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Unraveling the meiotic puzzle: chromosome count, meiotic behaviour, and reproductive challenges in *Phlomis cashmeriana* Royle ex Benth. from the Kashmir Himalaya

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Meiotic stability is crucial for maintaining reproductive success and genetic diversity in plants, especially in montane regions like the Himalaya, where fluctuating environmental conditions can disrupt normal chromosome behavior. *Phlomis cashmeriana* Royle ex Benth., a medicinally important species, has not previously been studied for the meiotic behavior and its impact on reproductive output. This study presents the first comprehensive meiotic analysis of *P. cashmeriana* across three populations in the Kashmir Himalaya, focusing on chromosome count, meiotic behavior, pollen fertility, and seed set. While most of the Pollen Mother Cells (PMCs) exhibited normal meiosis, several meiotic abnormalities were recorded, including chromosome stickiness, laggards, unoriented bivalents, and interchromosomal connections. Chromosome stickiness (11.48%) was the most prominent abnormality, particularly during diakinesis and metaphase I across all the study sites. These irregularities, likely influenced by high UV radiation and low temperatures characteristic of the region, were associated with reduced pollen viability (67.65–74.50%) and seed set (54.40–59.75%) across the studied populations. Such reproductive impairments may compromise the long-term survival and genetic resilience of *P. cashmeriana*, potentially limiting its adaptive capacity under ongoing changing environmental conditions. These findings highlight the broader ecological significance of meiotic behavior as a determinant of reproductive fitness and evolutionary potential in Himalayan flora. Understanding these cytological constraints is vital for developing informed, long-term conservation and management strategies for *P. cashmeriana* and other threatened montane species. Future research should explore the genetic basis of these abnormalities and assess population viability under shifting climate conditions.

KEYWORDS

meiotic behavior, abnormalities, pollen fertility, seed set, conservation

Introduction

The genus *Phlomis* L. is one of the largest genera of the subfamily Lamioideae (Lamiaceae) with about 93 species distributed throughout the world (POWO, 2024). These species have been divided into two main sections: *Phlomoides* and *Phlomis* (Rechinger, 1982; Albaladejo et al., 2005). The diagnostic character for separating the sections is corolla morphology. The species of genus *Phlomis* have corolla with a curved upper lip and trifid lower lip with large median and smaller lateral lobes while the species belonging to *Phlomoides* have corolla with straight upper lip and trifid lower lip with subequal lobes (Azizian and Moore, 1982).

In Kashmir Himalaya the genus *Phlomis* is represented by two species namely, *Phlomis cashmirica* Wells and *Phlomis cashmeriana* Royle ex Benth (POWO, 2024). The later species native to Kashmir Himalaya has been selected for the present study. *P. cashmeriana*, locally known as Darshol is used in traditional medicine systems to treat wounds and bone fractures. Phytochemical analyses have identified a wide range of bioactive compounds, including flavonoids, triterpenes, alkaloids, phenolics, tannins, coumarins, shikimic acid derivatives, and steroids (Qadir et al., 2022, 2024a; Hussain et al., 2024). Consequently, this plant species is reported to have different biological activities, including antioxidant, anti-inflammatory, anti-bacterial anti-fibrillation, immunosuppressive, and antidiabetic properties (Qadir et al., 2024b; Hussain et al., 2024). In addition to its medicinal value, *P. cashmeriana* is also used as an ornamental plant (Sarkhail et al., 2007; Shang et al., 2016).

Over the past few decades, *P. cashmeriana* has experienced severe anthropogenic pressures like habitat degradation, and overharvesting due to its high therapeutic demand leading to a rapid decline in its populations across natural habitats (Ganie et al., 2019; Wani et al., 2022). These pressures often result in habitat fragmentation and population isolation, which limit gene flow and reduce genetic diversity. Small and fragmented populations are more prone to inbreeding, which can lead to the accumulation of deleterious alleles and heightened expression of meiotic abnormalities. Such genetic consequences impair reproductive success by reducing pollen viability, seed set, and overall fitness. As a result, conserving this species has become a critical priority. For developing effective conservation strategies, detailed knowledge about the meiotic behavior of a plant species is crucial as it offers valuable scientific insights into identifying genetic factors influencing reproduction, viability, and long-term survival (Armstrong and Jones, 2003).

