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Meiotic behavior and its implications on the reproductive success of *Arnebia euchroma* (Royle ex Benth.) I.M.Johnst. (Boraginaceae), an important medicinal plant of Trans-Himalaya

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Abstract. The present study reports the chromosome number, meiotic behavior and its relation with pollen fertility and seed set of *Arnebia euchroma* (Royle ex Benth.) I.M.Johnst. The species shows a chromosome count of $2n = 2x = 14$. The meiotic abnormalities such as chromatin stickiness, cytomixis, laggard formation, chromosomal bridges, were also observed in the Pollen Mother Cells (PMCs) of the target plant species. The linear model of regression showed a significant reduction of seed set with increasing meiotic abnormality and correlation analysis highlighted positive relationship between pollen viability and seed set. Meiotic abnormalities within the species hinder its reproductive process, causing a decline in reproductive efficiency. This study highlights the importance of addressing these intrinsic factors in future conservation programs to prevent a decline in the species population in nature.

Keywords: chromosome number, meiotic abnormalities, pollen viability, seed set.

INTRODUCTION

Reproduction is an essential and vital stage in the life history of plants and a necessary natural process for survival, multiplication and evolution (Wani et al. 2022). The meiotic abnormalities are one of the factors that affect the reproductive success of plant species (Wani et al. 2022). The decline in seed set and loss of genetic variability, are some of the repercussions of the meiotic depression (Cohen et al. 2021). The studies on reproduction, meiotic behaviour and seed biology may aid in identifying the key factors that affect the reproductive success of species as well as sustenance or survival of its population (Gan et al. 2013). The study is also critical for developing strategies for sustainable utilisation and effective conservation measures of threatened species (Rashid et al. 2022a).

The restricted distribution pattern of *Arnebia euchroma* (Royle ex Benth.) I.M.Johnst., in Himalaya is further declining and this species is at risk of disappearing because of habitat deterioration, fragmentation and climate change (Lal et al. 2020; Sofi et al. 2022a). Therefore, understanding of the meiotic behavior may improve the knowledge about reproduction and inherent bottlenecks of the species. The foundation of conservation biology, reintroduction, and mass production and multiplication rely on quality of germplasm. Understanding the biological characteristics of the species will unveil the attributes of the germplasm (Ma et al. 2022).

Therefore, the present study was conducted to study the meiotic behaviour and its impact on pollen fertility and seed set. Understanding the aspects of meiotic behaviour, pollen fertility and seed production may provide vital clues for sustainable development of this important medicinal plant species of the Trans-Himalaya.

MATERIAL AND METHODS

Study area

The study was conducted in the four studied sites (Table 1) of the trans-Himalayan range of Ladakh, India. The area is dominated by mountains and harsh climatic conditions, however, regarded as cold biodiversity hot-spot because of presence of rich species diversity (Sofi et al. 2022b).

Analysis of pollen mother cell (PMC) meiosis

During the present investigation, floral buds of *A. euchroma* from four different natural populations were fixed for meiotic studies. Young, unopened flower buds of suitable sizes were randomly collected from various plants within each studied population. The collected buds were preserved for 24 hours in Carnoy's fixative, which is a mixture of ethanol, chloroform, and glacial acetic acid (6: 3: 1 v/v). The materials were then transferred to 70% ethanol and kept at 4°C under refrigeration until use. Anthers were squashed in 1% propio-carmine and slides were observed under microscope. Pollen mother Cells (PMCs) were observed to count the chromosome number and meiotic abnormalities if any. Photomicrographs of chromosomes were taken from freshly prepared slides using an EVOS XL microscope. Chiasmata number was counted for cells at diplotene.

Pollen fertility estimation

Pollen fertility was estimated by collecting 10-15 fresh floral buds with dehiscing anthers followed by squashing in 2% acetocarmine and glycerol (Marks 1954). Well-filled pollen grains with uniformly stained

Table 1. Proportion of Meiotic abnormalities in PMCs of *Arnebia euchroma*.

