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Exploring Meiosis Stability and Pollen Fertility of *Nepeta racemosa* Lam. and *Nepeta pungens* (Bunge) Benth.

Running Title: Meiosis Stability and Pollen Fertility of *Nepeta* Species

Fatemeh Hajmoradi[1*] and Mehdi Kakaei[2]

1 Department of Biology, Payame-Noor University, Tehran, Iran
2 Department of Agricultural Sciences, Payam-Noor University, Tehran-Iran

*Corresponding Author: Email: f.hajmoradi@pnu.ac.ir

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ABSTRACT

Nepeta pungens (Bunge) Benth. and *Nepeta racemosa* Lam., both members of the Lamiaceae family, are valuable medicinal plants in Qazvin Province, Iran. This study investigated their meiotic behavior and pollen fertility due to their medicinal importance. Meiosis was studied in pollen mother cells using the squash technique, while pollen viability was assessed based on staining ability. Chromosomal analysis revealed that both species are diploid, with a chromosome number of 2n=2x=18. Both species exhibited meiotic abnormalities, including chromosomal stickiness, laggards, bridges, cytomixis, non-synchronous segregation, micronuclei, tripolar and pentapolar spindles, at varying frequencies. The meiotic index was 94% for *N. racemosa* and 86% for *N. pungens*. Pollen fertility tests showed that *N. racemosa* (89%) had a higher proportion of fertile pollen grains compared to *N. pungens* (83%). These findings enhance our understanding of the fundamental biological and genomic characteristics of these species, providing a basis for future research. Additionally, they highlight the potential implications of meiotic instability for plant breeding and conservation strategies, particularly in medicinally important species.
Keywords: Chromosome aberrations, Herbal medicine, Meiosis, *Nepeta*

INTRODUCTION

The genus *Nepeta* L. (Lamiaceae) comprises approximately 300 species, making it one of the largest genera in the family. These herbaceous annual or perennial plants are predominantly distributed across Eurasia. In Iran, *Nepeta* is the most significant genus within the Lamiaceae family, with 39 endemic species. Rechinger [1] documented 63 *Nepeta* species in Iran, while subsequent systematic studies have identified approximately 17 additional species [2].

In recent years, geneticists and botanists have increasingly focused on studying meiosis to assess genomic diversity in plant species. Due to their accessibility, pollen mother cells (PMCs) are commonly used in meiotic investigations. Previous chromosomal studies on *Nepeta* have reported base chromosome numbers of x = 6, 7, 8, 9, 11, 12, 13, 15, 17, and 18 [3-7]. The pre-meiotic, meiotic, and post-meiotic stages are tightly regulated by various genes [8]. Proper execution of meiosis ensures accurate chromosome segregation, whereas errors can lead to genomic instability, including structural chromosomal alterations and ploidy variations [9]. Non-genetic factors may also induce meiotic abnormalities and reduce pollen viability by causing anatomical, structural, and metabolic disruptions [10-12]. One common meiotic aberration is chromosome stickiness, which ranges from mild to severe during pachytene and may lead to chromatin degradation [13]. Lagging chromosomes, often caused by delayed karyokinesis, can result in micronuclei formation and aneuploid gametes due to unequal chromosome distribution [8, 14]. Micronuclei enclosed by the microspore wall may develop into non-viable pollen grains [15]. Meiotic irregularities can also disrupt microsporogenesis, producing abnormal dyads, triads, and tetrads (with or without micronuclei), while cytomixis may lead to triads and pentads [16]. Despite extensive research on *Nepeta's* mitotic chromosomes and karyotypes, studies on its meiotic behavior remain limited [17-19].

The medicinal plant *Nepeta pungens* (Bunge) Benth. is a widely distributed species of *Nepeta*, found in Iran, Afghanistan, Turkmenistan, and Central Asia [11]. Another medicinally important species in Iran, *N. racemosa* Lam., is traditionally valued for its fragrance and used to treat ailments such as abdominal bloating, digestive disorders, stomach pain, and infections [20].

Despite their medicinal significance, chromosomal and meiotic data for *N. pungens* and *N. racemosa* in Iran remain limited. This study therefore aims to investigate the meiotic behavior and pollen viability of these two species in Qazvin Province, Iran, to fill critical gaps in their cytogenetic characterization.