Meiosis is fundamental for regular cell division, gamete formation, and the maintenance of chromosomal stability—all crucial for sustaining plant populations, especially in challenging montane environments (Golubovskaya, 1979; Pagliarini, 2000). Disturbances (mutations) during meiosis result in abnormalities, leading to variations in the genetic constitution (Kaul and Murthy, 1985; Jiang et al., 2009). Studies on other plant species have shown that such abnormalities—like chromosomal stickiness, laggards, bridges, and micronuclei—can significantly impair gamete viability and seed production, ultimately affecting population sustainability (Pagliarini, 2000; Singhal et al., 2018; Kaur and Singhal, 2019). These cytogenetic disruptions are often associated with environmental stress, polyploidy, or genomic instability and have been widely used as indicators of reproductive constraints in threatened and endemic species. In fragile ecosystems like the Kashmir Himalaya, where climatic extremes and anthropogenic disturbances can intensify reproductive limitations, understanding the meiotic behavior of *P. cashmeriana* is particularly important. Such insights can help identify the reproductive constraints contributing to its reduced reproductive success and inform targeted conservation strategies.

Although the Kashmir Himalaya harbors a rich diversity of medicinal plants, *Phlomis cashmeriana* was chosen for this study due to its restricted geographic distribution, high medicinal importance, and lack of prior cytogenetic research. Its rapid population decline makes it a priority species for understanding reproductive constraints among Himalayan medicinal plants.

This study does have certain limitations, including a focus on a limited number of populations and an emphasis solely on cytological parameters. Broader ecological interactions, genetic diversity assessments, and long-term population monitoring were beyond the current scope. Nonetheless, this work establishes a cytogenetic baseline and highlights key reproductive bottlenecks that can guide future conservation efforts for this and other threatened species in the region.

To date, *Phlomis cashmeriana* has not been investigated for its chromosome count, meiotic behavior, and the effect of meiotic irregularities on pollen fertility and seed set. Given the ecological and medicinal significance of this species, we hypothesize that disturbances in meiotic processes contribute to reduced pollen fertility and seed set, thereby impacting the reproductive success and long-term survival of *P. cashmeriana* in its natural habitat. Based on this hypothesis, we addressed the following research questions: (a) What is the chromosome number and meiotic behavior of *Phlomis cashmeriana*? (b) What are the different meiotic abnormalities in *P. cashmeriana* across selected sites? (c) How do meiotic abnormalities affect pollen viability, fruit, and seed set in the target plant?

By addressing these questions, this study provides essential cytogenetic insights that can aid in understanding the reproductive constraints of *P. cashmeriana*, ultimately informing its conservation and management strategies.

Materials and methods

Study area

Kashmir Himalaya is located at the north-western extremes of India and lies between latitude 32°17′ and 37°05′ North and longitude 72°31′ and 80°20′ East. This Himalayan region has a wide elevational gradient, diverse geological formations, and varied climatic zones, supporting a rich diversity of medicinal plants (Tali et al., 2019; Ganie et al., 2020). For the current study, 3 sites *viz*., Daksum (Site 1), Hillar Naar (Site 2), and Jawahar Tunnel (Site 3) were selected across the Kashmir Himalaya based on the ease of access, habitat characteristics, and abundance of the target plant population (Figure 1). Herbarium specimens of *P. cashmeriana*

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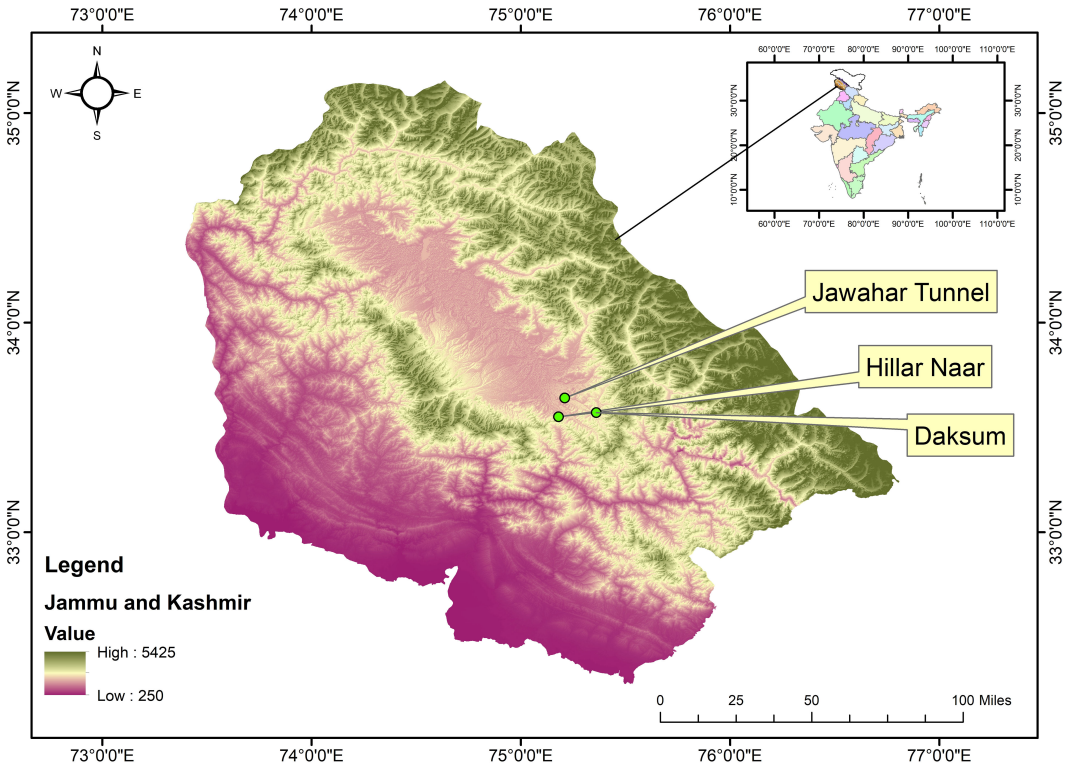