Population /Site	Meiotic stage	No. of PMCs	Normal PMCs	PMCs with stickiness/ clumping	PMCs with laggards	PMCs with chromatin bridges	PMCs with cytoplasmic channels	PMCs with satellite chromosomes
Matayen	Diplotene	52	52	-	-	-	-	-
	Diakinesis	47	47	-	-	-	-	-
	Metaphase-I	93	56	7	9	7	14	-
	Anaphase-I	20	-	11	6	3	-	-
Karpokhar	Diplotene	24	24	-	-	-	-	-
	Diakinesis	17	13	-	-	-	-	-
	Metaphase-I	54	20	6	-	-	11	4
	Anaphase-I	13	-	3	-	10	-	-
	Telophase-I	6	-	-	-	-	-	-
Changoyal	Diplotene	34	34	-	-	-	-	-
	Metaphase-I	42	16	9	6	-	-	-
	Anaphase-I	16	-	16	-	-	-	-
Rungdum	Diplotene	19	19	-	-	-	-	-
	Metaphase-I	29	21	8	-	-	-	-
	Anaphase-I	21	-	20	-	1(6:3)	-	-
	Telophase-I	7	7	-	-	-	-	-
	Telophase-II	5	5	-	-	-	-	-

cytoplasm were considered fertile while shrivelled and unstained pollen grains were counted as sterile. Percentage pollen fertility/viability was calculated as follows:

$$\text{Pollen viability} = \frac{\text{Number of fertile pollen grains}}{\text{Total number of pollen grains observed}} \times 100$$

Seed set calculation

Individual plants were randomly chosen, labelled, and tallied according to the Lubbers and Christensen (1986) technique for the quantity of seeds produced per plant in order to estimate the seed set.

$$\text{Seed set} = \frac{\text{Total number of seeds produced per flower}}{\text{Total number of ovules borne per flower}} \times 100$$

Statistical analysis

Statistical analysis including linear model regression and correlation analysis was used to depict relationship between different parameters; the analysis was carried using the software r.

RESULTS

All the four populations of *A. euchroma* matched in having 14 chromosomes in their PMCs revealing $2n = 14$ (diploid chromosome count) and $x=7$ (haploid chromosome count), i.e., $2n = 2x = 14$. The PMCs were analysed at diplotene, diakinesis, metaphase-I, anaphase-I and telophase I.