MATERIAL AND METHODS

Plant Materials

For chromosomal studies of *Nepeta pungens* (Bunge) Benth. and *N. racemosa* Lam., flower buds were collected from natural populations in Qazvin Province during spring 2021. Specimens were identified by the first author using available taxonomic resource [1]. *N. pungens* was collected from mountainous areas surrounding Meshaneh village in Avaj district (voucher no. 123; 35°35'46.1"N 49°07'02.2"E),

while *N. racemosa* was sampled near Peych Bon village (voucher no. 124; 36°23'56.6"N 50°46'37.1"E). All collected specimens were preserved as herbarium vouchers at the Herbarium of Qazvin Payame Noor University.

Cytogenetic Study

For meiotic chromosome studies, approximately 15 flower buds were collected from each species (minimum five plants per species) at optimal developmental stages. Samples were immediately fixed in Carnoy's solution (ethanol: chloroform: propionic acid, 6:3:2 v/v) for 24 hours at room temperature (20-25°C). Following fixation, specimens were rinsed with distilled water and preserved in 70% ethanol at 4°C until processing.

For meiotic stage analysis, anthers were dissected from fixed buds and stained with 1-2 drops of 2% acetocarmine. Chromosomal spreads were prepared using standard squash techniques, with permanent mounts created using Entellan mounting medium. Meiotic observations were conducted using an Olympus BX-41 research microscope. High-resolution micrographs were captured using an integrated Olympus digital camera system.

Meiotic Index

About 300 cells in the tetrad stage were observed in both species. Based on these counts, the Meiotic Index (MI) was calculated by dividing the number of normal tetrads by the total number of observed tetrads and multiplying the result by 100 [4]. The deviation of the standard and the mean was calculated using Excel software.

Pollen Fertility

To evaluate pollen fertility, pollen stainability was carefully examined. Pollen grains were collected from floral structures of the studied herbarium specimens and stained using a 1:1 acetocarmine-glycerin mixture. After storage at ambient temperature for 24-48 hours, approximately 500 pollen grains per flower were assessed for stainability. Slide documentation and analysis were performed using an Olympus BX-51 photomicroscope. Pollen grains showing intense staining were classified as fertile, while unstained or empty grains were considered infertile. Pollen fertility percentage was calculated by dividing the total number of fertile pollen grains by the total pollen grains counted, then multiplying by 100.

RESULTS AND DISCUSSION

Chromosome Studies and Meiotic Behavior

Chromosome studies of pollen mother cells in *N. pungens* and *N. racemosa* at the first meiotic division showed that both species are diploid with a chromosome number of 2n = 18. Previous studies have suggested 8, 9, and 17 as the basic chromosome numbers for the genus *Nepeta* [11, 17]. The chromosome counts of pollen mother cells in *N. racemosa* and *N. pungens* indicated that both species are diploid with 9 bivalents in their diakinesis cells, confirming a basic chromosome number of x = 9 (2n = 18). This is consistent with previous reports for these two species [12], although some studies have reported 2n = 22 for *N. pungens* [10]. The present study, along with previous reports, confirms x = 9 as the most common basic chromosome number in the genus *Nepeta*. [10]. The present study, along with previous reports, confirms x = 9 as the most common basic chromosome number in the genus *Nepeta*.

The meiotic behavior was studied for the first time in *N. pungens* and *N. racemosa*. A total of approximately 2,195 pollen mother cells were analyzed: 672 cells at diakinesis/metaphase I, 525 cells at anaphase I/telophase I, 398 cells at metaphase II, and 600 cells at anaphase II/telophase II. Observed abnormalities in both species included chromosome stickiness, lagging chromosomes, bridges, cytomixis, asynchronous chromosome separation, micronuclei, and tri-polar and penta-polar formations (Table 1, Fig. 1).

Table 1 The number of mother pollen cells studied at different meiotic stages, meiotic abnormalities, meiotic index, and chromosome count in *N. racemosa* and *N. pungens*.