FIGURE 1
Map showing study area and sampling sites in Kashmir Himalaya.

collected from selected sites were deposited in the University of Kashmir Herbarium (KASH) under voucher specimen numbers 2941, 2942, and 2943.

Study species

Phlomis cashmeriana (Cashmere Sage) is a densely woolly multi-stem perennial herb native to Tadzhikistan, Pakistan, Afghanistan, and West Himalaya (POWO, 2024). The species mainly grows on open exposed slopes at an elevational range of 2000—2800 m asl. The stem is simple or branched, and the rootstock is woody. Leaves are lanceolate-oblong and leathery, covered with hairs. The inflorescence is verticillaster with dense lilac-purple flowers, and corolla lobes pale purple.

Analysis of pollen mother cells

For meiotic studies, young floral buds were randomly collected during the early morning hours in April 2023 from three wild populations—Daksum, Hillar Naar, and Jawahar Tunnel. This period coincides with the pre-anthesis stage of *P. cashmeriana*, when meiotic activity is at its peak, allowing accurate observation of meiotic stages. The buds were fixed in fresh Carnoy's solution (3 ethanol: 1 glacial acetic acid) for 24 hours, and then stored in freshly prepared 70% ethanol at 4°C in a refrigerator till further analysis. The squashing technique involving crushing individual anthers in 2% acetocarmine was used for slide preparation. To ensure high-quality slide preparations, only appropriately sized buds were selected, and squashing was performed gently to minimize mechanical damage and avoid overlapping cells. Each slide was carefully screened, and only well-spread, clearly stained PMCs were used for meiotic analysis. The anther squash method is widely used for its reliability in visualizing meiotic stages and chromosomal behavior. To reduce potential errors such as poor staining, cell overlap, or mechanical damage, buds were carefully staged, and squashes were performed gently and consistently. Multiple cells from each stage were analyzed to ensure reproducibility and accuracy.

The freshly prepared slides from each population were analyzed for chromosome counts and meiotic behaviour in Pollen Mother Cells (PMCs) at different stages (diplotene, diakinesis, metaphase I, II, anaphase I, II, telophase and tetrad stage). This procedure was repeated for two consecutive years (2023–2024). Only good preparations were used for chromosome counts and analyzing meiotic behavior. Photomicrographs of PMCs with ideal stages (for chromosome counts, meiotic abnormalities, and sporads) were taken using a trinocular microscope (Leica) integrated with Leica software (magnification 100x). The percent meiotic abnormality (stickiness, laggards, unoriented bivalents, interchromosomal connections) was calculated by the following formula:

$$\text{Meiotic abnormality (\%)} = \frac{\text{Total number of abnormal cells (particular stage) observed}}{\text{Total number of cells observed}} \times 100$$

From each population, buds were collected from 25 plants and the anthers from these floral buds were squashed to ascertain the meiotic behavior and the results were obtained from about 50 slides with different meiotic stages from each population.

Pollen fertility estimation

To determine pollen viability, three methods were employed, in the first method; the mature pollen grains were mounted in Fluorescein diacetate (FDA) solution and incubated for 3–5 min. The pollens with fluorescent and non-fluorescent cytoplasm were treated as fertile and non-fertile, respectively. In the second method, the ready-to-dehisce mature anthers were placed in 1% tetrazolium chloride for one hour and squashed to check for viability. In the third method, mature and undehisced anthers were squashed in 1% aniline blue-lactophenol and observed after 15 minutes. These methods have been widely validated in previous studies for their reliability in evaluating pollen viability. FDA staining is sensitive to enzymatic activity in viable pollen (Heslop-Harrison and Heslop-Harrison, 1970), while tetrazolium chloride assesses metabolic activity through dehydrogenase function (Norton, 1966). Aniline blue-lactophenol has been effectively used to identify viable pollen based on the intensity of cytoplasmic staining (Shivanna and Rangaswamy, 2012). The use of multiple techniques enhances the accuracy and robustness of pollen fertility estimation.