In Matayen population, a total of 212 cells were scanned with 52 cells (24.52%) at diplotene (Fig. 1 A), 47 cells (22.16%) at diakinesis (Fig. 1 B), 93 cells (43.86%) at metaphase and 20 cells (9.43%) at anaphase I (Table 1). At diakinesis, we found perfect 7IIs and 6 cells had 2IVs+4IIs (Fig. 1 B). At metaphase-I, studied PMCs with6 IIs (Figs. 1 C, D) perfect 7IIs (Figs. 1 E, F), clumping (Fig. 1 G), laggards, chromatin bridges and cytoplas-
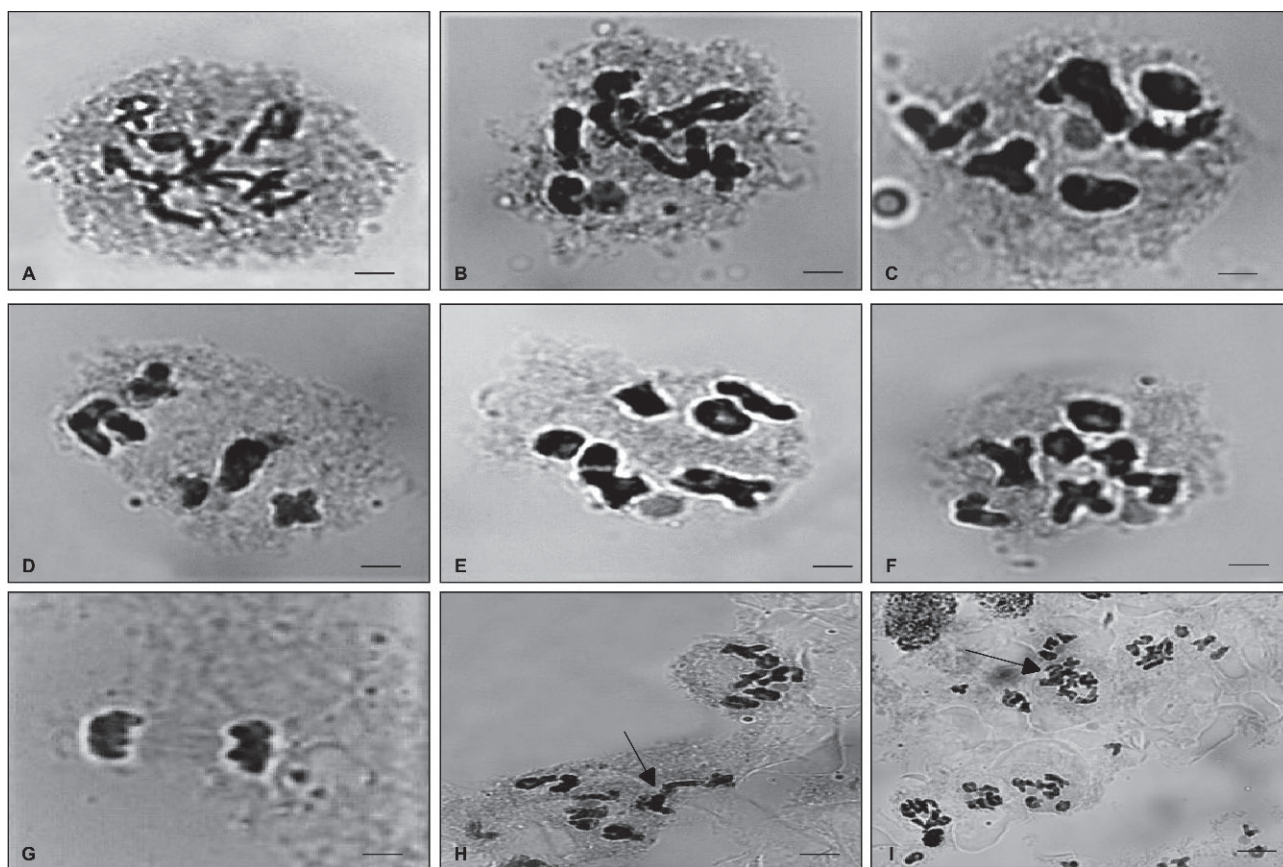