Species/Feature	*N. racemosa*	*N. pungens*
Total number of cells	1092	1103
Diakinesis/Metaphase I	321	351
% Diakinesis/Metaphase I	29.3	31.8
% Chromosome stickiness	10.78 ± 0.02	14.21 ± 0.14
% Cytomixis	5.32 ± 0.50	-
Anaphase I/Telophase I	287	247
% Anaphase I/Telophase I	26.2	22.3
% Cytomixis	-	4.31 ± 0.1
% Bridge	4.8 ± 0.21	2.51 ± 0.09
% Laggards	3.19 ± 0.13	6.30 ± 0.05
% Asynchronous separation	2.6 ± 0.15	3.41 ± 0.19
Metaphase II	193	205
% Metaphase II	17.6	18.5
% Chromosome stickiness	7.13 ± 0.12	06.12 ± 0.08
Anaphase II/Telophase II	300	300
% Anaphase II/Telophase II	27.4	27.1
% Cytomixis	2.53 ± 0.03	3.7 ± 0.05
% Bridge	-	2.29 ± 0.03
% Laggards	0.62 ± 3.1	-

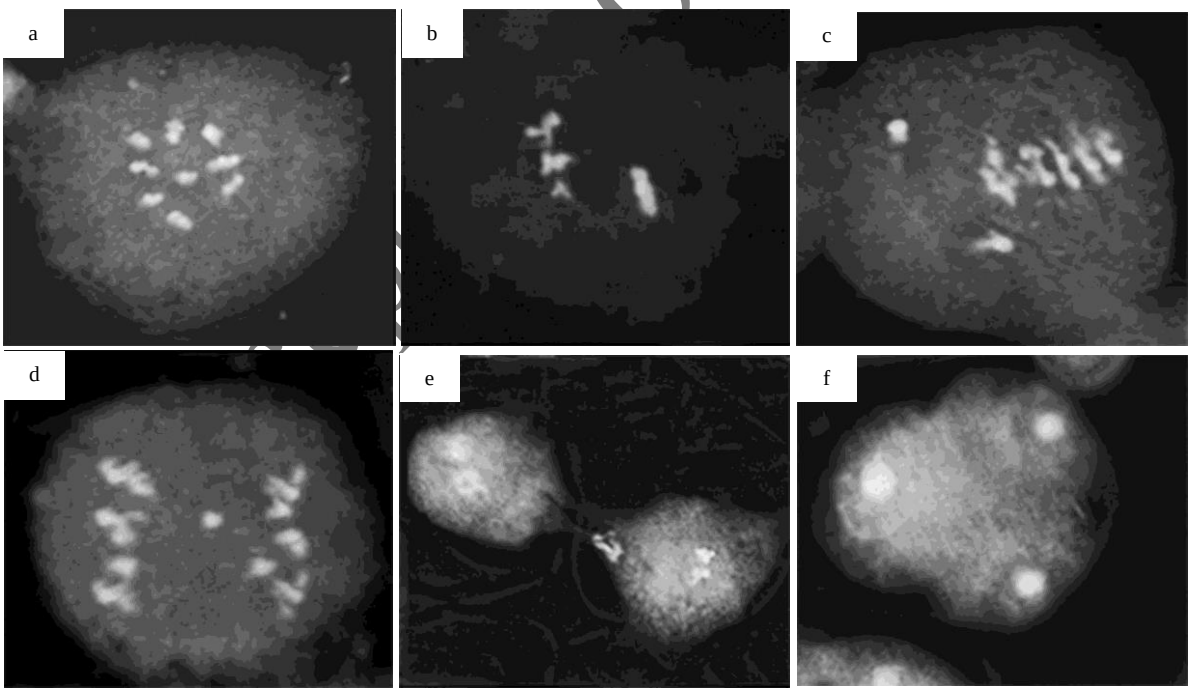
Species/Feature	*N. racemosa*	*N. pungens*
% Asynchronous separation	-	1.12 ± 0.02
% Micronucleus	2.61 ± 0.04	3.83 ± 0.02
% Tri-polar	3.12 ± 0.02	1.4 ± 0.04
% Penta-polar	-	5.78 ± 0.12
% Meiotic index	94	86
% Pollen fertility	89	83
Base chromosome number	9	9

All values are expressed as mean ± SE (standard error).

Chromosome stickiness, the most frequently observed aberration in both *N. pungens* and *N. racemosa* (Figures A1 and H1, respectively), manifests as sticky chromatin masses initially documented in wheat during pachytene. This phenomenon displays variable severity, from mild chromosomal entanglement to extreme cases involving pycnotic nuclei formation that may affect the entire genome and potentially lead to chromatin loss [21].Our data revealed *N. pungens* exhibited greater stickiness frequency than *N. racemosa* (Table 1), consistent with Malik et al.'s [19] findings in *N. cataria* and *N. nervosa*.