The percentage pollen fertility was determined by the following formula:

$$\text{Pollen fertility (\%)} = \frac{\text{Number of pollen grains stained}}{\text{Total number of pollen grains observed}} \times 100$$

Calculation of fruit set

To assess the fruit set, we recorded the total number of flowers at the peak blooming period and the number of mature fruits post-fruiting period on selected, tagged plants. The fruit set percentage was calculated using the formula:

$$\text{Fruit set(\%)} = \frac{\text{Number of fruits}}{\text{Number of fowers}} \times 100$$

Calculation of seed set

For estimating the seed set, plants were selected randomly, tagged, and scored for the number of seeds produced per plant following Lubbers and Christensen (1986).

$$\text{Seed set(\%)} = \frac{\text{Total number of seeds produced}}{\text{Total number of ovules borne by the plant}} \times 100$$

Results

The meiotic analysis of *P. cashmeriana* revealed that the species is diploid, possessing a chromosome number of 2n = 2x = 20 at all three study sites. This was confirmed by the presence of 10 bivalents during diplotene, diakinesis, and metaphase-I (Figures 2a-d). The bivalents exhibited both terminal and interstitial chiasmata, and with ring-shaped bivalents particularly evident at diakinesis (Figure 2d). The present investigation revealed normal anaphasic segregation of 10:10 chromosomes in pollen mother cells (PMCs) at anaphase I and II (Figures 2g, n), followed by normal telophase and tetrad formation (Figure 2o). In addition to normal meiosis, a range of abnormalities were identified in PMCs, including chromosomal stickiness, lagging chromosomes, chromosome bridges, out-of-plate bivalents, and inter-chromosomal connections.

Among 3,648 PMCs analyzed at different stages, chromosome stickiness was the most prominent meiotic abnormality observed, occurring mainly at diakinesis and metaphase I (Figure 2e), in all the 3 selected sites. Stickiness caused chromosomes to form compact masses, losing their distinct morphology and appearing as dense clumps in affected PMCs. At metaphase I, the frequency of stickiness was highest at Site 3 (31.12 ± 1.15%), followed by Site 2 (23.03 ± 1.81%) and Site 1 (20.59 ± 1.03%) (Table 1). Out-of-plate bivalents observed during metaphase I did not show significant variation among sites. This abnormality was recorded in 3.81 ± 1.46%, 4.61 ± 0.62%, and 5.87 ± 1.87% of PMCs at Sites 1, 2, and 3, respectively (Figure 2f, Table 1). Likewise, PMCs with Lagging chromosomes were recorded during anaphase I and II (Figures 2k-m), with the highest frequencies observed at Site 3 (17.14 ± 1.28% and 11.91 ± 1.80%, respectively), followed by Site 2 (13.18 ± 0.59% and 5.36 ± 2.77%) and Site 1 (9.64 ± 1.21% and 4.36 ± 1.60) (Table 1). Lagging chromosomes are those that fail to migrate properly to the poles during anaphase, often due to spindle defects or chromosomal adhesions. Chromosome bridges, resulting from incomplete separation of chromatids or unresolved chiasmata, were most prevalent at Site 3 (18.24 ± 0.61% in anaphase I, 12.64 ± 1.31% in anaphase II), followed by Site 2 (16.55 ± 1.99% and 6.07 ± 1.80%) and Site 1 (11.09 ± 1.92% and 5.03 ± 1.44%) (Figures 2h-k; Table 1). Similarly, inter-chromosomal connections were observed in PMCs during diplotene and diakinesis, occurring at frequencies of 13.43 ± 1.47%, 15.77 ± 1.20%, and 17.39 ± 0.69% at Sites 1, 2, and 3, respectively. All these meiotic irregularities resulted in the formation of non-fertile pollen grains and reduced seed set.

The pollen fertility (Figure 2p), fruit set, and seed set of *P. cashmeriana* varied significantly among study sites (Table 2; Figure 2n). Pollen viability was assessed using three staining techniques: aniline blue, 2,3,5-triphenyl tetrazolium chloride (TZ), and fluorescein diacetate (FDA). Viability was consistently highest at Site 1 and lowest at Site 3 across all staining methods. At Site 1, pollen viability was maximum, with 79.57 ± 2.28% for aniline blue, 73.70 ± 1.57% for

