00
ZF-HD	4	4.4	0.91

Shaded boxes indicate <1 fold change.



5



6

Figs 5 & 6: Frequency of putative TF binding sites of TF families with stress-responsive function in least expressed and most expressed 5. gametophyte-specific genes 6. stress-responsive genes responsive functions, the expression levels under various biotic and abiotic stress conditions were also considered for the study (Fig. 4). It was observed that most genes expressed more in the male gametophyte category under developmental/ tissue-specific function. Five genes, *ATAGP24, AGP23, AtVEX1, TAG1* and *TUA1* had more expression under biotic and abiotic stress conditions, especially abiotic. While *ATAGP24* and *AtVEX1* showed expression in roots, *AGP23* expression was more in the shoot under abiotic stress. *TAPNAC* was exceptional because, though being a GS gene, it had the highest expression under biotic stress (virulent *P.s*).

Since the GS gene promoters carried numerous putative stress-responsive TFBSs and also showed high expression under biotic/abiotic stresses, it could be possible that there was some correlation between TF site density and transcription levels. To check this hypothesis, the frequencies of putative binding sites of 29 stress-responsive TFs were compared based on the expression levels of the GS genes under biotic/abiotic stresses. For this, the GS genes were grouped into 2, least and most expressing under biotic and abiotic stresses. Then, the putative binding sites of stress-responsive TFs on these were compared (Table 4). Ratio of these sites from the least and most expressing groups were then calculated and compared. Ratios with >1 value indicate that these TFs have more putative sites on least expressing genes. This calculation indicated that a majority 18 of 29 sites were present more on least expressing genes (Fig. 5), suggesting an inverse correlation between the gene expression under biotic and abiotic stresses and the number of putative binding sites of biotic and abiotic stress-responsive TFs.

To see if this was the situation with other stress-responsive genes, similar analysis was conducted on the previously known 20 stress-responsive genes (Table 4). Expression levels of the genes were retrieved and sorted on the basis of their sum. Frequency of putative TFBSs in the most and least expressed genes from the stress-responsive genes group were compared and fold changes calculated (Table 3). The ratios here revealed that only 10 of 29 sites were present more on least expressing genes (Fig. 6). Hence, unlike the GS genes, a direct correlation between the gene expression under biotic/abiotic stresses and the number of putative binding sites of biotic/abiotic stress-responsive TFs was seen in other stress-responsive genes. This comparison of GS genes with other stress-responsive genes reveals an opposite pattern between the 2 groups.

DISCUSSION

ERF and NAC TF families were the most frequently found putative TFBSs in the GS genes of *A. thaliana*, with ERF having 4.5-fold greater numbers than NAC. ERF TFs play crucial roles in regulating plant responses to both biotic and abiotic stresses. Overexpression of genes such as *Pti4*, *ERF1*, *OPBP1* and *TSRF1* which come under ERF family, has been reported to significantly enhance resistance against fungal and bacterial pathogens, highlighting their importance in improving plant defense mechanisms (Berrocal-Lobo et al. 2002, Gu et al. 2002, Guo et al. 2004, Oñate-Sánchez & Singh. 2002, Zhang et al. 2004). Similarly, members of the NAC TF family are involved in abiotic stress tolerance. Genes such as, *OsNAC2/6*, *OsNAC10* and *SNAC1* have been shown to improve drought and salt-stress-tolerance in plants, while *ANAC092* is known to regulate complex gene networks associated with salt-induced senescence (Hu et al. 2006, Nakashima et al. 2009, Jeong et al. 2010, Balazadeh et al. 2010). While *AGP11* had the highest number of putative TFBSs, these were most diverse in *AtVEX1*. *ATVEX1*, also referred to as *AtSWEET5*, encodes a Nodulin MtN3 family protein belonging to the *SWEET* family of sugar transporters. These transporters play vital roles in carbohydrate transport and overall plant development (Engel et al. 2005). When the functions of TF families were studied, 29 TFs among 43 were stress-responsive. For checking the correlation between putative TF binding sites and expression in *Arabidopsis,* expression level values under 3 sets of conditions were studied. It showed that 5 of 20 genes had more expression in biotic and abiotic stress conditions (*ATAGP24, TAG1, TUA1, AGP23* and *AtVEX1*). *AtAGP24* exhibited enhanced expression in the roots of *A. thaliana* following infection by the necrotrophic fungal pathogen, *Plectosphaerella cucumerina*. Localization of the AtAGP24-GFP fusion protein near the plasma membrane and increase in pathogen susceptibility by its over-expression suggest that *AtAGP24* functions as a disease susceptibility factor during host–pathogen interactions (Dobon et al. 2015). Similarly, *TAG1* which is involved in triacylglycerol biosynthesis, was reported to play an important role in abiotic stress responses. Mutants deficient in *TAG1* exhibited increased sensitivity to abscisic acid, sugars, and osmotic stresses during germination and early seedling development, (Lu & Hills 2002). *AtVEX1* also contributes to stress responses, particularly under biotic stress. Several *AtSWEET* genes, including *AtSWEET5* were induced upon *P. syringae* infection, facilitating sugar efflux and pathogen nutrition (Chen et al. 2010). *AGP23* encodes an arabinogalactan protein and plays a crucial role in many plant processes, including growth, development and stress response, by influencing the structure and integrity of the cell wall (Pereira et al. 2014). In contrast, *TUA1* encoding alpha-tubulin 1, is predominantly expressed in pollen and does not show major involvement in stress responses (Carpenter et al. 1992). But there was no valid pattern in their putative TFs. Similarly, the frequencies of TFBSs for genes that had higher expression in different conditions were also studied, but no valid data were obtained. We hypothesized that the genes with higher expression would have more TFBSs. When 20 GS genes were compared between those showing the most and least expression, it was found to have more TFBSs for genes that showed lesser expression. To check the condition in stress-responsive genes, we did the same with a new set of stress-responsive genes. But an opposite relation was seen, where the genes with more expression have more TFs, which supports our hypothesis. This contradiction opens a new window for further such studies in other groups of genes responsible for different pathways, which will help to identify the relation between TFBSs and the expression of genes. Since no previous studies have been reported on this topic, the findings of the current work provide new insights into this area of research.

ACKNOWLEDGEMENT

Authors wish to express sincere gratitude to the Department of Plant Science, Central University of Kerala, for giving an opportunity to complete this work.

Declaration

Authors have no conflict of interest

REFERENCES

ARIEL F D, MANAVELLA P A, DEZAR C A & CHAN R L 2007 The true story of the HD-Zip family *Trends Plant Sci* **12** 419–426

ALVARADO V Y, TAG A & THOMAS T L 2011 A *cis* regulatory element in the *TAPNAC* promoter directs tapetal gene expression *Plant Mol Biol* **75** 129–139

ATKINSON N J, LILLEY C J & URWIN P E 2013 Identification of genes involved in the response of *Arabidopsis* to simultaneous biotic and abiotic stresses *Plant Physiol* **162** 2028–2041

BALAZADEH S, W U A & MUELLER-ROEBER B 2010 Salt-triggered expression of the ANAC092-dependent senescence regulon in *Arabidopsis thaliana Plant Signal Behav* **5** 733–735

BERROCAL-LOBO M, MOLINA A & SOLANO R 2002 Constitutive expression of *ETHYLENE-RESPONSE-FACTOR1* in *Arabidopsis* confers resistance to several necrotrophic fungi *Plant J* **29** 23–32

BHATTACHARJEE B & HALLAN V 2023 NF-YB family transcription factors in *Arabidopsis* structure phylogeny and expression analysis in biotic and abiotic stresses *Front Microbiol* **13** 1067427 doi:10.3389/fmicb.2022.1067427

BROWNFIELD L, HAFIDH S, DURBARRY A, KHATAB H, SIDOROVA A, DOERNER P & TWELL D 2009 *Arabidopsis* DUO POLLEN3 is a key regulator of male germline development and embryogenesis *Plant Cell* **21** 1940–1956

CAO H, GLAZEBROOK J, CLARKE J D, VOLKO S & DONG X 1997 The *Arabidopsis NPR1* gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats *Cell* **88** 57–63

CARPENTER J L, PLOENSE S E, SNUSTAD D P & SILFLOW C D 1992 Preferential expression of an alpha-tubulin gene of *Arabidopsis* in pollen *Plant Cell* **4** 557–571

CHAMPIGNY M J, ISAACS M, CARELLA P, FAUBERT J, FOBERT P R & CAMERON R K 2013 Long-distance movement of DIR1 and investigation of the role of DIR1-like during systemic acquired resistance in *Arabidopsis Front Plant Sci* **4** 230 doi: 10.3389/fpls.2013.00230

CHEN F, HU Y, VANNOZZI A, WU K, CAI H, QIN Y, MULLIS A, LIN Z & ZHANG L 2017 The WRKY transcription factor family in model plants and crops *Crit Rev Plant Sci* **36** 311–335

CHEN L Q, HOU B H, LALONDE S, TAKANAGA H, HARTUNG M L, QU X Q, GUO W J, KIM J G, UNDERWOOD W, CHAUDHURI B & CHERMAK D 2010 Sugar transporters for intercellular exchange and nutrition of pathogens *Nature* **468** 527–532

CHEVALIER F, PERAZZA D, LAPORTE F, LE HÉNANFF G, HORNITSCHEK P, BONNEVILLE J M, HERZOG M & VACHON G 2008 GeBP and GeBP-like proteins are noncanonical leucine-zipper transcription factors that regulate cytokinin response in *Arabidopsis Plant Physiol* **146** 1142–1154

COIMBRA S, COSTA M, JONES B, MENDES M A & PEREIRA L G 2009 Pollen grain development is compromised in *Arabidopsis agp6 agp11* null mutants *J*

Exp Bot **60** 3133–3142

DAI Y, WANG Y, ZENG L, JIA R, HE L, HUANG X, ZHAO H, LIU D, ZHAO H, HU S & GAO L 2023 Genomic and transcriptomic insights into the evolution and divergence of MIKC-type MADS-box genes in *Carica papaya Int J Mol Sci* **24** 14039 doi:10.3390/ijms241814039

DAUDI A, CHENG Z, O'BRIEN J A, MAMMARELLA N, KHAN S, AUSUBEL F M & BOLWELL G P 2012 The apoplastic oxidative burst peroxidase in *Arabidopsis* is a major component of pattern-triggered immunity *Plant Cell* **24** 275–287

DOBON A, CANET J V, GARCIA-ANDRADE J, ANGULO C, NEUMETZLER L, PERSSON S & VERA P 2015 Novel disease susceptibility factors for fungal necrotrophic pathogens in *Arabidopsis PLoS Pathog* **11** 1004800 doi: 10.1371/journal.ppat.1004800

DUBOS C, STRACKE R, GROTEWOLD E, WEISSHAAR B, MARTIN C & LEPINIEC L 2010 MYB transcription factors in *Arabidopsis Trends Plant Sci* **15** 573–581

ENGEL M L, HOLMES-DAVIS R & MCCORMICK S 2005 Green sperm Identification of male gamete promoters in *Arabidopsis Plant Physiol* **138** 2124–2133

FANG Y, YOU J, XIE K, XIE W & XIONG L 2008 Systematic sequence analysis and identification of tissue-specific or stress-responsive genes of NAC transcription factor family in rice *Mol Genet Genomics* **280** 547–563

FINKLER A, ASHERY-PADAN R & FROMM H 2007 CAMTAs calmodulin-binding transcription activators from plants to human *FEBS Lett* **581** 3893–3898

FITTER D W, MARTIN D J, COPLEY M J, SCOTLAND R W & LANGDALE J A 2002 *GLK* gene pairs regulate chloroplast development in diverse plant species *Plant J* **31** 713–727

FORSTHOEFEL N R, KLAG K A, MCNICHOL S R, ARNOLD C E, VERNON C R, WOOD W W & VERNON D M 2018 *Arabidopsis PIRL6* is essential for male and female gametogenesis and is regulated by alternative splicing *Plant Physiol* **178** 1154–1169

GALLI M, THERIAULT A, LIU D & CRAWFORD N M 2003 Expression of the *Arabidopsis* transposable element *Tag1* is targeted to developing gametophytes *Genetics* **165** 2093–2105