Another aberration observed in both species under investigation was the presence of directionless chromosomes on the cell plate, referred to as laggards. In *N. racemosa*, this occurred during the anaphase I/telophase I and anaphase II/telophase II stages (Figure D1), whereas in *N. pungens*, it was only observed during the first meiotic division. Laggards may form due to improper kinetochore attachment to the spindle fibers [22]. Malik et al. [19] reported chromosome stickiness in *N. nervosa* and *N. cataria*, asynchronous chromosome separation in *N. erecta* and *N. nervosa*, and laggards in *N. govaniana*.

Asymmetrical chromosomal bridges represent another aberration observed in the pollen mother cells of both studied species. In *N. pungens*, these bridges were detected during both anaphase I and II (Figure I1), while *N. racemosa* exhibited them exclusively during anaphase I. Wani et al., [23] suggested that both genetic and environmental factors may contribute to the formation of chromosomal bridge aberrations. Saggoo et al., [17] investigated meiotic behavior in 14 *Nepeta* species from India. All examined species were diploid (2n = 2x = 18), with the exception of the polyploid *N. leucophylla*. Their study documented various chromosomal abnormalities including chromosome stickiness, pole deviations, and laggard chromosomes across multiple species. Cytomixis was observed during metaphase I and telophase II in *N. racemosa* (Figure E1) and during telophase I and telophase II in *N. pungens* (Figure J1). The transfer of chromosomes through this phenomenon was observed to be partial to complete, involving the entire genome. In *N. pungens*, cytomixis involved two or more pollen mother cells, while in *N. racemosa*, chromosomal transfer was strictly limited to pairs of cells. Kaur and Singhal [18] investigated cytomixis during meiosis in pollen mother cells of *N. govaniana*, documenting chromosomal exchanges between multiple cells during prophase. Their study also reported additional meiotic abnormalities including chromosome stickiness, asynchronous chromosome segregation, and laggard chromosomes in this species.