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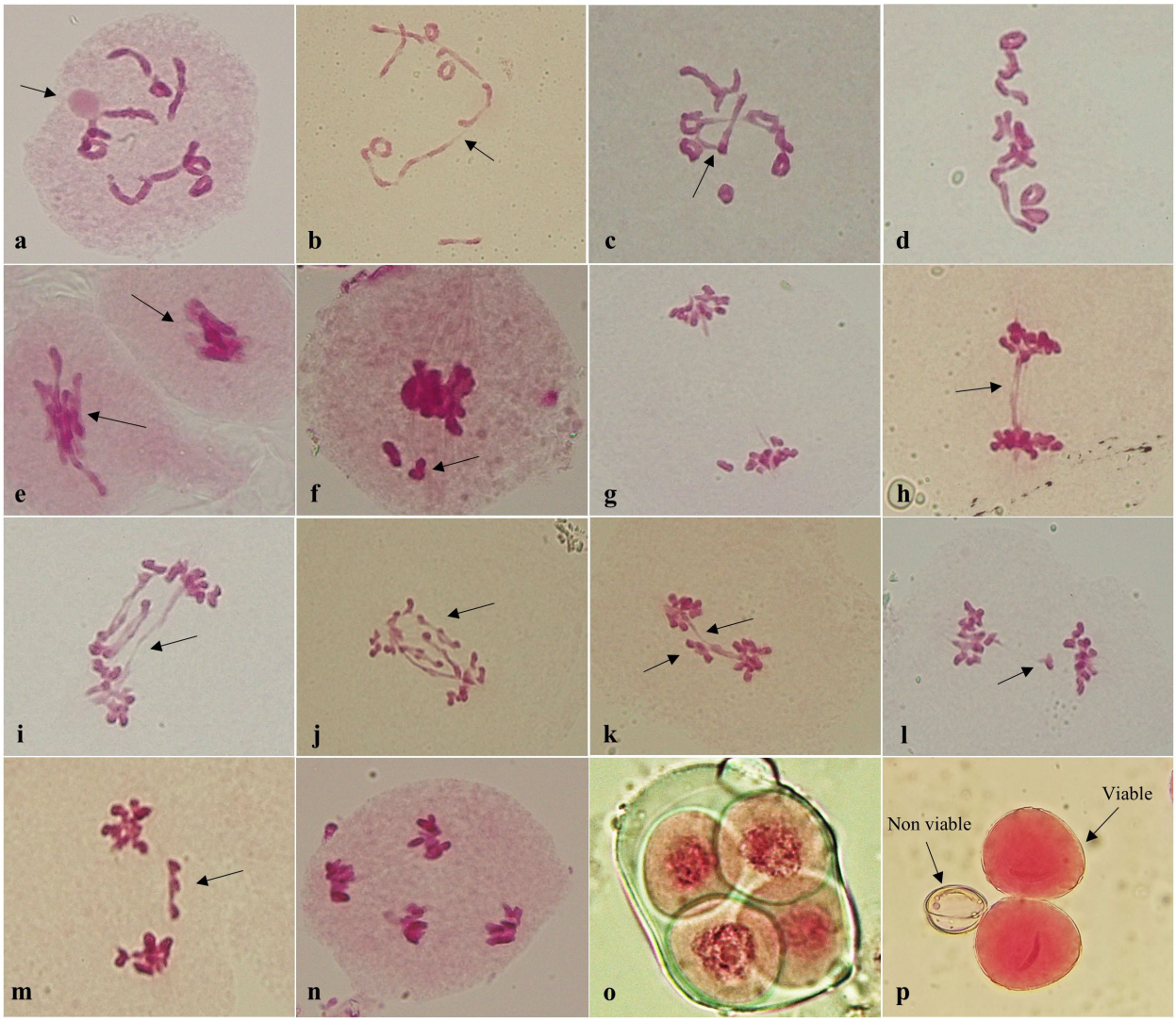

FIGURE 2
(a-p) Meiotic behavior of *Phlomis cashmeriana*. **(a)** PMC at diplotene with 10 bivalents and prominent nucleolus (arrowed); **(b, c)** PMC at diakinesis with interchromosomal connections (arrowed); **(d)** PMC showing 10 bivalents at metaphase I; **(e)** PMCs with chromatin stickiness at metaphase I (arrowed); **(f)** Out of plate bivalent at metaphase I (arrowed); **(g)** Anaphase I showing 10:10 chromosome distribution at each pole; **(h)** PMC with single bridge at anaphase I (arrowed); **(i, j)** PMCs with multiple bridges at anaphase I (arrowed); **(k)** A bridge and laggards at anaphase I (arrowed); **(l, m)** PMCs with laggards at anaphase I (arrowed); **(n)** PMC at Anaphase II, **(o)** Tetrad, **(p)** Viable and non-viable pollens.