GANGULY D R, LI Y, BHAT S S, TIWARI S, NG P J, GREGORY B D & SUNKAR R 2025 mRNA adenosine methylase promotes drought tolerance through N6-methyladenosine-dependent and independent impacts on mRNA regulation in *Arabidopsis New Phytol* **245** 183–199

GAWROŃSKI P, GÓRECKA M, BEDERSKA M, RUSACZONEK A, ŚLESAK I, KRUK J & KARPIŃSKI S 2013 Isochorismate synthase 1 is required for thylakoid organization, optimal plastoquinone redox status, and state transitions in *Arabidopsis thaliana J Exp Bot* **64** 3669–3679

GU Y Q, WILDERMUTH M C, CHAKRAVARTHY S, LOH Y T, YANG C, HE X, HAN Y & MARTIN G B 2002 Tomato transcription factors Pti4 Pti5 and Pti6 activate defense responses when expressed in *Arabidopsis Plant Cell* **14** 817–831

GUAN Y F, HUANG X Y, ZHU J, GAO J F, ZHANG H X & YANG Z N 2008 *RUPTURED POLLEN GRAIN1* a member of the MtN3/saliva gene family is crucial for exine pattern formation and cell integrity of microspores in *Arabidopsis Plant Physiol* **147** 852–863

GUO A Y, ZHU Q H, GU X, GE S, YANG J & LUO J 2008 Genome-wide identification and evolutionary analysis of the plant specific SBP-box transcription factor family *Gene* **418** 1–8

GUO Z J, CHEN X J, WU X L, LING J Q & XU P 2004 Overexpression of the AP2/EREBP transcription factor OPBP1 enhances disease resistance and salt tolerance in tobacco *Plant Mol Biol* **55** 607–618

GUPTA R, TING J T, SOKOLOV L N, JOHNSON S A & LUAN S 2002 A tumor suppressor homolog AtPTEN1 is essential for pollen development in *Arabidopsis Plant Cell* **14** 2495–2507

HAN G, LU C, GUO J, QIAO Z, SUI N, QIU N & WANG B 2020 C2H2 zinc finger proteins master regulators of abiotic stress responses in plants *Front Plant Sci* **11** 115 doi: 10.3389/fpls.2020.00115

HAN Z, XIONG D, SCHNEITER R & TIAN C 2023 The function of plant PR1 and other members of the CAP protein superfamily in plant–pathogen interactions *Mol Plant Pathol* **24** 651–668

HIRSCH S, KIM J, MUNOZ A, HECKMANN A B, DOWNIE J A & OLDROYD G E 2009 GRAS proteins form a DNA binding complex to induce gene expression during nodulation signaling in *Medicago truncatula Plant Cell* **21** 545–557

HONYS D, OH S A, RENAK D, DONDERS M, SOLCOVA B, JOHNSON J A & TWELL D 2006 Identification of microspore-active promoters that allow targeted manipulation of gene expression at early stages of microgametogenesis in *Arabidopsis BMC Plant Biol* **6** 1–9

HOSSAIN M F, SULTANA M M, TANAKA A, DUTTA A K, HACHIYA T & NAKAGAWA T 2022 Expression analysis of plant intracellular Ras-group related leucine-rich repeat proteins PIRLs in *Arabidopsis thaliana Biochem Biophys Rep* **30** 101241 101241 doi: 10.1016/j.bbrep.2022.101241

HSIN K T, YANG T J, LEE Y H & CHENG Y S 2021 Phylogenetic and structural analysis of NIN-like proteins with a type I II PB1 domain that regulates oligomerization for nitrate response *Front Plant Sci* **12** 672035 doi: 10.3389/fpls.2021.672035

HU H, DAI M, YAO J, XIAO B, LI X, ZHANG Q & XIONG L 2006 Overexpressing a NAM ATAF and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice *Proc Natl Acad Sci USA* **103** 12987–12992

ISHIHARA T, SEKINE K T, HASE S, KANAYAMA Y, SEO S, OHASHI Y, KUSANO T, SHIBATA D, SHAH J & TAKAHASHI H 2008 Overexpression of the *Arabidopsis thaliana EDS5* gene enhances resistance to viruses *Plant Biol* **10** 451–461

JEONG J S, KIM Y S, BAEK K H, JUNG H, HA S H, DO CHOI Y, KIM M, REUZEAU C & KIM J K 2010 Root-specific expression of OsNAC10 improves drought tolerance and grain yield in rice under field drought conditions *Plant Physiol* **153** 185–197

JIA Q S, ZHU J, XU X F, LOU Y, ZHANG Z L, ZHANG Z P & YANG Z N 2015 *Arabidopsis* AT-hook protein TEK positively regulates the expression of arabinogalactan proteins for nexine formation *Mol Plant* **8** 251–260

KAVIL S, OTTI G, BOUVAINE S, ARMITAGE A & MARUTHI M N 2021 *PAL1* gene of the phenylpropanoid pathway increases resistance to the Cassava brown streak virus in cassava *Virol J* **18** 184 doi: 1186/s12985-021-01649-2

KIM J H, CHOI D & KENDE H 2003 The AtGRF family of putative transcription factors is involved in leaf and cotyledon growth in *Arabidopsis Plant J* **36** 94–105

KOTAK S, PORT M, GANGULI A, BICKER F & VON KOSKULL-DORING P 2004 Characterization of C-terminal domains of *Arabidopsis* heat stress transcription factors Hsfs and identification of a new signature combination *Plant J* **39** 98–112

LÅNG V & PALVA E T 1992 The expression of a rab-related gene *rab18* is induced by abscisic acid during the cold acclimation process of *Arabidopsis thaliana* (L) Heynh *Plant Mol Biol* **20** 951–962

LEVITIN B, RICHTER D, MARKOVICH I & ZIK M 2008 Arabinogalactan proteins 6 and 11 are required for stamen and pollen function in *Arabidopsis Plant J* **56** 351–363

LI J, YU M, GENG L L & ZHAO J 2010 The fasciclin-like arabinogalactan protein gene *FLA3* is involved in microspore development of *Arabidopsis Plant J* **64** 482–497

LI Z & THOMAS T L 1998 PEI1 an embryo-specific zinc finger protein gene required for heart-stage embryo formation in *Arabidopsis Plant Cell* **10** 383–398

LIJAVETZKY D, CARBONERO P & VICENTE-CARBAJOSA J 2003 Genome-wide comparative phylogenetic analysis of the rice and *Arabidopsis Dof* gene families *BMC Evol Biol* **3** 1–11

LIN R, DING L, CASOLA C, RIPOLL D R, FESCHOTTE C & WANG H 2007 Transposase-derived transcription factors regulate light signaling in *Arabidopsis Science* **318** 1302–1305

LU C & HILLS M J 2002 *Arabidopsis* mutants deficient in diacylglycerol acyltransferase display increased sensitivity to abscisic acid, sugars, and osmotic stress during germination and seedling development *Plant Physiol* **129** 1352–1358

LU W, WANG Y, SHI Y, LIANG Q, LU X, SU D, XU X, PIRRELLO J, GAO Y, HUANG B & LI Z 2023 Identification of SRS transcription factor family in *Solanum lycopersicum* and functional characterization of responses to hormones and abiotic stresses *BMC Plant Biol* **23** 495 doi: 10.1186/s12870-023-04506-2

MANFRE A J, LANNI L M & MARCOTTE W R JR 2006 The *Arabidopsis* group 1 late embryogenesis abundant protein ATEM6 is required for normal seed development *Plant Physiol* **140** 140–14

MARTIN-TRILLO M & CUBAS P 2010 TCP genes a family snapshot ten years later *Trends Plant Sci* **15** 31–39

MOREIRA D, LOPES A L, SILVA J, FERREIRA M J, PINTO S C, MENDES S & PEREIRA A M 2022 New insights on the expression patterns of specific arabinogalactan proteins in reproductive tissues of *Arabidopsis thaliana Front Plant Sci* **13** 1083098 doi: 10.3389/fpls.2022.1083098

MSANNE J, LIN J, STONE JM & AWADA T 2011 Characterization of abiotic stress-responsive *Arabidopsis thaliana RD29A* and *RD29B* genes and evaluation of transgenes *Planta* **234** 97–107

NAIN V, VERMA A, KUMAR N, SHARMA P, RAMESH B & KUMAR P A 2008 Cloning of an ovule specific promoter from *Arabidopsis thaliana* and expression of beta-glucuronidase *Indian J Exp Biol* **46** 207–211

NAKANO T, SUZUKI K, FUJIMURA T & SHINSHI H 2006 Genome-wide analysis of the ERF gene family in *Arabidopsis* and rice *Plant Physiol* **140** 411–432

NAKASHIMA K, ITO Y & YAMAGUCHI-SHINOZAKI K 2009 Transcriptional regulatory networks in response to abiotic stresses in *Arabidopsis* and grasses *Plant Physiol* **149** 88–95

NGUEMA-ONA E, COIMBRA S, VICRE-GIBOUIN M, MOLLET J C & DRIOUICH A 2012 Arabinogalactan proteins in root and pollen-tube cells distribution and functional aspects *Ann Bot* **110** 383–404

NONOMURA K I, MIYOSHI K, EIGUCHI M, SUZUKI T, MIYAO A, HIROCHIKA H & KURATA N 2003 The MSP1 gene is necessary to restrict the number of cells entering into male and female sporogenesis and to initiate anther wall formation in rice *Plant Cell* **15** 1728–1739

OIDE S, BEJAI S, STAAL J, GUAN N, KALIFF M & DIXELIUS C 2013 A novel role of PR2 in abscisic acid (ABA)-mediated pathogen-induced callose deposition in *Arabidopsis thaliana New Phytol* **200** 1187–1199

OKUSHIMA Y, OVERVOORDE P J, ARIMA K, ALONSO J M, CHAN A, CHANG C, ECKER J R, HUGHES B, LUI A, NGUYEN D & ONODERA C 2005 Functional genomic analysis of the *AUXIN RESPONSE FACTOR* gene family members in *Arabidopsis thaliana*: Unique and overlapping functions of ARF_7 and ARF_{19} *Plant Cell* **17** 444–463

OÑATE-SÁNCHEZ L & SINGH K B 2002 Identification of *Arabidopsis* ethylene-responsive element binding factors with distinct induction kinetics after pathogen infection *Plant Physiol* **128** 1313–132

PAILLARD G & LAVERY R 2004 Analyzing protein-DNA recognition mechanisms *Structure* **12** 113–122

PEREIRA A M, MASIERO S, NOBRE M S, COSTA M L, SOLÍS M T, TESTILLANO P S, SPRUNCK S & COIMBRA S 2014 Differential expression patterns of arabinogalactan proteins in *Arabidopsis thaliana* reproductive tissues *J Exp Bot* **65** 5459–5471

PNUELI L, HAREVEN D, ROUNSLEY S D, YANOFSKY M F & LIFSCHITZ E 1994 Isolation of the tomato AGAMOUS gene TAG1 and analysis of its homeotic role in transgenic plants *Plant Cell* **6** 163–173

POULAKI E G, TSOLAKIDOU M D, GKIZI D, PANTELIDES I S & TJAMOS S E 2020 The ethylene biosynthesis genes *ACS2* and *ACS6* modulate disease severity of *Verticillium dahliae Plants* **9** 907 doi: 10.3390/plants9070907

ROHS R, JIN X, WEST S M, JOSHI R, HONIG B & MANN R S 2010 Origins of specificity in protein-DNA recognition *Annu Rev Biochem* **79** 233–269

RONG M, GAO S X, WEN D, XU Y H & WEI J H 2024 The LOB domain protein a novel transcription factor with multiple functions a review *Plant Physiol Biochem* **214** 108922 doi: 10.1016/j.plaphy.2024.108922

SAFI A, MEDICI A, SZPONARSKI W, RUFFEL S, LACOMBE B & KROUK G 2017 The world according to GARP transcription factors *Curr Opin Plant Biol* **39** 159–167

SAHU A, SINGH R & VERMA P K 2023 Plant BBR BPC transcription factors unlocking multilayered regulation in development stress and immunity *Planta* **258** 31 doi: 10.1007/s00425-023-04188-y