Figure 1. (A) A PMC at diplotene, (B) A PMC at diakinesis, (C & D) A PMC at metaphase with 6 IIs, (E & F) A PMC at metaphase with 7 IIs, (G) A PMC at anaphase showing clumping of chromosomes, (H, & I) PMCs at metaphase showing migration of chromatin material. Scale bars =10 μm.
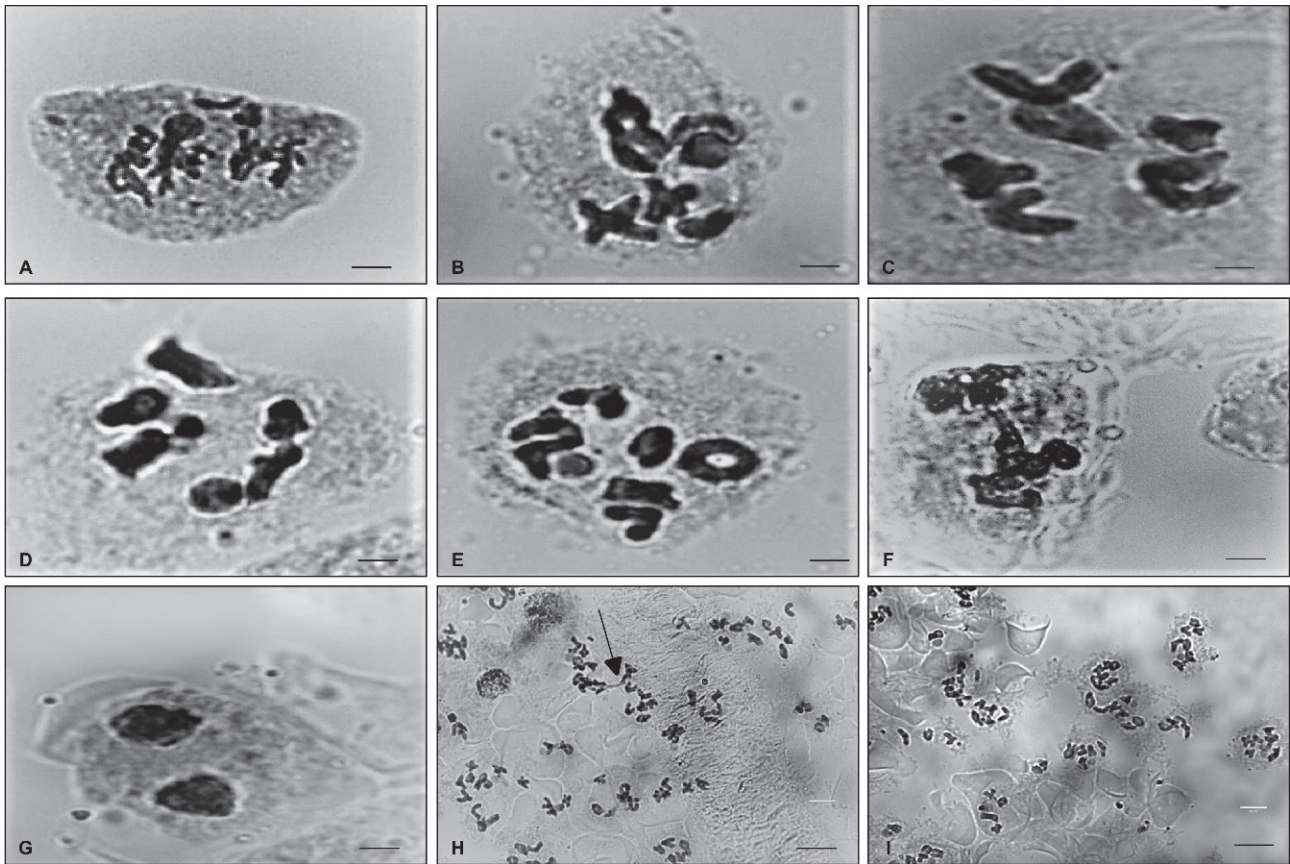
Figure 2. (A) A PMC at diplotene, (B) A PMC at diakinesis, (C) A PMC at metaphase with 5 IIs, (D) A PMC at metaphase with 6 IIs and 1 satellite, (E) A PMC at metaphase with 7 IIs, (F) A PMC at anaphase with chromatin bridge formation, (G) A PMC at telophase, (H & I) PMCs showing migration of chromosomes through cytoplasmic channels. Scale bars =10 μm.

mic channels (Figs. 1 H, I) were observed. At anaphase-I no PMC with normal segregation was observed out of 20 cells scanned (Table 1). Chiasmata frequency per PMC calculated at diplotene in this population is 10.9 while RI calculated is 17.9.

In Karpokhar population, a total of 114 cells were scanned at different stages of meiosis with 24 cells (21.05%) at diplotene (Fig. 2 A), 17 cells (14.91%) at diakinesis, 54 cells (47.36%) at metaphase, 13 cells (11.40%) at anaphase I and 6 