TZ, and 73.70 ± 1.57% for FDA. In contrast, Site 3 exhibited the lowest pollen viability, with 65.83 ± 1.04%, 64.67 ± 1.45%, and 62.30 ± 3.11% for aniline blue, TZ, and FDA, respectively (Table 2). Similarly, both fruit set and seed set showed site-dependent variation. Site 1 showed the highest fruit set (69.77 ± 2.84%) and seed set (62.43 ± 1.85%), whereas Site 3 exhibited the lowest values, with 59.40 ± 1.25% fruit set and 49.43 ± 2.79% seed set (Table 2). These findings indicate a progressive decline in reproductive success from Site 1 to Site 3, coinciding with increased frequencies of meiotic abnormalities.

Discussion

In the present study, we determined the chromosome number and detailed meiotic behaviour of *Phlomis cashmeriana* from Kashmir Himalaya, India. This study presents the first report on chromosome count and meiotic analysis for this species. Our results revealed a basic chromosome number of x=10 in the studied populations. Plant populations from all three study sites showed the same chromosome count of 2n=2x=20, confirming the diploid nature of the species. Previous reports reveal that all the studied *Phlomis* species have chromosome counts of 2n=2x=20 (Aparicio and Albaladejo, 2003; Ozdemir et al., 2014; Yousefi et al., 2018; Sadeghian et al., 2021). Azizian and Moore (1982) reported that *Phlomis* species in the section *Phlomis* are characterized by having 2n=20 chromosomes, while those in the section *Phlomoides* have 2n=22 chromosomes. Ozdemir et al. (2014) also reported a chromosome count of 2n=20 for *P. grandiflora* H.S. Thomps. and *P. lunariifolia* Sm., both members of the *Phlomis* section *Phlomis*.

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TABLE 1 Percentage of PMCs with normal and irregular meiotic behavior observed at different meiosis stages in *Phlomis cashmeriana*.

Population	Meiotic stages	Total PMCs	Normal PMCs	PMCs with Stickiness	Out of Plate Bivalents	PMCs with Laggards	PMCs with Bridges	Inter-chromosomal Connections
Site 1	Diplotene/ Diakinesis	350	81.29 ± 1.44*ab	5.28 ± 1.18^{f}	0	0	0	13.43 ± 1.47^{a}
	Metaphase I	315	75.6 ± 2.88^{c}	20.59 ± 1.03^{b}	3.81 ± 1.46^{a}	0	0	0
	Anaphase I	318	73.61 ± 2.33^{cd}	5.66 ± 0.95^{f}	0	9.64 ± 1.21^{bc}	11.09 ± 1.92^{bc}	0
	Anaphase II	298	82.89 ± 2.00^{a}	7.71 ± 0.96^{de}	0	4.36 ± 1.60^{e}	5.03 ± 1.44^{e}	0
Site 2	Diplotene/ Diakinesis	317	76.03 ± 1.86^{bc}	8.2 ± 1.26^{cd}	0	0	0	15.77 ± 1.20^{a}
	Metaphase I	304	72.37 ± 1.89^{cde}	23.03 ± 1.81^{ab}	4.61 ± 0.62^{a}	0	0	0
	Anaphase I	296	63.51 ± 1.65^{ef}	6.76 ± 1.58^{ef}	0	13.18 ± 0.59^{ab}	16.55 ± 1.99^{ab}	0
	Anaphase II	280	81.07 ± 2.67^{ab}	7.5 ± 1.23^{de}	0	5.36 ± 2.77^{de}	6.07 ± 1.80^{de}	0
Site 3	Diplotene/ Diakinesis	310	70.29 ± 2.00^{de}	12.32 ± 2.03^{c}	0	0	0	17.39 ± 0.69^{a}
	Metaphase I	295	63.1 ± 2.27^{ef}	31.12 ± 1.15^{a}	5.87 ± 1.87^{a}	0	0	0
	Anaphase I	290	57.34 ± 0.55^{f}	7.26 ± 1.23^{de}	0	17.14 ± 1.28^{a}	18.24 ± 0.61^{a}	0
	Anaphase II	275	67.09 ± 2.92^{e}	7.46 ± 1.42^{de}	0	11.91 ± 1.80^{b}	12.64 ± 1.31^{b}	0

*Mean ± SD, means with the different superscript letters in the same column are significantly different at $p \leq 0.05$.

Other studies have also confirmed a chromosome count of 2n=20 in other species within the genus *Phlomis*, including *P. italica* L., *P. lychnitis* L., *P. herba-venti* L. var. *tomentosa*, and *P. purpurea* L., Boiss (Mateu, 1986); *P. cypria* Post var. *cypria* (Yildiz and Gücel, 2006); *Phlomis composita* (Aparicio and Albaladejo, 2003); *Phlomis olivieri* Benth. (Yousefi et al., 2018) and *Phlomis anisodonta* and *Phlomis pachyphylla* (Sadeghian et al., 2021).