SCHOLZ-STARKE J, BUTTNER M & SAUER N 2003 *AtSTP6* a new pollen-specific H+- monosaccharide symporter from *Arabidopsis Plant Physiol* **131** 70–77

SIEGFRIED K R, ESHED Y, BAUM S F, OTSUGA D, DREWS G N & BOWMAN J L 1999 Members of the *YABBY* gene family specify abaxial cell fate in *Arabidopsis Development* **126** 4117–4128

TAWAB F, MUNIR I, NASIM Z, KHAN M S, TAWAB S, NASIM A, IQBAL A, AHMAD M A, ALI W, MUNIR R & MUNIR M 2020 Identification and characterization of a novel multi-stress responsive gene in *Arabidopsis PLoS One* **15** e0244030 doi: 10.1371/journal.pone.0244030

TEAKLE G R, MANFIELD I W, GRAHAM J F & GILMARTIN P M 2002 *Arabidopsis thaliana* GATA factors organisation expression and DNA-binding characteristics *Plant Mol Biol* **50** 43–56

TRUERNIT E, SCHMID J, EPPLE P, ILLIG J & SAUER N 1996 The sink-specific and stress-regulated *Arabidopsis STP4* gene: enhanced expression of a gene encoding a monosaccharide transporter by wounding elicitors and pathogen challenge *Plant Cell* **8** 2169–2182

ULUISIK S, KIYAK A, KURT F & FILIZ E 2023 Genome-wide identification of the VOZ transcription factors in tomato *Solanum lycopersicum J Hortic Sci Biotechnol* **98** 468–482

VAN DEN HEUVEL S & DYSON N J 2008 Conserved functions of the pRB and E2F families *Nat Rev Mol Cell Biol* **9** 713–724

VAN DER GRAAFF E, LAUX T & RENSING S A 2009 The WUS homeobox-containing WOX protein family *Genome Biol* **10** 1–9

VON BESSER K, FRANK A C, JOHNSON M A & PREUSS D 2006 *Arabidopsis HAP2 GCS1* is a sperm-specific gene required for pollen tube guidance and fertilization *Development* **133** 4761–4769

WANG J, YU Y C, LI Y & CHEN L Q 2022 Hexose transporter SWEET5 confers galactose sensitivity to *Arabidopsis* pollen germination via a galactokinase *Plant Physiol* **189** 388–401

WANG S, ZHANG Q, SHEN Z, HE Y, CHEN Z H, LI J & HUANG D S 2021 Predicting transcription factor binding sites using DNA shape features based on shared hybrid deep learning architecture *Mol Ther Nucleic Acids* **24** 154–163

WANG Y & LIU A 2020 Genomic characterization and expression analysis of basic helix-loop-helix bHLH family genes in *Dendrobium officinale Plants* **9** 1044 doi: 10.3390/plants9081044

WANG Y, ZHANG Y, ZHOU R, DOSSA K, YU J, LI D, LIU A, MMADI M A, ZHANG X & YOU J 2018

Identification and characterization of the bZIP transcription factor family and its expression in response to abiotic stresses in sesame *PLoS One* **13** e0200850doi: 10.1371/journal.pone.0200850

WEIGEL R R, PFITZNER U M & GATZ C 2005 Interaction of NIMIN1 with NPR1 modulates *PR* gene expression in *Arabidopsis Plant Cell* **17** 1279–1291

WHITEHEAD A & CRAWFORD D L 2005 Variation in tissue-specific gene expression among natural populations *Genome Biol* **6** 1–14

WILLIAM D A, SU Y, SMITH M R, LU M, BALDWIN D A & WAGNER D 2004 Genomic identification of direct target genes of LEAFY *Proc Natl Acad Sci USA* **101** 1775–1780

WINDHOVEL A, HEIN I, DABROWA R & STOCKHAUS J 2001 Characterization of a novel class of plant homeodomain proteins that bind to the C4 phosphoenolpyruvate carboxylase gene of *Flaveria trinervia Plant Mol Biol* **45** 201–214

WRAY G A, HAHN M W, ABOUHEIF E, BALHOFF J P, PIZER M, ROCKMAN M V & ROMANO L A 2003 The evolution of transcriptional regulation in eukaryotes *Mol Biol Evol* **20** 1377–1419

XIA F, SUN T, YANG S, WANG X, CHAO J, LI X, HU J, CUI M, LIU G, WANG D & SUN Y 2019 Insight into the B3Transcription factor superfamily and expression profiling of *B3* genes in axillary buds after topping in tobacco (*Nicotiana tabacum* L) *Genes* **10** 164 doi: 10.3390/genes10020164

XU H, DAVIES S P, KWAN B Y, OBRIEN A P, SINGH M & KNOX R B 1993 Haploid and diploid expression of a *Brassica campestris* anther-specific gene promoter in *Arabidopsis* and tobacco *Mol Gen Genet* **239** 58–65

XU H, KNOX R B, TAYLOR P E & SINGH M B 1995 *Bcp1* a gene required for male fertility in *Arabidopsis Proc Natl Acad Sci USA* **92** 2106–2110

XU H, SHI X, HE L, GUO Y, ZANG D, LI H, ZHANG W, WANG Y 2018 *Arabidopsis thaliana* trihelix transcription factor AST1 mediates salt and osmotic stress tolerance *Plant Cell Physiol* **59** 946–965

YAMASAKI K, KIGAWA T, INOUE M, YAMASAKI T, YABUKI T, AOKI M, SEKI E, MATSUDA T, TOMO Y, TERADA T & SHIROUZU M 2005 Solution structure of the major DNA-binding domain of *Arabidopsis thaliana* ethylene-insensitive3-like3 *J Mol Biol* **348** 253–264

YANG X, GUO T, LI J, CHEN Z, GUO B & AN X 2021 Genome-wide analysis of the MYB-related transcription factor family and associated responses to abiotic stressors in *Populus Int J Biol Macromol* **191** 359–376

YANG Z, GU S, WANG X, LI W, TANG Z & XU C 2008 Molecular evolution of the *CPP-like* gene family in plants insights from comparative genomics of *Arabidopsis* and rice *J Mol Evol* **67** 266–277

YIN Y, VAFEADOS D, TAO Y, YOSHIDA S, ASAMI T & CHORY J 2005 A new class of transcription factors mediates brassinosteroid-regulated gene expression in *Arabidopsis Cell* **120** 249–259

YOU S H, CHEN Y P, SHI W J, LI X, WU Z & YAO Q H 2025 Genome-wide analysis of *OPR* family genes in *Vitis vinifera* and the role of *VvOPR1* in copper zinc tolerance *Front Plant Sci* **16** 1509472 doi: 10.3389/ fpls. 2025.1509472

ZHANG H, ZHANG D, CHEN J, YANG Y, HUANG Z, HUANG D, WANG X C & HUANG R 2004 Tomato stress-responsive factor TSRF1 interacts with ethylene responsive element GCC box and regulates pathogen resistance to *Ralstonia solanacearum Plant Mol Biol* **55** 825–834

ZHOU D X, BISANZ-SEYER C & MACHE R 1995 Molecular cloning of a small DNA binding protein with specificity for a tissue-specific negative element within the *rps1* promoter *Nucleic Acids Res* **23** 1165–1169

Journal of Cytology and Genetics 2025 VOL. **26** (NS): 115–132

REVIEW ARTICLE

DNA METHYLATIONAIDING RESILIENCE AND TRANSGENERATIONAL INHERITANCE IN PLANTS**

A.T. MEHARBAN AND JASMINE M. SHAH*
Department of Plant Science, Central University of Kerala, Periye, Kasargod 671 320, India
*For correspondence. Email: jasmine@cukerala.ac.in

(Received 22 October 2025, revised accepted 12 November 2025)

SUMMARY In the plant genome, DNA methylation is an epigenetic modification that involves transferring a methyl group to the fifth position of the cytosine, resulting in the formation of 5-methylcytosine. DNA methylation can regulate gene expression by recruiting proteins that activate gene transcription or by inhibiting the binding of transcription factors, leading to gene silencing. During plant development or in response to biotic and abiotic stress conditions, the DNA methylation pattern in the genome changes due to either de novo DNA methylation or demethylation. These changes in DNA methylation enable the plant to respond to and survive changing external or internal conditions, ultimately developing a stable, unique DNA methylation pattern that regulates specific gene transcription. In this paper, we review the processes of DNA methylation and demethylation in plants, describe the machinery involved in these processes, and discuss the role of DNA methylation in response to biotic and abiotic stress conditions. Furthermore, these epigenetic changes, which regulate gene expression patterns and plant growth, are heritable. Threfore, the plant's epigenetic response is significant; epigenetic editing can improve plant genome efficiency by targeting and editing genome-specific loci. Here, we summarize the development of epigenome editing and its future prospects, including precision breeding, climate-resilient agriculture, and the integration of methylation data into predictive models for sustainable crop improvement. We look forward to the future development of this system.

Keywords: Epigenetics, DNA methylation, RdDm pathway, inheritance, epigenome editing, crop improvement.

INTRODUCTION

The epigenetic study deals with changes in gene expression due to non-genetic influences that do not alter the DNA sequence. Plants undergo various epigenetic modifications that alter gene expression during normal development or in response to environmental influences, which sometimes are transmissible to subsequent generations as well. these modifications can activate or repress genes in response to environmental stimuli. This aids the

---

** Paper presented at the XV All India Conference on Cytology and Genetics held on 11th and 12th September 2025.

plants in adapting to the conditions and managing biotic and abiotic stressors such as, pathogen attack, drought, heat, or salinity (Kinoshita & Seki 2014). These stressors can bring numerous changes to plants, resulting the gene regulation (upregulation/downregulation) of specific genes and alters the cell function by epigenetic mechanisms (Doddavarapu et al. 2024).

Epigenetics is broadly divided into 2 categories: (1) Regulation of selective gene transcription, which includes DNA methylation, histone modification, and chromatin remodeling, and (2) post-transcriptional gene regulation, which includes regulation by non-coding RNAs, RNA modification, and nucleosome positioning (Willbanks et al. 2016). So far, the DNA methylation/demethylation is a major epigenetic modification. Moreover, the epigenetic regulation by DNA methylation is well known for its heritable capacity in eukaryotes. The DNA methylation improves plant growth and development. During unfavorable conditions, DNA methylation in plants helps them cope with the situation, but it also renders the plants more susceptible. The DNA methylation process reversible and sensitive to each environmental stimulus and also the same stressor can induce different epigenetic changes in individuals or populations. Furthermore, the DNA methylation and other epigenetic modifications cross-talk with other signaling pathways, such as hormonal responses, like abscisic acid during water stress, and such interactions synchronize the stress response and improve plant survival in unfavorable conditions (Lucibelli et al. 2022). Additionally, these types of modifications can install a type of "memory" within the plant. For example, when a plant is exposed to a stressor, specific epigenetic modifications happen on the DNA that can change the expression of genes responsive to stress. The next generation can inherit these epigenetic modifications in their genome, providing an advantage for adaptation. Moreover, it allows the plants to respond and adapt to future events of the same stress conditions (Hemenway & Gehrig 2023).

Epigenetics and gene regulation are now emerging as a potential technology. The trans-generational memory of DNA methylation can contribute to the development of biotic and abiotic stress tolerance in crops, making it a potential technology for creating biotic and abiotic stress-resilient crops. It can be utilized for the targeted epigenomic changes (Doddavarapu et al. 2024). Moreover, the development of epigenetic genome editing technology provides technical support for epigenetic genetic improvement of biotic and abiotic stress resistance. Therefore, in this review we have summarized the DNA methylation and demethylation mechanisms which include the RdDm pathway and DNA methylation maintenance across the genome and to the next generations and discussed the emerging epigenome editing technology that are being developed to find the potential epigenome candidates across the genome in order to produce stress-resilient plants.

DNA methylation and demethylation: Cytosine methylation of DNA is a common epigenetic mark.