cells at telophase-I (5.26%) (Table 1). At diakinesis stage perfect 7IIs and 4 cells with 1IV+5IIs (Fig. 2 B) were found. At metaphase-I, 5IIs (Fig. 2 C), 6IIs (Fig. 2 D), cells with perfect 7IIs (Fig. 2 E), clumping, satellite chromosomes (Fig. 2 D) and cytoplasmic channels (Figs. 2 H, I) were observed. At anaphase-I no PMC with normal segregation was observed, PMC's with chromatin bridges (Fig. 2 F) and clumping (Fig. 2 F) were recorded (Table 1). Chiasmata frequency per PMC calculated at diplotene in this population is 11.3 while RI calculated is 18.3.

In Changoyal, a total of 92 cells were scanned at different stages of meiosis with 34 cells (36.95%) at diplotene (Fig. 3 A), 42 cells (45.65%) at metaphase and 16 cells (17.39%) at anaphase I (Table 1). At metaphase-I, perfect 7IIs (Fig. 3 B), 5IIs (Fig. 3 C), 14 cells with Is (Fig. 3 D), clumping (Fig. 3 E) and laggards (Fig. 3 F) were observed. At anaphase-I no PMC with normal segregation was observed out of 16 cells scanned, all the cells with huge clumping (Fig. 3 G) were recorded. Chiasmata frequency per PMC calculated at diplotene in this population is 12.4 while RI calculated is 19.4.