The present study revealed that meiosis was normal in most (about 80%) of the observed PMCs; however, some PMCs showed meiotic abnormalities such as stickiness, laggards, unoriented bivalents, inter-chromosomal connections, resulting in sterile pollen grains and low seed set. The meiotic abnormalities were also observed in some other species of the family Lamiaceae and genus *Phlomis* growing in India (Singh et al., 2018). Chromosome stickiness was the most prominent abnormality observed across all three populations of *P. cashmeriana* and has been widely reported in various flowering plants, including species from the western Himalaya (Tripathi and Kumar, 2010; Kaur and Singhal, 2012, 2014; Rana et al., 2013; Rashid et al., 2021: Rashid et al., 2022; Wani et al., 2022). Chromosome stickiness is thought to arise from defects in the function of specific non-histone proteins, such as DNA topoisomerase II and peripheral proteins, which are essential for proper chromatid segregation (Gaulden, 1987; Azad et al., 2022). Some researchers attribute chromosome stickiness to genetic and environmental factors (Nirmala, 1996; Baptista-Giacomelli et al., 2000; Bione et al., 2000; Saggoo and Farooq, 2011; Jeelani et al., 2012; Rashid et al., 2021). In the alpine to sub-alpine regions of the Himalaya, harsh environmental conditions such as low temperatures, high UV radiation, and shorter growing seasons likely intensify meiotic stress, contributing to chromosomal stickiness, failure of segregation at anaphase I, and other irregularities (Weitz et al., 2021; Fu et al., 2024). The formation of laggards and bridges during meiosis is considered a syndrome indicating reduced control over the meiotic process (Jones and Brumpton, 1971; Sofi et al., 2023). Laggards may result from delayed terminalization and stickiness at chromosome ends (Kaur and Grover, 1985; Rashid et al., 2022), while bridges are likely caused by chiasma interlocking in bivalents, chromatin stickiness, and late disjunction of bivalents (Tarar, 1980; Rashid et al., 2022). The presence of laggards may also be attributed to irregular spindle formation, cytoskeletal disruptions, and other cellular changes (Vasek, 1962; Potapova and Gorbsky, 2017).

The irregularities in the meiotic course—such as chromatin stickiness, interchromosomal connections, and formation of lagging chromosomes and bridges can lead to defective sporad formation and reduced pollen viability (Risso-Pascotto et al., 2005; Kumar and

TABLE 2 Pollen fertility, fruit set and seed set of *Phlomis cashmeriana* recorded during the present study.

Characters	Study Sites		
	Site 1	Site 2	Site 3
Pollen viability (%) i) Aniline blue	79.57 ± 2.28*a	72.90 ± 2.15^{b}	65.83 ± 1.04^{c}
ii) TZ	74.97 ± 1.69^{a}	67.17 ± 2.58^{ab}	64.67 ± 1.45^{b}
iii) FDA	73.70 ± 1.57^{a}	68.70 ± 1.98^{b}	62.30 ± 3.11^{c}
Fruit set (%)	69.77 ± 2.84^{a}	67.03 ± 3.20^{b}	59.40 ± 1.25^{c}
Seed set (%)	62.43 ± 1.85^{a}	58.93 ± 1.0^{b}	49.43 ± 2.79^{c}

*Mean ± SD, means with the different superscript letters in the same column are significantly different at $p \leq 0.05$.

Singhal, 2008, 2012; Rashid et al., 2021). These meiotic abnormalities disrupt microsporogenesis and contribute to pollen sterility, negatively impacting the reproductive success of species in natural habitats (Lattoo et al., 2006; Kumar and Singhal, 2008, 2012; Kumar, 2010; Rashid et al., 2021). In this study, a clear link was observed between the frequency of meiotic abnormalities and reduced reproductive success in *P. cashmeriana*. Site 1, with the highest incidence of abnormalities, showed the lowest pollen viability, fruit set, and seed set, whereas Site 3, with more regular meiotic behavior, exhibited higher reproductive output. This highlights the direct impact of meiotic stability on fertility and subsequent fruit and seed production (Pagliarini, 2000; Souza et al., 2006; Kumar and Singhal, 2012). Such abnormalities can result in the formation of abnormal gametes, leading to reduced genetic recombination, impaired gene flow, and lower genetic diversity among populations—ultimately threatening long-term survival and adaptability.

Furthermore, in the context of environmental change, factors such as habitat degradation, climate-induced stress, and pollinator decline may intensify reproductive constraints. Pollen sterility, combined with declining pollinator populations due to habitat fragmentation, and global climate change can significantly reduce fertilization success and gene exchange between fragmented populations.