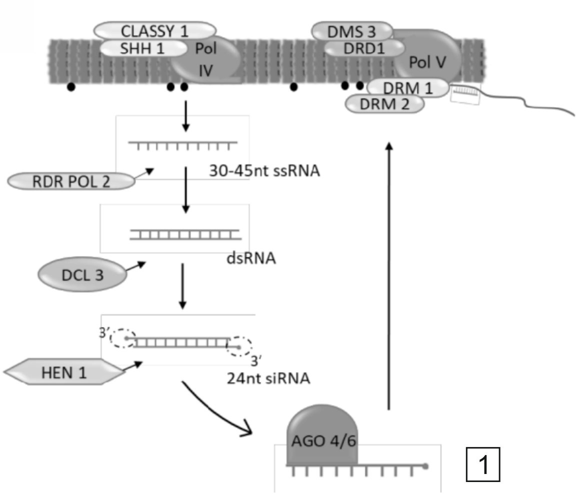
The extent of DNA methylation regulates plant gene expression on a broad scale. DNA methylation reforms the chromatin structure, suppressing gene transcription by preventing certain transcriptional factors from binding to DNA. However, some specific group of transcription factors shows an enhanced binding affinity for methylated motifs, so that promoter hypermethylation in those cases can stimulate transcription (Willbanks et al. 2016, Hagmann et al. 2015). The DNA methyltransferases (MTases) and DNA demethylases control methylation and demethylation, which keep the DNA methylation system in balance (Agius et al. 2023). The methylation in plants occurs at CG, CHG, and CHH regions, where H can be any nucleotide except G. The simultaneous presence of CG and non-CG methylation usually occurs in transposable elements (TEs) and other sequences derived from or related to repeats (Hemenway & Gehring 2023, Stroud et al. 2014). In plants, cytosine-specific DNA MTases are responsible for methylation, which adds a methyl group to the fifth carbon of cytosine, There are 2 classes of DNA methyltransferases: 1) The de novo class or enzymes that create new methylation marks on the DNA (DRM 1/ DRM 2) A maintenance class that recognizes the methylation marks on the parental strand of DNA and transfers new methylation to the daughter strands after DNA replication (MET 1/CMT 2/ CMT 3) (Cao & Jacobsen 2002). Furthermore, in plants, the TEs and repetitive sequences undergo significant cytosine methylation across all three contexts, and they are abundant in heterochromatic regions (Agius et al. 2023, Ashapkin et al. 2020). Cytosine methylation causes transcriptional silencing of TEs. The spread of cytosine methylation from gene-adjacent TEs to the gene can methylate cytosine residues in transcription factor binding motifs, which makes the TFs less likely to bind. Additionally, epigenetic changes resulting from DNA methylation enhance heterosis in genetically identical chromosomes across generations in *Oryza sativa* (Hagmann et al. 2015, Ashapkin et al. 2020).

RdDm pathway: The RNA-directed DNA methylation pathway (RdDm) is the only pathway that can add a methyl group to cytosine regardless of any sequence context and the other context-specific pathways can only maintain existing DNA methylation patterns in the genome (Matzke & Mosher, 2014). In *Arabidopsis,* RNA-directed DNA methylation could occur through canonical and non-canonical pathways. In this process, DNA-dependent RNA Polymerase IV (Pol IV) interacts with the Chromatin remodeler classy 1 (CLSY1) and its partner Sawadee homeodomain homolog 1 (SHH1). This complex binds to heterochromatin, where RNA Pol IV generates short single-strand RNAs (ssRNAs) of about 30–45 nucleotides. These ssRNAs are then converted into double-stranded (dsRNAs) by RNA-dependent RNA polymerase 2 (RDR2) and RNA Pol IV (Erdmann & Picard 2020). The endoribonuclease Dicer-like 3 (DCL3) processes the dsRNAs into 24-nucleotide small RNAs (siRNAs). The siRNAs are then methylated on their 3' ends by *HEN1*. The Argonaute proteins

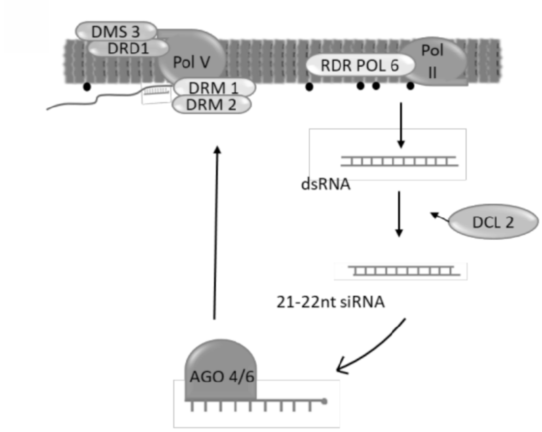
(AGO4, AGO6) guide the single strands of these siRNAs to their complementary RNAs transcribed by the plant-specific RNA Polymerase V, which recruits the methyltransferase Domain rearranged methyltransferases (DRM 1) to methylate the adjacent DNA. Pol V probably produces non-coding transcripts from siRNA-targeting regions or intergenic areas adjacent to these regions, with the help of other established RdDm components, such as Defective in RNA-directed DNA methylation 1 (DRD1) and Defective in meristem silencing 3 (DMS3) (Willmann et al. 2011, Agius et al. 2023, Chan et al. 2004) (Fig. 1).

The non-canonical pathway encompasses various modifications of the canonical pathway, typically leading to de novo DNA methylation, such as the methylation of TEs. In this pathway, the production of siRNAs is not solely reliant on Pol IV, RDR2, and *DCL3*; instead, siRNAs can also be generated through other mechanisms, including transcription by RNA Pol II, and the transcripts of RNA Pol II can be amplified by RNA-dependent RNA polymerase 6 (RDR6). The endoribonuclease DICER-LIKE 2 (DLC2) processes the siRNA into 21-22-nucleotide siRNAs. These siRNAs induce DNA methylation via DNA-dependent RNA Pol V and DNA methyltransferases Domain rearranged methyltransferases 2 (DRM2) (Cuerda-Gil & Slotkin 2016, Wendte & Pikaard 2017, Chan et al. 2004) (Fig. 2).

DNA methylation maintenance: The enzymes involved in the DNA methylation, which all establish methylation, recognize and remove methylation are categorized into 3 classes writers, erasers and readers: writers are the enzymes which catalyze and add methyl groups to the cytosine residues, erasers are responsible for modification and removal of methyl groups and readers identify



1



2

Figs 1 & 2: 1. Canonical RdDm pathway. 2. Non-canonical RdDm pathway.

and bind to methyl groups to influence the gene expression (Moore et al. 2012).

De novo DNA methylation is controlled by siRNAs. Methylation is primarily established by domains rearranged methyltransferase 1 and 2 (DRM1 and DRM2), and is guided by siRNAs. The methylation is maintained and managed by methyltransferase 1 (MET1), which works in connection with variant in methylation (VIM) family proteins *(*VIM1-VIM3*)* that specifically preserve CG site methylation. MET1 identifies hemi-methylated CG dinucleotides after DNA replication and methylates the cytosine on the daughter strand. The chromomethylase 2 (CMT2) is associated with the asymmetric CHH methylation of constitutive heterochromatin, and the chromomethylase 3 (CMT3) is more involved with the CHG methylation (Cuerda-Gil & Slotkin 2016, Agius et al. 2023) (Fig. 3).

DNA demethylation: In plants, demethylation can occur passively, through the loss of the methylation signal during DNA replication, or actively, through specific enzymes that facilitate the process via DNA base excision repair (BER). Certain plant DNA glycosylases identify methylated cytosine in various sequence contexts and cleave the bond with the deoxyribose sugar, creating an abasic site (Fig. 4) that is subsequently repaired by DNA polymerase and ligase. The repressor of silencing 1 (ROS1), demeter (DME), and demeter-like protein 2 and 3 (DML2 and DML3) are the 4 DNA demethylases (DNA glycosylases/DNA glycolyases) found in *Arabidopsis* (Parrilla-Doblas et al. 2019, Niederhuth et al. 2014, Erdmann & Picard 2020). Active demethylation was found to be a key regulatory mechanism for plant disease resistance and adaptation (Halter et al. 2021). In the previous studies, an improved susceptibility to *Hyaloperonospora arabidopsidis* and Pt0 DC3000 was reported in *ros1* mutants, as well as disease susceptibility to *Fusarium oxysporum* was found in the *Arabidopsis ros1 dml2 dml3 (rdd)* mutant (Yu et al. 2013, López Sánchez et al. 2016, Le et al. 2014, Schumann et al. 2019). Moreover, this evidence indicates that the demethylase promotes resistance against pathogens.

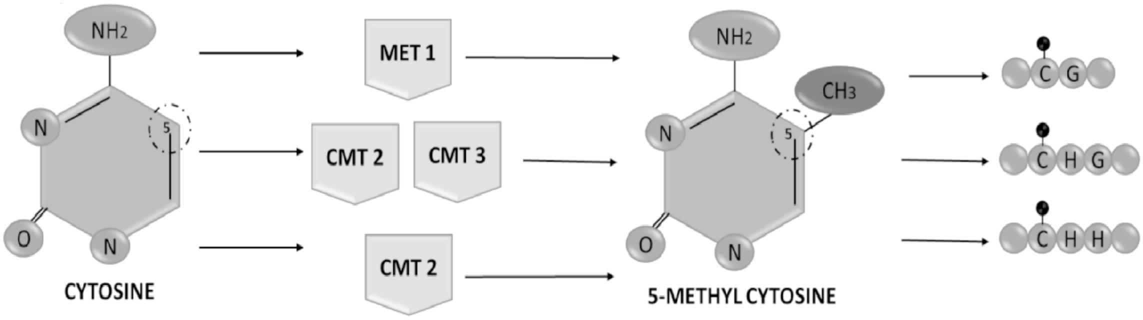


Fig. 3: The enzymes that maintain the methylation during replication in all three contexts of plant DNA methylation

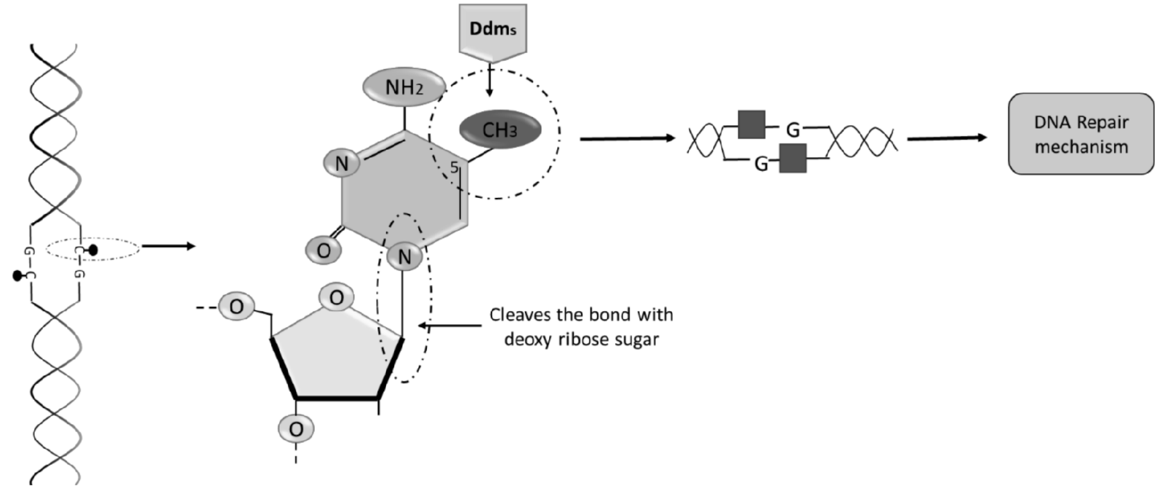


Fig. 4: The removal of methylated cytosine by the DNA repair mechanism

Role of DNA methylation in stress response: DNA methylation plays a vital role in helping plants respond to different stress contexts by regulating the expression of specific genes. DNA methylation suppresses potentially harmful genomic elements, including repeat sequences and transposable elements (TEs), while the significant molecular result is regulating gene expression at the transcriptional level (Lei et al. 2015).