Similarly, in Rungdum population, a total of 81 cells were scanned at different stages of meiosis with 19 cells (23.45%) at diplotene (Fig. 4 A), 29 cells (35.80%) at metaphase, 21 cells (25.92%) at anaphase I, 7 cells (8.64%) at telophase-I and 5 cells (6.17%) at telophase-II (Table 1). At metaphase-I, perfect 7IIs (Figs. 5 B-E) and clumping (Figs. 4 F, G) containing 7IIs were observed. At anaphase-I a PMC with abnormal segregation of 6:3 (Fig. 4 G) with a chromatin bridge was observed. However,
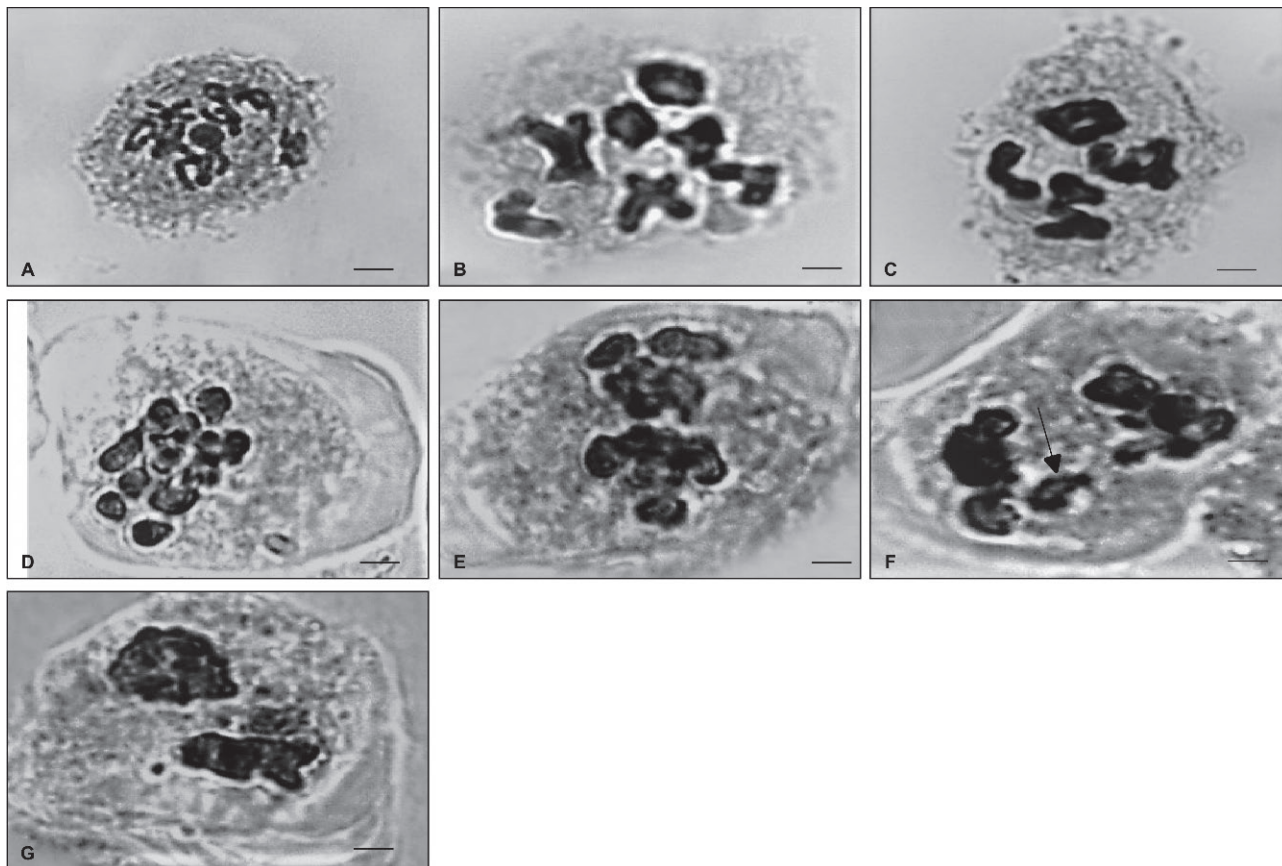
Figure 3. (A) A PMC at diplotene, (B) A PMC at metaphase with 7 IIs, (C) A PMC at metaphase with 5 II s, (D) A PMC at metaphase with 14 Is, (E) A PMC at metaphase showing clumping of chromosomes, (F) A PMC at metaphase showing laggards, (G) A PMC at anaphase. Scale bars =10 µm.