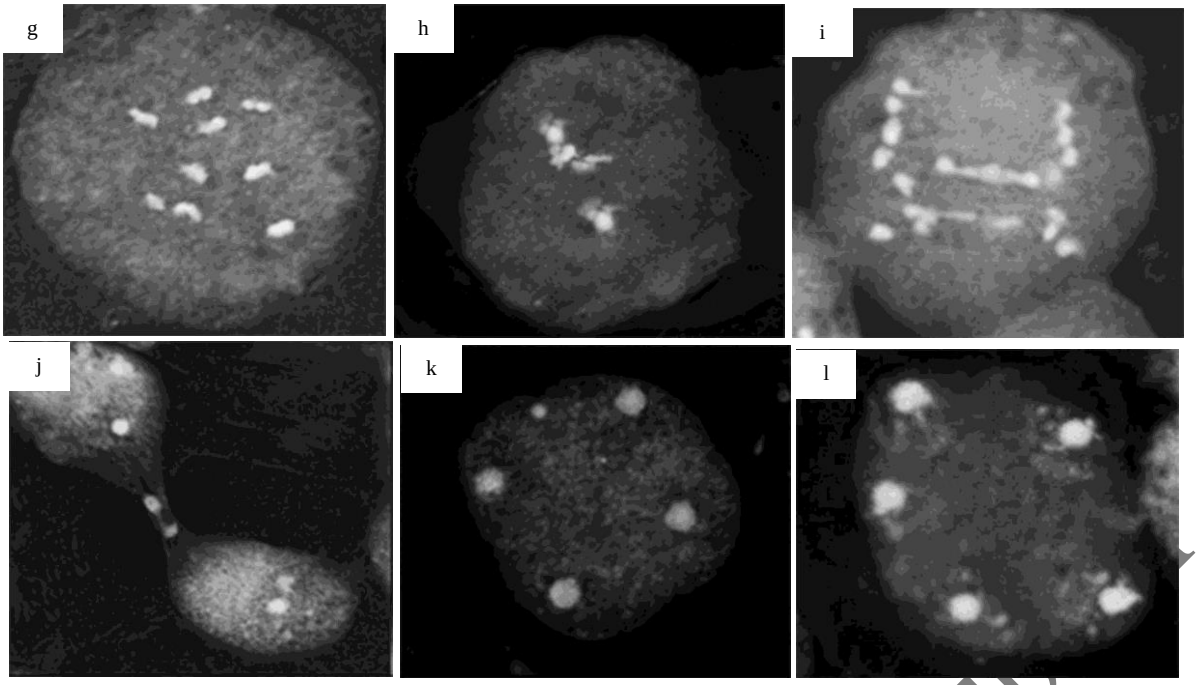


Fig. 1 Chromosomal aberrations at different stages of meiosis in *N. racemosa* (a-f) and *N. pungens* (g-l). a) Dyad with 9 bivalents. b) Chromosome stickiness. c) Premature chromosome separation. d) Laggard. e) Cytomixis in dyad. f) Tripolar. g) Dyad with 9 bivalents. h) Chromosome stickiness. i) Bridge. j) Cytomixis during telophase I. k) Micronucleus at telophase II. l) Pentapolar.

Asynchronous chromosome segregation was observed in both study species, manifesting as both premature and delayed separation events. While *N. racemosa* exhibited this abnormality exclusively during anaphase I, *N. pungens* displayed asynchronous segregation during both anaphase I and II. Representative premature chromosome separation during anaphase I in *N. racemosa* is shown in Figure C1. Asynchronous chromosome segregation may result in micronucleus formation during subsequent meiotic stages. This phenomenon was observed in both species, occurring with greater frequency in *N. pungens* (Figure K1).While normal meiosis produces four poles during telophase II, we observed abnormal tripolar and pentapolar configurations in *N. racemosa* at this stage (Figure F1). In contrast, *N. pungens* exhibited exclusively pentapolar formations (Figure L1), with no tripolar cells detected.

This study confirms the diploid nature of both *N. racemosa* and *N. pungens*. While the majority of pollen mother cells in both species exhibited normal meiotic divisions, we observed chromosomal abnormalities occurring at varying frequencies in each species. These meiotic irregularities may reduce seed viability and promote chromosomal diversity through aneuploidy and polyploidy. Frequency analysis revealed distinct aberration patterns:

In *N. racemosa* (descending order): chromosomal stickiness > cytomixis > laggard chromosomes > tripolar configurations > micronuclei > asynchronous separation

In *N. pungens* (descending order): chromosome stickiness > cytomixis > pentavalent formations > bridges > asynchronous separation > micronuclei > laggard chromosomes > tripolar configurations

The greater prevalence of meiotic abnormalities in *N. pungens* correlated with its significantly lower meiotic index compared to *N. racemosa*.

Meiotic Index

In this study, 300 cells from each species were analyzed. Tetrads with equal-sized cells were classified as normal, while those with unequal volumes or aberrations (e.g., micronuclei) were deemed abnormal. The meiotic index was calculated as 94% for *N. racemosa* and 86% for *N. pungens* (Table 1).

In Malik et al.'s [19] study, the meiotic index was calculated for *N. cataria*, *N. erecta*, *N. govaniana*, *N. laevigata*, and *N. nervosa*, with a reported value of 100 for all species. However, meiotic aberrations do not always affect tetrad formation. Occasionally, these aberrations are corrected during meiosis. However, when abnormal tetrads form, they may produce aneuploid and sterile pollen grains. This can ultimately reduce pollen germination capacity and viability [19].

Pollen Fertility

A study of pollen fertility in two *Nepeta* species showed that *N. racemosa* (89% fertility) produced more viable pollen grains than *N. pungens*, which exhibited 83% pollen fertility (Figure 2, Table 1). The regular and standard segregation processes of chromosomes, which occur without any structural modifications or alterations, play a crucial role in the successful development of normal and viable pollen grains that are essential for plant reproduction. The observed decline in pollen fertility among the studied plant specimens can be attributed to the occurrence of chromosomal aberrations, which were found in both species. Species that exhibit the most significant chromosomal irregularities during meiosis tend to demonstrate a marked reduction in their pollen fertility levels, while those with minimal chromosomal abnormalities generally maintain higher fertility rates. Previous studies have consistently supported this perspective, indicating that reduced pollen fertility directly results from chromosomal anomalies. These genetic alterations lead to the formation of aberrant and non-

viable pollen grains [24]. Jabeen *et al*. [25], in their study of pollen grains across several *Nepeta* species, reported that *N. laevigata* (90.9%) showed the highest pollen fertility percentage, whereas *N. hindostana* (64.2%) exhibited the lowest.