DNA methylation changes can activate or silence drought-responsive genes in response to drought. During times of water scarcity, methylation can suppress the activation of genes that are not needed and enhance the activation of genes that help plants survive in dry conditions. The methylation levels correlate with changes in the expression profiles of several drought stress-responsive genes in *Populus trichocarpa* subjected to drought conditions (Liang et al. 2014). *RD29A* and *RD20* are essential genes in the drought stress response; they exhibit high expression levels when the plant experiences drought conditions, enabling it to activate specific pathways that enhance its capacity to manage water scarcity. Modifications in DNA methylation can trigger the activation of *RD29A* and *RD20*, allowing the plant to initiate protective responses against dehydration. By regulating the expression of *RD29A* and *RD20* by DNA methylation, the plants can successfully adapt to water-deficient conditions. Moreover, these protein-encoding genes will participate in the production of osmo-protectants that help preserve cellular function and integrity during low water availability periods (Ashapkin et al. 2020, Liang et al. 2014).

In the context of salinity stress, plants encounter difficulties due to high salt concentrations in the environment, which affect the expression of genes essential for ion transport and the maintenance of osmotic balance within plant cells. The variations in the methylation status of specific genes allow plants to withstand high salinity (Kinoshita & Seki 2014). Studies on salt-tolerant and salt-sensitive rice varieties have revealed the links between global DNA methylation levels and salt stress response. The variations in DNA methylation play a crucial role in regulating gene expression. Under high salinity stress, promoter and gene body methylation levels are critical in regulating gene expression in a genotype- and organ-specific manner. Additionally, the plants responded to salinity by reducing DNA methylation levels, which is associated with the upregulation of the *DNA demethylase* (*DRM2*) gene. The upregulation of *DRM2* was only observed in salt-sensitive cultivars. This indicates that salt-sensitive plants may rely on changes in DNA demethylation to manage stress, while salt-tolerant plants do not exhibit the same response (Ferreira et al. 2015). *Salt overly sensitive 1 (SOS1)* is an essential gene for salt tolerance because it encodes a sodium/proton exchanger. The DNA methylation regulates the expression of the *SOS1* gene and allows the plant to adjust its ion transport process and increase its survival capacity in response to salinity stress (Ashapkin et al. 2020).

When plants get exposed to extreme temperature conditions, whether high or low, their physiological processes and health can be affected. Changes in the DNA methylation can be induced, which in turn regulate the expression of heat shock proteins and cold-responsive genes. These epigenetic modifications enable the plant to adapt to temperature fluctuations, adapting to changing thermal conditions and retaining cellular function (Kinoshita & Seki 2014). The *Heat shock protein 70 (HSP70)* gene plays a crucial role in supporting proper protein folding and defending the plant against heat stress. Its expression is affected by changes in DNA methylation that occur when the plant is exposed to high temperatures, upregulating its ability to cope with stress and preventing potential damage to proteins that could disrupt cellular function. The *COR15A* gene plays a crucial role in cold adaptation, enabling the plant to acclimate to low temperatures. The *COR15A* gene expression is regulated by DNA methylation during cold stress, and allows the plant to respond effectively to cold conditions and improve its chances of survival in cold environments (Ashapkin et al. 2020).

During a pathogen attack (such as bacteria, fungi, or viruses), the plants can raise a more effective defense by regulating gene expression through DNA methylation. They can enhance the activation of the defense-related genes, allowing the plant to identify and respond to pathogens more rapidly (Alonso et al. 2018). The *PR1* gene is one of the significant genes in the plant's defense system. The changes influence the regulation of the *PR1* gene in DNA methylation. When DNA methylation change happens in response to pathogen presence, they can enhance the transcription of the *PR1* gene and increase the production of PR1 protein, which is crucial for the plant's immune response, helping to protect against infections. By elevating *PR1* levels, the plant improves its ability to resist pathogens, thereby reducing the severity of the infection and aiding in recovery (Ashapkin et al. 2020).

When plants experience a nutrient deficiency in their environment, it also leads to stress in the plant and can result in alterations in the DNA methylation patterns. It also affects the expression of subsequent genes important for nutrient uptake and metabolism. The plant's response to nutrient stress involves modifying how genes are turned on or off to adapt to the deficiency and optimize nutrient absorption and use (Kinoshita & Seki 2014). The *Nitrate transporter 1.1(NRT1.1)* gene has a role in the nitrate uptake from the soil. In response to nitrogen deficiency, DNA methylation can regulate the expression of the *NRT1.1* gene by methylation changes, which can help the plant's absorption and utilization of nitrate, thereby helping it cope with the shortage of nitrogen (Ashapkin et al. 2020). Furthermore, under normal circumstances, epigenetic repression mechanisms such as DNA methylation keep transposons in check, preventing their unwanted activation and movement. When these repression systems fail, transposons can become derepressed (turned on) and mobilize, leading to potential genomic instability and disease. They also have a crucial role in losing various repression systems, commonly the transposon derepression system. (Bošković & Rando 2018).

Common phenotypes associated with DNA methylation/demethylation-related genes: In *A. thaliana*, the genome-wide DNA methylation exhibits high levels of methylation in heterochromatin, which is rich in TEs (transposons) and other repetitive DNA sequences. Interspersed transposon-associated DNA methylation also exists in euchromatic chromosome arms (Zhang et al. 2006). The change in DNA methylation patterns, specifically at CG and CHH islands, was linked to the reduced RNA levels and more biomass and seed yield heterosis (Kinoshita et al. 2004). In specific genes, the RNA-directed DNA methylation is responsible for DNA methylation, promoting growth vigor in hybrid *Arabidopsis* (Begum et al. 2022). The demethylase DME is essential for establishing epigenetic influence in gametophytes, which is advantageously expressed in central cells of female gametophytes, where it induces maternal allele-specific DNA demethylation of imprinted genes such as *Medea (MEA)* and *Flowering Wageningen (FWA)* and also in vegetative cells of pollen (Choi et al. 2002). The DNA methylation suppresses the genes that cause delayed flowering. Also, DNA methylation influences controlling leaf morphology, where it represses the genes responsible for shaping and sizing of leaves, and forming smaller leaves (Lee et al. 2023).

Methylation can occur on different regions of the same chromosome, and the effect also varies depending on the region and the methylation level. Typically, base modifications occurring in the promoter, nearby regions, or within the gene body might affect gene expression. Generally, DNA methylation represses gene transcription. On rarer occasions, DNA methylation can also activate gene transcription, but in some instances, DNA methylation or a certain level of demethylation may also promote gene transcription (Kumar &

Mohapatra 2021). Cytosine methylation in the *ROS1* promoter enhances its transcription in *Arabidopsis* (Williams et al. 2015). Moreover, gene expression regulation results from the interplay of various epigenetic mechanisms that affect each other, such as DNA methylation and histone modifications. Generally, regions with low DNA methylation levels also exhibit other modifications that promote transcription, such as histone acetylation. In some cases, hypermethylated and actively transcribed regions can show less histone acetylation, indicating that loss of DNA methylation alone is a mechanism that promotes activation of transcription. In addition to the DNA methylation in the promoter, methylation can also occur in the full-length coding region. Methylation in the coding regions suppresses transcript elongation. Unlike promoter methylation, methylation in the coding region hinders transcript elongation. As a result, transcription proceeds from the start site to the methylation site, leading to the production of a shorter, potentially inactive transcript (Gehrin & Henikoff 2007).

DNA methylation of transposons: As evidenced by previous studies, transposons are now considered an integral part of the genome, comprising repetitive DNA sequences. In most eukaryotic genomes, the transposons are distributed across the genome. They are unstable within the genome and interfere with the typical genome sequence. Moreover, in plant stress conditions, they can significantly contribute to adaptation. The activity of transposons and DNA methylation are inter-connected. In plants, the transposon methylation, specifically at the CHG and CHH context, can suppress the activation (Ritter & Niederhuth 2021, Nuthikattu et al. 2013).

Crop plants with larger genomes tend to have more TEs, and most of these TEs are located near genes, thereby influencing their gene expression. Consequently, DNA modification has a more pronounced impact on gene expression in these crops than in *Arabidopsis*. Additionally, DRM2 specifically targets TEs located on the promoters to regulate genes involved in stress responses (Le et al. 2014, Lucibelli et al. 2022). In *Arabidopsis*, about one-third of the genes have methylation in their gene bodies. Generally, TEs and repetitive regions are heavily methylated in all cytosine contexts, while gene body methylation is relatively rare in non-CG contexts (Zhang et al. 2006). Some introns within a gene can contain TEs or repeat sequences that are hypermethylated across all cytosine contexts and play a role in regulating mRNA processing. For instance, the loss of DNA methylation from a retrotransposon located in an intron of a developmental regulatory gene has been reported to lead to alternative splicing and premature transcript termination (Zhang et al. 2018).

Inheritance of DNA methylation: A key finding in epigenetics is the concept of epigenetic memory, which suggests that plants can retain the epigenetic imprints of past experiences and utilize them for future stress responses. Plants do not develop stress memory during an initial exposure to stress, but struggle to survive subsequent stress events. Plants that acquire short-term memory from the stress event can improve their resistance to a second stress, but this memory lasts only temporarily. Future generations cannot inherit it. In contrast, plants that exhibit intergenerational memory display stress memory in the first generation of offspring that are free from stress. This can be observed in at least 2 successive generations for plants capable of transgenerational memory. It is inherited across generations and potentially influences the phenotype of the progeny (Sun et al. 2022). The dynamics and stability of transgenerational epigenetic effects are crucial for developing strategies for crop improvement. This transgenerational memory enables plants to better adapt to continuous stress conditions and provides a mechanism for rapidly adapting crops to changing environments. An actively regulated balance between methylation and demethylation activities is necessary to ensure the long-term stable inheritance of epigenetic information (Hemenway& Gehring 2023).

Cytosine methylation is an important base modification that can be passed down through mitotic and meiotic cell divisions in plant genomes. Heritable cytosine methylation mainly occurs within the symmetric CpG dinucleotide context. During replication, this results in 2 daughter genomes, each with a hemimethylated CpG. This hemimethylated CpG serves as a target for methyltransferases (Met1 in *Arabidopsis*) to preserve the methylation pattern (Williams & Gehring 2017, Bošković & Rando 2018, Hagmann et al. 2015). MET 1 exhibits a catalytic preference for hemimethylated DNA substrates, making it well-suited for the efficient maintenance of CG methylation following DNA replication and throughout cell division. Most non-CG methylation is redundantly maintained by DRM2 and the plant-specific protein chromomethylase 2 (CMT2) and chromomethylase 3 (CMT3) (Henderson & Jacobsen 2007, Hagmann et al. 2015, Law & Jacobsen 2010).

Imprinting of genes: In *Arabidopsis*, parent-of-origin-specific gene expression, also known as genomic imprinting, mainly occurs in the endosperm. This means specific genes are expressed differently depending on whether they come from the mother or the father. 'Imprinting' refers to heritable epigenetic methylation marks in the germline. An epigenetic allele, or epiallele, is an allele that exhibits a heritable change in expression due to epigenetic modifications rather than alterations in the DNA sequence. During gametogenesis, the alleles of imprinted genes are epigenetically silenced either by the mother or the father. This epigenetic memory of the parental origin continues after fertilization, leading to varying transcriptional activity between maternal and paternal alleles in the developing endosperm. Because the endosperm is a terminal tissue, the imprinting characteristics of specific genes cannot be passed on to the next generation and, therefore, are not reset. Cytosine methylation patterns are predominantly preserved in the egg and sperm nuclei, which likely explains the stability of their cytosine methylation epialleles. (Iwasaki &

Paszkowski. 2014, Sontag et al. 2006). The somatic cytosine methylation patterns are primarily, but not entirely, preserved in plant germ cells. In *Arabidopsis*, CG and CHG methylation patterns are retained in the sperm nuclei, while CHH-context RNA-dependent DNA methylation at retrotransposons is largely erased. This minimal reprogramming of cytosine methylation during gametogenesis contributes to the unusual stability of epialleles in various plant species (Bošković & Rando 2018, Henderson & Jacobsen 2007).