in rest of cells clumping were observed. Chiasmata frequency per PMC calculated at diplotene in this population is 12 while RI calculated is 19.

The average proportion of meiotic irregularity, pollen fertility, and seed set observed in the four studied populations of the *Arnebia euchroma* is shown in Table 2. It was evident from results that the percentage of pollen fertility and seed set declined with increase in percentage of meiotic anomalies in the four populations under study.

The linear regression between seed set (%) and meiotic abnormality (%) revealed a significant (p<0.001) decline of seed set with the increase in meiotic abnormality in the studied sites of target plant species (Fig. 5). The correlation analysis also depicted negative relationship between meiotic abnormality and pollen viability (r = -0.96), meiotic abnormality and seed set (r = -0.99) and positive correlation between pollen viability and seed set (r = 0.98), (Fig. 6).

DISCUSSION

The present study has documented chromosome number and meiotic behaviour of *Arnebia euchroma* from the four natural populations. The study confirms chromosome number $2n = 2x = 14$ in accordance with previous studies (Sharma et al. 2013). The presence of chromosomal stickiness, cytomixis, laggard formation and other chromosomal abnormalities have been observed in all the studied populations. The most prevalent chromosome abnormality observed was chromosomal stickiness and chromosomal clumping in all studied sites. During the current study, cytomixis which involves the transfer of chromatin material primarily between proximal PMCs (Guan et al. 2012) was also observed. As a result of the chromatin material being transferred between PMCs, the irregularities associated with this transfer including chromosomes stickiness, sterility of pollen grains (Páez et al. 2021) was observed. This phenomenon functions as an additional potential
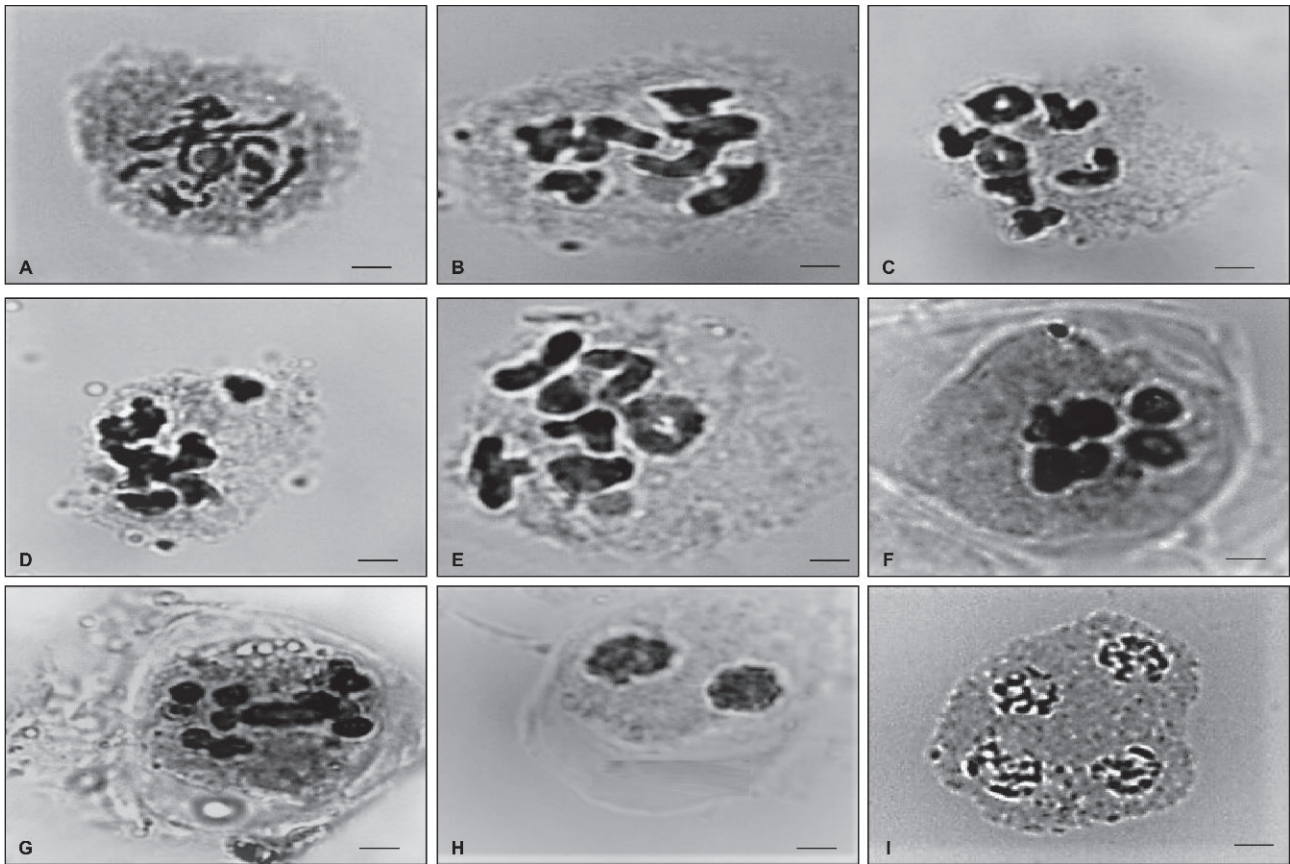
Figure 4. (A) A PMC at diplotene, (B-E) PMCs at metaphase with 7 IIs, (F) A PMC at metaphase showing clumping, (G) A PMC at anaphase showing chromatin bridge, (H) A PMC at telophase I, (I) A PMC at telophase II. Scale bars =10 μm.