While meiotic abnormalities appear to be a key factor, other ecological and genetic influences—such as pollinator limitation, environmental stress, and low genetic diversity—may also contribute to reduced reproductive output (Ashman et al., 2004; Barrett, 2010). Limited seed dispersal and low seedling recruitment caused by reproductive bottlenecks could further restrict gene flow among fragmented populations, increasing inbreeding and reducing population connectivity—factors that must be considered in conservation planning (Aguilar et al., 2006; Browne and Karubian, 2018).

Understanding the causes of the meiotic instability along with the breeding behavior of the species is crucial for the conservation and management of threatened and native plants (Souza et al., 2006). Pollination plays a crucial role in determining fruit and seed set in *P. cashmeriana*. The species is pollinated by four different insect species, including *Xylocopa* sp., *Bombus tunicatus*, *Apis mellifera*, and a wasp. Although ambophilous, *P. cashmeriana* primarily relies on entomophily (Roof, 2024). Given its dependence on insect pollination, pollen sterility caused by meiotic irregularities could limit successful fertilization, ultimately reducing reproductive success. The potential decline in pollinator populations may exacerbate these challenges, especially in sensitive ecosystems where both plants and pollinators are vulnerable to environmental pressures. The effectiveness of pollinators, along with the availability of viable pollen, significantly influences seed production. In species exhibiting high pollen sterility, even efficient pollinators may fail to ensure adequate seed set, leading to lower reproductive output. The long-term survival of a plant species depends on effective reproduction and constant recruitment of new individuals to maintain healthy populations (Corlett, 2007). In this study, significant meiotic irregularities observed in the species could contribute to low seed production, leading to a gradual decline in its population size. Previous studies on some species have shown that various meiotic abnormalities can reduce plant fertility or even lead to complete male sterility (Pagliarini, 2000; Wani et al., 2022). From an evolutionary perspective, persistent meiotic instability can reduce fitness and limit adaptive potential, making species more susceptible to environmental change. Given the restricted distribution of the target species, increasing anthropogenic pressures and the presence of meiotic bottlenecks could significantly contribute to a decline in its population size within its native range in the Kashmir Himalaya, thereby increasing the risk of extinction (Najar et al., 2024) within its native range in the Kashmir Himalaya, which may decline further in the near future. Therefore, it is important to devise conservation strategies for this valuable medicinal plant species.

Conclusions and conservation implications

This study presents the first comprehensive meiotic analysis of *Phlomis cashmeriana*, a valuable Himalayan medicinal herb, confirming its diploid chromosome number (2n = 2x = 20) and identifying critical meiotic irregularities that may compromise reproductive success. Although most Pollen Mother Cells (PMCs) exhibited normal meiosis, abnormalities such as chromosomal stickiness, laggards, unoriented bivalents, and interchromosomal connections were observed—among which chromosomal stickiness was most frequent. These disruptions were associated with reduced pollen viability and seed set, indicating potential reproductive bottlenecks that could limit natural regeneration.

Environmental factors characteristic of high-altitude habitats, including low temperatures and elevated ultraviolet radiation, are likely contributors to these meiotic disturbances. Given the species' medicinal significance and the increasing anthropogenic pressures on its native habitats, such reproductive challenges may critically threaten population viability and long-term survival. Thus, integrating cytogenetic insights into broader conservation frameworks is vital.

The observed meiotic irregularities—particularly chromosomal stickiness, laggards, bridges, and interchromosomal connections—impair microsporogenesis and reduce reproductive efficiency. In the montane ecosystems of the Kashmir Himalaya, where populations are already impacted by habitat degradation and unsustainable harvesting, these reproductive constraints pose a serious risk to species persistence. Limited seed output and reduced genetic recombination due to meiotic instability may further decrease genetic diversity, increasing vulnerability to environmental stressors and human activities.

Urgent conservation interventions are warranted, including habitat protection, regulation of wild harvesting, and promotion of *in situ* conservation measures. Simultaneously, the development of *ex situ* strategies—such as seed banking and micropropagation—will be crucial for safeguarding germplasm and supporting restoration initiatives. Future research should integrate cytogenetic assessments with studies on breeding systems, genetic diversity, and ecological adaptability to inform effective conservation and sustainable utilization. This study underscores

the importance of reproductive biology in shaping conservation priorities for *P. cashmeriana* and other ecologically significant Himalayan endemics.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

RQ: Data curation, Formal analysis, Methodology, Software, Visualization, Writing – original draft. HJ: Data curation, Formal analysis, Methodology, Software, Visualization, Writing – review & editing. AG: Methodology, Supervision, Validation, Writing – original draft. BW: Data curation, Writing – review & editing. IN: Conceptualization, Investigation, Methodology, Supervision, Validation, Writing – review & editing. JM: Conceptualization, Data curation, Methodology, Visualization, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The author(s) declare that no Generative AI was used in the creation of this manuscript.

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