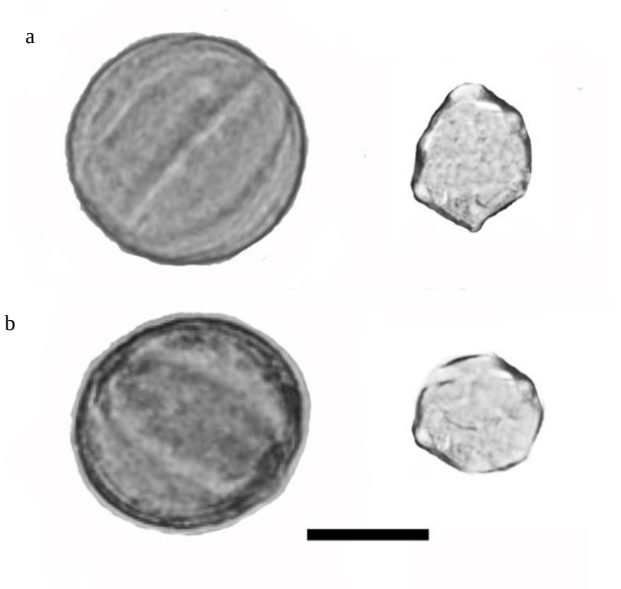


Fig. 2 The images show a fertile pollen grain on the left and a sterile pollen grain on the right. a: *N. racemosa*; b: *N. pungens*

CONCLUSION

Thorough cytological investigations conducted on species within the genus *Nepeta*, including *N. racemosa* and *N. pungens*, revealed the presence of a diploid prophase characterized by nine fundamental chromosomes. Nevertheless, the examined species exhibited a range of chromosomal irregularities, such as chromosomal stickiness, disruptions in normal separation during anaphase, cytomixis, and other anomalies, resulting in reduced pollen fertility. Notably, *N. pungens* showed a lower percentage of pollen fertility compared to *N. racemosa*, correlating with a higher frequency of meiotic abnormalities and a reduced meiotic index. It is important to note that meiotic irregularities do not always lead to adverse outcomes and may instead contribute to the emergence of genetic diversity. However, further research is essential to achieve a deeper understanding of these complex interactions. Given the well-documented variation in chromosome counts and ploidy levels among *Nepeta* species, such meiotic irregularities could potentially drive the evolution of aneuploidy and polyploidy within the genus. Studying meiotic abnormalities and their effects on pollen fertility is not only critical for advancing our knowledge of plant reproductive processes but also holds practical significance in applied fields like plant breeding and species conservation.