A key role of methylation-based epigenetic imprinting is establishing a cell's transcriptional state and offering a mechanism for regulating the activation or deactivation of regulatory genes during development and differentiation (Sontag et al. 2006). Interestingly, while cytosine methylation dynamics in plant germ cells are relatively stable, the companion cells of gametes show distinct methylation patterns. For example, global demethylation occurs in the central cell of ovules, which develops into the placenta-like endosperm, leading to the expression of imprinted genes specifically in this tissue. In seed-bearing plants, changes in cytosine methylation in the embryo are relatively minor. CHH methylation, which is missing in pollen, is reestablished in the paternal genome. Additionally, any loss of methylation in the egg cell is quickly restored (Bošković & Rando 2018, Calarco et al. 2012). Cytosine demethylation in the central cell of female gametophytes leads to a genome in the endosperm that is globally undermethylated, which in turn supports imprinted gene expression in this tissue (Bošković & Rando 2018, Kawashima & Berger 2014). The DNA demethylase demeter (DME), which exhibits DNA glycosylase activity targeting methylated cytosines, is found in the central cell where it removes methylated cytosines from maternally expressed genes *(MEGs)* such as *MEA, FIS2,* and *FWA*. This process results in the transcriptional activation of their maternal alleles (Choi et al. 2002, Gehring et al. 2006, Schoft et al. 2011). Additionally, the DNA methyltransferase MET1 is involved in regulating maternally imprinted genes. While MET1 maintains DNA methylation in somatic tissues, its expression is suppressed in the central cell during female gametogenesis, which likely contributes to the hypomethylation of *MEGs*. For instance, imprinted genes like *FWA, MEA, FIS2,* and *PHERES1* are expressed only from the maternal copy of the genome in the endosperm, while the corresponding paternal copies of these genes are silenced. This selective expression ensures that only the maternal alleles are active in regulating certain aspects of seed development (Choi et al. 2002, Gehring et al. 2006, Iwasaki & Paszkowski 2014).

In *Arabidopsis*, (Molinier et al. 2006) research has found that UV-C radiation or the addition of the bacterial component flagellin (a component from bacteria which boosts the frequency of somatic homologous recombination) increases the rate of somatic homologous recombination. The offspring inherits this change as a dominant trait. A systemic signal induced by tobacco mosaic virus (TMV) enhances somatic recombination rates. The offspring of plants infected with TMV also exhibited a higher frequency of recombination. Additional research indicated that systemic acquired resistance (SAR) can be passed on to the next generation in tomato and *Arabidopsis* (Boyko et al. 2007). Furthermore, there are stable epialleles which occur naturally. Different flower shapes range from bilaterally symmetrical to radial forms, in toadflax (*Linaria vulgaris*). This phenotypic variability is caused by the methylation of the promoter of the *CYCLOIDE* gene (Cubas et al. 1999). A natural epiallele was revealed from examining genetic incompatibility in different *Arabidopsis* accessions. The incompatibility stemmed from the epigenetic traits of duplicated *AtFOLT* genes. In this case, a specific change in one *AtFOLT* gene led to DNA methylation of the other copy through RdDM (Durand et al. 2012).

Epigenetic marks affect the following generations by inheritance of epigenetic variants and sometimes by reprogramming events. Some epigenetic variants can be stably inherited and influence subsequent generations' traits. These inherited epigenetic changes can affect the expression of specific genes and may contribute to traits or predispositions in the offspring. However, because most epigenetic marks are reset, only a subset of the epigenetic information is typically passed on. After fertilization, the epigenetic marks in the early embryo are significantly reprogrammed. This reprogramming process is crucial for resetting the epigenetic landscape to a state appropriate for development and differentiation into various tissues. As a result, many of the epigenetic marks present in the germline cells are erased or modified during this reprogramming phase. While some epigenetic information is inherited, a substantial amount of the original epigenetic marks is not maintained in the subsequent generation's germline. Instead of inheritance or reprogramming of epigenetic marks, sometimes they are not copied and maintained in the subsequent germline. The resetting process ensures that each generation starts with a relatively clean slate regarding epigenetic marks. This allows for the proper development of the embryo and ensures that epigenetic modifications from one generation do not directly dictate the epigenetic state of the next generation's germline. This resetting is important for normal development and helps prevent the accumulation of potentially harmful or maladaptive epigenetic changes across generations (Bošković & Rando 2018, Iwasaki & Paszkowski 2014).

Epigenome editing: The sequencing and genome editing technology has evolved rapidly until now. Whole-genome sequencing, has now been done in several crops. As research progresses, epigenome editing can enhance the genetic efficiency of plants in responding to and adapting to various environmental conditions, and at the genome level, can elucidate the relationship between epigenetic modifications and gene expression. The epigenomic alteration can design chosen qualities and activate genes to perform new functions during plant development and stress. Epigenome editing targets alteration at specific genome loci, and the invention of DNA nucleases, which recognize specific sequences, allows targeted genes at a particular site within the genome to be targeted for modification. The DNA nucleases, such as Zinc finger (ZF), Transcription activator-like effector (TALE), and CRISPR/Cas9 complex, are used for epigenome editing (Kungulovski & Jeltsch 2016). Fusing the DNA nucleases with the methylation-modifying enzymes can directly regulate the expression of the targeted gene by the epigenetic modification of the specific loci (Qi et al. 2023).

The successful DNA methylation epigenome editing was done by the fusion of ZF and SUVH9 at an *FWA* gene to the epiallele *fwa-4* in *Arabidopsis*, which led to the gene silencing, and the corresponding phenotype was early flowering. These modifications were transferred to the next generation (Johnson et al. 2014). The demethylation of *FWA* gene promoter in *Arabidopsis* by the fusion of the catalytic domain of ten-eleven translocation 1 (TET1cd) demethylase with an artificial ZF, upregulated gene expression with late flowering phenotype. Moreover, the targeted editing based on the CRISPR/Cas9 system, which combines the TET1cd and an improved sun-tag system, can also demethylate and activate the *FWA* gene. Both systems achieved targeted demethylation, which was heritable (Gallego-Bartolomé, 2018). Based on the CRISPR/Cas9 Sun-tag system, utilizing the transcriptional activator VP64 drives robust expression with the catalytic domain of the *Nicotiana tabacum* DRM methyltransferase, which efficiently targets DNA methylation to specific loci, resulting in the *FWA* promoter methylation modification and early flowering phenotype (Papikian et al. 2019). Epigenome editing, as it does not alter the DNA sequences, is an ethically favorable technique for crop improvement. However, the epigenome editing mainly targets the promoter regions. However, the promoter region sequences are not always unique, making it difficult to design a gene-specific epigenome editing construct. Furthermore, the epigenome editing technique needs more development to bring a beneficial transformation in crop improvement (Doddavarapu et al. 2024).

Future prospects: The study of DNA methylation as an epigenetic mechanism in plants is rapidly evolving and offering transformative potential across agriculture, biotechnology, and genetics. Recent technological developments, such as the use of CRISPR/dCas9-based systems combined with methyltransferases or demethylases, will enable precise control over gene expression, aiming to shift plant improvement strategies towards targeting and modulating specific epigenetic marks without altering the underlying DNA sequence. This provides non-GMO crop approaches where stress-resilient traits can be induced epigenetically, allowing for the selective activation or silencing of genes. Future tools may enable tissue-specific or inducible epigenetic editing, thereby enhancing resilience to biotic and abiotic stresses, such as drought, heat, salinity, or pathogens.

Epigenetic profiling can help conserve

endangered species by identifying stress-adaptive epialleles. In restoration projects, epigenetically primed plants can be used to rehabilitate degraded ecosystems, especially in extreme environments. Additionally, the transgenerational inheritance of DNA methylation provides a means to precondition crops for future stress events, potentially reducing yield losses. Epigenetic markers can be used to screen and select climate-resilient genotypes, thereby accelerating breeding programs. Harnessing this mechanism can help develop crops that thrive in the face of climate change-induced stressors. These markers reflect environmentally induced traits, allowing breeders to select for stress tolerance, flowering time, and biomass production with greater precision.

Furthermore, emerging technologies such as single-cell epigenomics and multi-omics platforms will enable high-resolution mapping of methylation patterns. This will uncover complex gene regulatory networks and their interactions with histone modifications and non-coding RNAs. Additionally, the future development of machine learning models trained on epigenomic data can predict plant responses to stress and guide breeding decisions. Integration of methylation data into AI-driven prediction could revolutionize precision agriculture.

The future of the plant DNA methylation research is rich with possibilities–from enhancing crop resilience and productivity to unlocking new layers of biological regulation. As tools become more precise and datasets more comprehensive, epigenetics will become a cornerstone of next-generation agriculture and environmental stewardship. Moreover, the technology is promising; several challenges remain, including achieving specificity in targeting unique genomic loci for epigenome editing, understanding the long-term stability and heritability of induced epigenetic changes, and integrating high-throughput methylome data with functional outcomes at the phenotypic level. Overcoming these challenges should be a key focus for researchers, encouraging innovation in computational biology, molecular techniques, and translation of discoveries into field trials.

ACKNOWLEDGEMENTS

ATM would like to acknowledge the CSIR for award of the Junior Research Fellowship. JMS thanks the Department of Biotechnology for funding.

Declaration

Authors have no conflict of interest.

REFERENCES

AGIUS D R, KAPAZOGLOU A, AVRAMIDOU E, BARANEK M, CARNEROS E, CARO E, CASTIGLIONE S, CICATELLI A, RADANOVIC A, EBEJER J P, GACKOWSKI D, GUARINO F, GULYÁS A, HIDVÉGI N, HOENICKA H, INÁCIO V, JOHANNES F, KARALIJA E, LIEBERMAN-LAZAROVICH M & VASSILEVA V 2023 Exploring the crop epigenome: A comparison of DNA methylation profiling techniques *Front Plant Sci* **14** 1181039

ALONSO C, RAMOS-CRUZ D & BECKER C 2018 The role of plant epigenetics in biotic interactions *New Phyto* **221** 731–737

ASHAPKIN V V, KUTUEVA L I, ALEKSANDRUSHKINA N I, & VANYUSHIN B F 2020 Epigenetic mechanisms of plant adaptation to biotic and abiotic stresses *Int J Mol Sci* **21** 7457 doi:10-3390/jms21207457

BEGUM Y 2022 Regulatory role of microRNAs (miRNAs) in the recent development of abiotic stress tolerance of plants *Gene* **821** 146283 doi:10.1016/j.gene.2022.146283

BOŠKOVIĆ A & RANDO O J 2018 Transgenerational epigenetic inheritance *Ann Rev Genet* **52** 21–41

BOYKO A, KATHIRIA P, ZEMP F J, YAO Y, POGRIBNY I & KOVALCHUK I 2007 Transgenerational changes in the genome stability and methylation in pathogen-infected plants: (virus-induced plant genome instability) *NucleicAcids Res* **35** 1714– 1725

CALARCO J P, BORGES F, DONOGHUE M T, VAN EX F, JULLIEN P E, LOPES T, GARDNER R, BERGER F, FEIJÓ J A, BECKER J D & MARTIENSSEN R A 2012 Reprogramming of DNA methylation in pollen guides epigenetic inheritance via small RNA *Cell* **151** 194–205

CAO X & JACOBSEN S E 2002 Locus-specific control of asymmetric and CpNpG methylation by the DRM and CMT3 methyltransferase genes *Proc Natl Acad Sci USA* **99** 16491–16498

CHAN S W, ZILBERMAN D, XIE, Z, JOHANSEN L K, CARRINGTON J C & JACOBSEN S E 2004 RNA silencing genes control de novo DNA methylation *Sci New York NY* **303** 1336 doi:10.1126/science.1095989

CHOI Y, GEHRING M, JOHNSON L, HANNON M, HARADA J J, GOLDBERG R B, JACOBSEN S E & FISCHER R L 2002 DEMETER a DNA glycosylase domain protein is required for endosperm gene imprinting and seed viability in *Arabidopsis Cell* **110** 33–42

CUBAS P, VINCENT C & COEN E 1999 An epigenetic mutation responsible for natural variation in floral symmetry *Nature* **401** 157–161

CUERDA-GIL D & SLOTKIN R K 2016 Non-canonical RNA-directed DNA methylation *Nature Plants* **2** 161163doi:10.1038/nplants.2016.163

DODDAVARAPU B, LATA C & SHAH J M 2024 Epigenetic regulation influenced by soil microbiota and nutrients: Paving road to epigenome editing in plants *Biochem Biophys Acta Gen Subj* **1868** 130580 doi:1016/jbbagen.2024.130580

DURAND S, BOUCHE N, PEREZ-STRAND E, LOUDET O & CAMILLERI C 2012 Rapid establishment of genetic incompatibility through natural epigenetic variation *Curr Biol* **22** 326–331

ERDMANN R M & PICARD C L 2020 RNA-directed DNA methylation *PLOS Genetics* **16** 1009034 doi:1371/journal.pgen.1009034

FERREIRA L J, AZEVEDO V, MAROCO J, OLIVEIRA M M & SANTOS A P 2015 Salt tolerant and sensitive rice varieties display differential methylome flexibility under salt stress *PLoS ONE* **10** 0124060 doi:10.1371/journal.pone.0124060

GALLEGO-BARTOLOMÉ J, GARDINER J, LIU W, PAPIKIAN A, GHOSHAL B, KUO H Y, ZHAO J M, SEGAL D J & JACOBSEN S E 2018 Targeted DNA demethylation of the *Arabidopsis* genome using the human TET1 catalytic domain *Proc Natl Acad Sci U S A* **115** 2125–2134

GEHRING M, HUH J H, HSIEH T F, PENTERMAN J, CHOI Y, HARADA J J, GOLDBERG R B & FISCHER R L 2006 DEMETER DNA glycosylase establishes MEDEA polycomb gene self-imprinting by allele-specific demethylation *Cell* **124** 495–506

GEHRING M & HENIKOFF S 2007 DNA methylation dynamics in plant genomes *Biochim Biophys Acta* **1769** 276–286

HAGMANN J, BECKER C, MÜLLER J, STEGLE O, MEYER R C, WANG G, SCHNEEBERGER K, FITZ J, ALTMANN T, BERGELSON J, BORGWARDT K & WEIGEL D 2015 Century-scale methylome stability in a recently diverged *Arabidopsis thaliana* lineage *PLoS Genet* **11** 1004920 doi:10.1371/journal.pgen.1004920

HALTER T, WANG J, AMESEFE D, LASTRUCCI E,

CHARVIN M, SINGLA RASTOGI M & NAVARRO L 2021 The *Arabidopsis* active demethylase ROS1 *cis*-regulates defence genes by erasing DNA methylation at promoter-regulatory regions *eLife* **10** 62994 doi: 10.7554/eLife.62994

HEMENWAY E A & GEHRING M 2023 Epigenetic regulation during plant development and the capacity for epigenetic memory *Ann Rev Plant Biol* **74** 87–109

HENDERSON I R & JACOBSEN S E 2007 Epigenetic inheritance in plants *Nature* **447** 418–424

IWASAKI M & PASZKOWSKI J 2014 Epigenetic memory in plants *EMBO J* **33** 1987–1998

JOHNSON L M, DU J, HALE C J, BISCHOF S, FENG S, CHODAVARAPU R K, ZHONG X, MARSON G, PELLEGRINI M, SEGAL D J, PATEL D J & JACOBSEN S E 2014 SRA- and SET-domain-containing proteins link RNA polymerase V occupancy to DNA methylation *Nature* **507** 124–128

KAWASHIMA T & BERGER F 2014 Epigenetic reprogramming in plant sexual reproduction *Nat Rev Genet* **15** 613–624

KINOSHITA T, MIURA A, CHOI Y, KINOSHITA Y, CAO X, JACOBSEN S E, FISCHER R L & KAKUTANI T 2004 One-way control of FWA imprinting in *Arabidopsis* endosperm by DNA methylation *Science* **303** 521–523

KINOSHITA T & SEKI M 2014 Epigenetic memory for stress response and adaptation in plants *Plant Cell Phys* **55** 1859–1863

KUMAR S & MOHAPATRA T 2021 Dynamics of DNA methylation and its functions in plant growth and development *Front Plant Sci* **12** 596236 doi:10.3389/ fpls.2021.596236–236

KUNGULOVSKI G & JELTSCH A 2016 Epigenome editing: state of the art, concepts, and perspectives *Trends Genet* **32** 101–113

LAW J A & JACOBSEN S E 2010 Establishing maintaining and modifying DNA methylation patterns in plants and animals *Nat Rev Genet* **11** 204–220

LE T N, SCHUMANN U, SMITH N A, TIWARI S, AU P C, ZHU Q H, TAYLOR J M, KAZAN K, LLEWELLYN D J, ZHANG R, DENNIS E S & WANG M B 2014 DNA demethylases target promoter transposable elements to positively regulate stress responsive genes in *Arabidopsis Genome Biol* **15** DOI:10.1186/s13059-014-0458-3

LEE S, CHOI J, PARK J, HONG C P, CHOI D, HAN S, CHOI K, ROH T-Y, HWANG D & HWANG I 2023 DDM1-mediated gene body DNA methylation is associated with inducible activation of defense-related genes in *Arabidopsis Genome Biol* **24** 106 doi:10.1186/ s13059-023-02952-7

LEI M, ZHANG H, JULIAN R, TANG K, XIE S & ZHU J K 2015 Regulatory link between DNA methylation and active demethylation in *Arabidopsis PNAS* **112** 53–57

LIANG D, ZHANG Z, WU H, HUANG C, SHUAI P, YE C Y, TANG S, WANG Y, YANG L, WANG J, YIN W & XIA X 2014 Single-base-resolution methylomes of Populus trichocarpa reveal the association between DNA methylation and drought stress *BMC Genet* **15** DOI:10.1186/ 1471–2156-15-S1-S9

LÓPEZ SÁNCHEZ A, STASSEN J H, FURCI L, SMITH L M & TON J 2016 The role of DNA (de)methylation in immune responsiveness of *Arabidopsis Plant J* **88** 361–374

LUCIBELLI F, VALOROSO M C & ACETO S 2022 Plant DNA methylation: An epigenetic mark in development, environmental interactions, and evolution *Int J Mol Sci* **23** 8299 doi:10.3390/ijms23158299

MATZKE M A & MOSHER R A 2014 RNA-directed DNA methylation: an epigenetic pathway of increasing complexity *Nat Rev Genet* **15** 394–408

MOLINIER J, RIES G, ZIPFEL C & HOHN B 2006 Transgenerational memory of stress in plants *Nature* **442** 1046 – 1049

MOORE L D, LE T & FAN G 2012 DNA methylation and its basic function *Neuropsychopharmacology* **38** 23–38

NIEDERHUTH C E & SCHMITZ R J 2014 Covering your bases: inheritance of DNA methylation in plant genomes *Mol Plant* **7** 472–480

NUTHIKATTU S, MCCUE A D, PANDA K, FULTZ D, DEFRAIA C, THOMAS E N & SLOTKIN R K 2013 The initiation of epigenetic silencing of active transposable elements is triggered by RDR6 and 21-22 nucleotide small interfering RNAs *Plant Phy* **162** 116–131

PAPIKIAN A, LIU W, GALLEGO-BARTOLOMÉ J & JACOBSEN S E 2019 Site-specific manipulation of *Arabidopsis* loci using CRISPR-Cas9 SunTag systems *Nat Commun* **10** 729 doi:10.1038/s1467-019-08736-7

PARRILLA-DOBLAS J T, ROLDÁN-ARJONA T, ARIZA R R & CÓRDOBA-CAÑERO D 2019 Active DNA demethylation in plants *Int J Mol Sci* **20** 4683 doi: 10.3390/ijms20194683

QI Q, HU B, JIANG W, WANG Y, YAN J, MA F, GUAN Q & XU J 2023 Advances in plant epigenome editing research and its application in plants *Int J Mol Sci* **24** 3442 doi:10.3390/ijms2094683

RITTER E J & NIEDERHUTH C E 2021 Intertwined evolution of plant epigenomes and genomes *Curr Opin Plant Biol* **61** 101990 doi:10.1016/jpbi.2020.101990

SCHOFT V K, CHUMAK N, CHOI Y, HANNON M, GARCIA-AGUILAR M, MACHLICOVA A, SLUSARZ L, MOSIOLEK M, PARK J S, PARK G T, FISCHER R L & TAMARU H 2011 Function of the DEMETER DNA glycosylase in the *Arabidopsis thaliana* male gametophyte *Proc Natl Acad Sci U S A* **108** 8042-8047

SCHUMANN U, LEE J M, SMITH N A, ZHONG C, ZHU J K, DENNIS E S, MILLAR A A & WANG M B 2019 DEMETER plays a role in DNA demethylation and disease response in somatic tissues of *Arabidopsis Epigenetics* **14** 1074–1087

SONTAG L B, LORINCZ M C & GEORG LUEBECK E 2006 Dynamics, stability, and inheritance of somatic DNA methylation imprints *J Theo Bio* **242** 890–899

STROUD H, DO T, DU J, ZHONG X, FENG S, JOHNSON L, PATEL D J & JACOBSEN S E 2014 Non-CG methylation patterns shape the epigenetic landscape in *Arabidopsis Nat Struct Mol Biol* **21** 64–72

SUN, MINGHUI, ZHUO YANG, LI LIU & LIU DUAN 2022 DNA methylation in plant responses and adaptation to abiotic stresses *Int J Mol Sci* **23** 6910 doi:10.3390/ijms23136910

WENDTE J M & PIKAARD C S 2017 The RNAs of RNA-directed DNA methylation *Biochimica et Biophysica Acta (BBA) - Gene Reg Mech* **1860** 140–148

WILLBANKS A, LEARY M, GREENSHIELDS M, TYMINSKI C, HEERBOTH S, LAPINSKA K, HASKINS K & SARKAR S 2016 The Evolution of epigenetics: from prokaryotes to humans and its biological consequences *Genet Epigenet* **8** 25–36

WILLIAMS B P & GEHRING M 2017 Stable transgenerational epigenetic inheritance requires a DNA methylation-sensing circuit *Nat Commun* **8** 21–24

WILLIAMS B P, PIGNATTA D, HENIKOFF S & GEHRING M 2015 Methylation-sensitive expression of a DNA demethylase gene serves as an epigenetic rheostat *PLoS Genet* **11** 1005142 doi:10.1371/journal/pgen.1005142

WILLMANN M R, ENDRES M W, COOK R T & GREGORY B D 2011 The functions of RNA-dependent RNA polymerases in *Arabidopsis The Arabidopsis Book* **9** 0146 doi:10.1199/tab.0146

YU A, LEPÈRE G, JAY F, WANG J, BAPAUME L, WANG Y, ABRAHAM A L, PENTERMAN J, FISCHER R L, VOINNET O & NAVARRO L 2013 Dynamics and biological relevance of DNA demethylation in *Arabidopsis* antibacterial defense *Proc Natl Acad Sci U S A* **110** 2389–2394

ZHANG Q, LIANG Z, CUI X, JI C, LI Y, ZHANG P, LIU J, RIAZ A, YAO P, LIU M, WANG Y, LU T, YU H, YANG D, ZHENG H & GU X 2018 N^6-Methyladenine DNA methylation in *Japonica* and *Indica* rice genomes and its association with gene expression plant development and stress responses *Mol Plant* **11** 1492–1508

ZHANG X, YAZAKI J, SUNDARESAN A, COKUS S, CHAN SW, CHEN H, HENDERSON I R, SHINN P, PELLEGRINI M, JACOBSEN S E & ECKER J R 2006 Genome-wide high-resolution mapping and functional analysis of DNA methylation in *Arabidopsis Cell* **126** 1189–201 doi:1016/j.Cell.2006.08.003

Journal of Cytology and Genetics 2025 VOL. **26** (NS): 133–135 (2025)

RESEARCH ARTICLE

Short communication:

NEW CHROMOSOME NUMBER REPORT OF *LEPTOCHILUS LANCEOLATUS* FÉE FROM WESTERN GHATS

SAMPADA KANK*AND A. BENNIAMIN
Botanical Survey of India, Western Regional Centre, Pune 411 001, India
*For correspondence. Email: kanksampada05@gmail.com

(Received 22 September 2025, revised accepted 20 November 2025)

SUMMARY The meiotic study was conducted on the fern *Leptochilus lanceolatus*, a member of the family Polypodiaceae. The analysis revealed the presence of 36 bivalents in each spore mother cell. Meiosis is normal. However, occasionally, laggards and sticky chromosomes were observed both in meiosis I and II. This is the first chromosome count reported for this species.