REFERENCES

1. Hedge C. Flora Iranica, Labiatae. In: Rechinger KH (ed) Academische Druck-U. Verlagsantalt Publication. 1982; 403-476.
2. Jamzad Z. Notes on the genus *Nepeta* L. (Lamiaceae, Nepetoideae). The Iranian Journal of Botany. 2009;15(2):141-145.
3. Love A. IOPB chromosome number reports LVII. Taxon. 1977;26(4):443-52.
4. Goldblatt P., Lowry Ii P. The index to plant chromosome numbers (Ipcn): Three decades of publication by the Missouri Botanical Garden come to An End. Annals of the Missouri Botanical Garden. 2011;98:226-27.
5. Bordbar F., Mahani Z.E., Mirtadzadini M. New chromosome counts in Lamiaceae from the flora of Iran-I. Journal of Applied Biological Sciences. 2021;15(3):310-317.
6. Hamant O., Ma H., Cande W. Genetics of meiotic prophase I in plants. Annual Review of Plant Biology. 2006;57:267-302.
7. Kharazian N., Shourabi S.Z., Yousefi M. Chromosome count and karyotype study of eleven *Nepeta* L. (Lamiaceae) species from Iran. Biological Diversity and Conservation. 2013;6(1):76-87.
8. Utsunomiya K.S., Bione N.C.P., Pagliarini M.S. How many different kinds of meiotic abnormalities could be found in a unique endogamous maize plant? Cytologia. 2002;67(2):69-76.
9. Cai X., Xu S.S. Meiosis-driven genome variation in plants. Current Genomics. 2007;8(3):151-61.
10. Chen Y.P., Zhao F., Peng H., Xiang C.L., Funamoto T. Chromosome numbers of 24 taxa of Lamiaceae from Southwest China. Caryologia. 2018;71(4):298-306.
11. Hasaninejad M., Jamzad Z., Afsharzadeh S., Saeedi H. Cytological studies on four endemic species of *Nepeta* L.(Lamiaceae). The Iranian Journal of Botany. 2020;26(1):62-66.
12. Hasaninejad M., Jamzad Z., Afsharzadeh S., Saeidi H. Chromosome counts of eight Iranian endemic species of *Nepeta* L. (Lamiaceae). Caryologia. 2021;74(1):53-61.
13. Pagliarini M.S. Meiotic behaviour of economically important plant species: the relationship between fertility and male sterility. Genetics and Molecular Biology. 2000;23:997-1002.
14. Defani-Scoarize M.A. Evaluation of meiotic behavior in double-cross maize hybrids and their parents. Maydica. 1995;40:319-24.
15. Baptists-Giacomoelli F.R., Pagliarini M.S., Almeida J.L. Elimination of micronuclei from microspores in a Brazilian oat (*Avena sativa* L.) variety. Genetics and Molecular Biology. 2000;23:681-84.
16. Tantray Y.R., Jan I., Wani M.S., Singhal V.K., Gupta R.C. Chromosome numbers and meiotic behavior in some species of Asteraceae from high altitudinal regions of Kashmir Himalayas. Journal of Asia-Pacific Biodiversity. 2021;14:590-606.

17. Saggoo M.I.S., Srivastava D.K., Grewal P. Meiotic studies in 14 species of the *Nepeta* L. (Lamiaceae) from cold desert regions of Lahaul-Spiti and adjoining areas of Northwest-Himalaya, India. Cytologia. 2011;76(3):231-36.
18. Kaur M., Singhal V.K. First report of cytomixis and meiotic abnormalities in *Nepeta govaniana* from Solang Valley, Kullu District, Himachal Pradesh. Cytologia. 2014;79(2):227-233.
19. Malik R.A., Gupta R.C., Singh V., Bala S., Kumari S. New chromosome reports in Lamiaceae of Kashmir (northwest Himalaya), India. Protoplasma. 2017;254:971-85.
20. Duda S.C., Marghitaş L.A., Dezmirean D., Duda M., Margaoan R., Bobiş O. Changes in major bioactive compounds with antioxidant activity of *Agastache foeniculum*, *Lavandula angustifolia*, *Melissa officinalis* and *Nepeta cataria*: Effect of harvest time and plant species. Industrial Crops and Products. 2015;77:499-507.
21. Consolaro M.E.L., Pagliarini M.S. Spontaneous chromosome stickiness in microsporocytes of *Centella asiatica* (L.) Urban (Umbelliferae). Cytologia. 1996;61(1):57-61.
22. Nicklas R.B., Ward S.C. Elements of error correction in mitosis: microtubule capture, release, and tension. The Journal of Cell Biology. 1994;126(5):1241-1253.
23. Wani B.A., Magray J.A., Qadir R.U., Javid H., Ganie A.H., Nawchoo I.A. Meiotic bottlenecks compromises reproductive success by impairing pollen fertility and seed set in *Swertia thomsonii* C.B. Clarke—an important endemic medicinal herb of western Himalaya. Cytologia. 2022;87(4):323-30.
24. Raina A.T.Y., Khan S. Assessment of bio-physiological damages and cytological aberrations in cowpea varieties treated with gamma rays and sodium azide. Plos One. 2023;18(7):e0288590.
25. Jabeen S.Z.M., Ahmad M., Ali M.A., Elshikh M.S., Makhkamov T., Mamarakhimov O., Yuldashev A., Khaydarov K., Gafforov Y., Baysunov B., Mammadova A.O., Botirova L., Sultana S., Majeed S., Rozina A.S, Abid A., Rahmatov A. Micrometer insights into *Nepeta* genus: pollen micromorphology unveiled. Micron. 2024;177:103574.