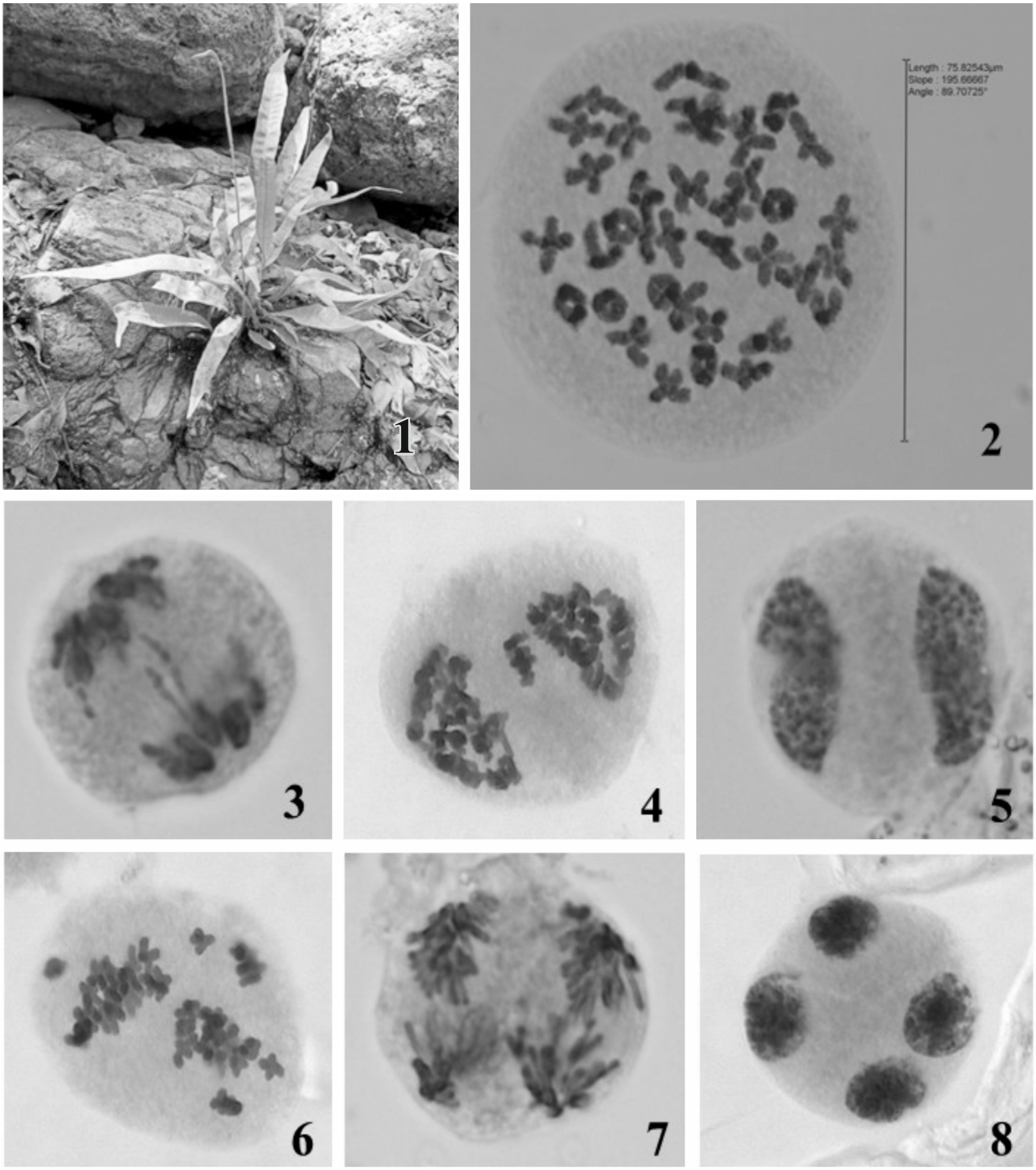
Keywords: *Leptochilus lanceolatus*, chromosome number, Western Ghats.

Leptochilus (Polypodiaceae), is distributed throughout tropical and subtropical regions. In India, the genus is represented by 12 species, 2 subspecies, and 1 hybrid (Fraser-Jenkins et al. 2020), of which 6 species and 2 subspecies occur in the Western Ghats (Benniamin & Sundari 2020). *Leptochilus lanceolatus* is distributed in Karnataka, Kerala, Maharashtra, Odisha, Tamil Nadu, and across parts of tropical Asia and Australasia, including Malaysia, Sri Lanka, Thailand, and New Guinea.

A perusal of literature reveals that there is no report of the chromosome count for *L. lanceolatus* so far, hence, the present study.

The material was collected from the Dhankal forest area of Chandoli National Park, Satara district, Maharashtra (Fig. 1). The collection site is situated at an altitude of 870 m (latitude 17°22′25″N, longitude 73°40′60″E). For chromosome analysis, fertile fronds bearing young sporangia were collected during the early morning hours between 6:00 a. m. and 8:00 a. m. and fixed in Carnoy's fluid (ethanol:chloroform:acetic acid in 6:3:1 ratio). Young sporangia were squashed in 1% aceto-carmine following the standard protocol outlined by Manton (1950). Photomicrographs of the meiotic stages were taken using an Olympus CX41 research microscope equipped with a digital imaging system. The Voucher specimen (BSI 202583) has been deposited in the Herbarium of the Botanical Survey of India, Western Regional Centre, Pune.

Meiotic analysis revealed 36 bivalents in each spore mother cell at diakinesis (Fig. 2). Both first and second meiosis are normal (Figs 3, 4). Occasionally, however, some abnormalities such as laggards and stickiness of chromosomes have been



Figs 1–8: 1. Plant habit. 2. Spore mother cell (SMC) at diakinesis showing 36 bivalents. 3. SMC at anaphase I showing a laggard. 4. SMC at telophase I showing a laggard. 5. SMC showing a dyad. 6. Abnormality at anaphase II. 7. SMC at anaphase II showing laggards. 8. SMC showing a tetrad.

observed at both first and second meiosis (Figs 5–8).

Cytological data available for the genus indicate that most of *Leptochilus* species are sexually diploid, although a few are polyploid (Bir & Verma 2010). Notably, *Leptochilus hemionitideus* (C. Presl) Noot. has been reported as both hexaploid (n = 108) and triploid (2n = 108) from Darjeeling (Bir & Trikha 1974, 1979). Additionally, *L. decurrens* has been reported as an octaploid (n = 144) from the Kolli Hills, Tamil Nadu (Sahaya & Vijayakanth 2015). In this context, the present finding of a diploid *L. lanceolatus* adds new and significant cytological data to the genus, especially considering its morphological intermediacy with *L. decurrens* and *L. thwaitesianus*. This further emphasizes the rarity of the species in the region and underlines the importance of cytological documentation for its taxonomic clarification and conservation assessment.

ACKNOWLEDGEMENTS

Authors are thankful to the Director, Botanical Survey of India, Kolkata for his support and encouragement. SK is thankful to SARATHI for providing financial assistance. Thanks, are also due to the Principal Chief Conservator of Forests, Maharashtra for granting permission to do field survey and collection of specimens.

Authors' contribution

SK was involved in collection of the plant material and conducted cytological experimental work. AB contributed to identification of the specimen and writing and reviewing of the manuscript.

Conflict of interest

Authors have no conflict of interest.

REFERENCES

BENNIAMIN A & SUNDARI M S 2020 *Pteridophytes of the Western Ghats: A pictorial guide* Bishen Singh Mahendra Pal Singh Dehradun

BIR S S & TRIKHA C K 1974 *In* Löve A (ed) IOBP chromosome number reports LIV *Taxon* **23** 376–377

BIR S S & TRIKHA C K 1979 Cytological evolution of polypodioid ferns with particular reference to the Himalayan forms In Nair P.K.K. (ed) *Glimpses in plant research* Vol IV Vikas publishing Pvt Ltd New Delhi pp 98–130

BIR S S & VERMA S C 2010 *Chromosome atlas of the Indian pteridophytes (1951–2009)* Bishen Singh Mahendra Pal Singh Dehradun

FRASER-JENKINS C R, GANDHI K N, KHOLIA B S & KANDEL D R 2020 *An annotated checklist of Indian pteridophytes Part-3 (Lomariopsidaceae to Salviniaceae)* Bishen Singh Mahendra Pal Singh Dehradun

MANTON I 1950 *Problems of cytology and evolution in the Pteridophyta* Cambridge University Press Cambridge

MANICKAM V S & IRUDAYARAJ V 1992 *Pteridophytic flora of South India* Today and Tomorrows publications New Delhi

VIJAYAKANTH P, SAHAYA SATHISH S, DOMINIC RAJKUMAR S, IRUDAYARAJ V, KAVITHA R & MAZUMDAR J 2018 Studies on the chromosome numbers of ferns from Kolli Hills Eastern Ghats Tamil Nadu India *Caryologia* **71** 380–396 doi: 10.1080/00087114.2018.1482151

SOCIETY OF CYTOLOGISTS AND GENETICISTS

No. 1387, 'Gayatri Nilaya', I 'D' Main, 11th Cross, Kengeri Upanagara, Bengaluru-560 060.
(gayatrimc2011@gmail.com)

Enrolment Proforma

I desire to enrol/renew* myself as a member of the Society of Cytologists and Geneticists, India for the year .... and shall abide by the constitution of the Society. The necessary particulars are given below:

Recent passport size photo

Name : ....

Date of Birth : ....

Qualifications : ....

Professional details : ....

Area/s of specialisation : ....

Address : ....

....

....

....

....Pin code..

E-mail : ....Ph. : (Mob.) ....

The subscription of Rs. 800/- (for new member) / Rs. 600/- (for renewal)* is sent by RTGS/NEFT in favour of **'TREASURER, SOCIETY OF CYTOLOGISTS AND GENETICISTS'** to **S.B. A/c No. 54047022485 on State Bank of India, Nagarabhavi Branch, Bengaluru.** IFSC : SBIN0040211. Branch code : 40211. (UTI No. & Date : ....)

Date : ....

Place : ....

Signature

* Delete as required.

STATEMENT ABOUT OWNERSHIP AND OTHER PARTICULARS ABOUT THE JOURNAL OF CYTOLOGY AND GENETICS

Place of publication	Bengaluru
Periodicity of publication	Yearly (may be issued in two parts)
Printer, publisher and editor	B. H. M. Nijalingappa
Nationality	Indian
Address	Flat No. 22114, Tower 22, Prestige Shantiniketan, Whitefield Road, Bengaluru - 560 048
Names and addresses of individuals who own the newspapers and partners or shareholders holding more than one per cent of the total capital	Society of Cytologists and Geneticists (Registered under the Bombay Public Trust Act XXIX of 1950) with Registration No. G. 399 (DWR)

I, B. H. M. Nijalingappa, hereby declare that the particulars given above are true to the best of my knowledge and belief.

(Sd/-)
Editor

31st December 2024

SUBSCRIPTION RATES

With Effect From 1st January 2026

INDIA

Personal

Ordinary membership	Rs. 600/- per year (plus admission fee of Rs. 200/- for new member)
Life membership (For Ordinary members of at least one year)	Rs. 6000/-
Institutional	Rs. 1000/- per year + Postage

ABROAD

Personal	US $ 50
Institutional	US $ 70

All correspondence regarding subscription should be addressed to the Hon. Secretary, Society of Cytologists and Geneticists, # 1387, `Gayatri Nilaya', I 'D' Main, Eleventh Cross, Kengeri Upanagara, Bengaluru 560 060, India. Email: gayatrimc2011@gmail.com. Ph.: +91 98455 08622.

The payment towards membership subscription should be made through R TGS/NEFT in favour of the **"Treasurer, Society of Cytologists and Geneticists"** payable to **S.B. A/c No. 54047022485, State Bank of India, Nagarabhavi Branch, Bengaluru. IFSC: SBIN0040211, Branch Code: 40211**, with an intimation to the Secretary through SMS or Email.