genetic recombination mechanism (Mursalimov and Deineko 2017; Rashid et al. 2022b) and is a natural meiotic aberration that may have evolutionary importance (Singhal et al. 2018). The cytoplasmic channels and chromatin migration has also been reported in meiocytes of *Arnebia hispidissima* (Baquar and Husain., 1969). The phenomenon of cytomixis and its effects on meiotic developments and pollen fertility has been reported in various taxa of Himalayan region (Tantary et al. 2021). Cytomixis causes various meiotic abnormalities which include interbivalent connections, chromosome stickiness, laggards, bridges, late disjunction, pyknotic chromatin and unorganized chromatin threads (Singhal and Kumar 2008) as observed in present study also. Unreduced gametes or aneuploids and polyploids plants with certain morphological traits can both result from cytomixis (Falistocco et al. 1995; Arabi et al. 2022) leading to increase or decrease of basic chromosome count of the species (Tantary et al. 2021).

Chromosome clustering in *A. euchroma* was associated with both the intense (entire genome affected) and mild (few chromosomes affected) chromosomal stickiness that was observed in some PMCs. The presence of clumping distorts the chromosome shape making it difficult to determine the chromosome count. In the majority of cases stickiness was observed at Metaphase-I, and Anaphase-I in the present study. The cause of chromosome stickiness in many plant species has been attributed to environmental and genetic causes, as well as the interplay between the two (Pessim et al. 2015; Arabi et al. 2022). The sticky chromatin in various flowering plants have been attributed to gene mutation that disrupts proteins which in normal circumstances helps the chromosomes stay apart and prevents adherence (Tantary et al. 2021). However, the low temperature and high UV exposure in the alpine habitats (Rashid et al. 2022b) may be responsible for the observed chromosomal stickiness in the *Arnebia euchroma*. Chromosomal stickiness and the ensuing lack of chromosomal segregation at anaphase I can be suspected as the cause of meiotic abnormalities in the current investigation as seen in case of other studies (Masoud et al. 2010; Sin-
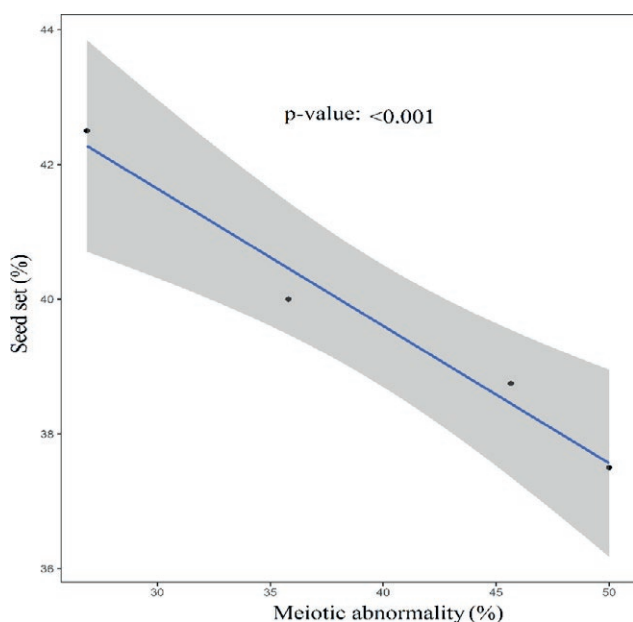
Figure 5. Relationship between seed set and meiotic abnormality as shown by linear model of regression with 95% confidence interval highlighted in grey shade.

Table 2. Coordinates of sites and mean meiotic irregularity, pollen fertility, and seed set observed in the 4 studied populations of the *Arnebia euchroma*

Population	Latitude	Longitude	Mean		
			Meiotic abnormality	Pollen viability	Seed set
Matayen	34.37	75.59	26.88	85	42.5
Karpokhar	34.24	75.97	50.00	67	37.5
Changoyal	34.35	76.13	45.65	75	38.75
Rungdum	34.05	76.20	35.80	78	40

Figure 6. Relationship between meiotic abnormalities, seed set, and pollen viability as depicted by correlation plot.

gh et al. 2022). The chromosome bridges may occur as a result of chiasma interlocking in bivalents, and laggards may develop as a result of delayed terminalisation of stickiness at the ends of chromosomes (Chaudhari and Chaudhary 2012). Pollen fertility may be totally or partially impacted by chromatin stickiness, depending on degree of presence (Rana et al. 2013). The presence of five and six bivalents and few quadrivalents in some cells of the studied populations as against the normal seven bivalent formation can lead to development of aneuploids in the target plant species. The rarity of quadrivalents points to translocation instead of segmental allopolyploidy as the cause (Dawson et al. 1993; Lattoo et al. 2006). Normal segregation of chromosomes occurs a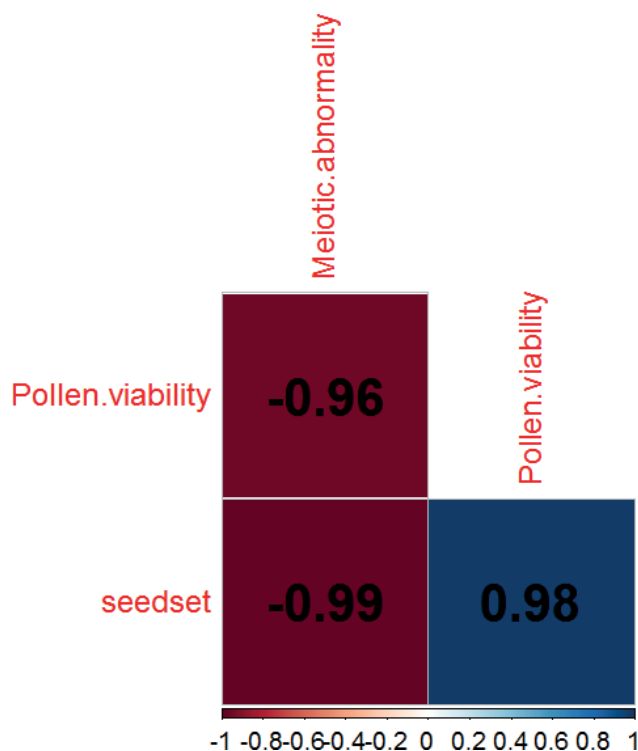s a result of optimal spindle orientation and chiasma development, while any deviation can lead to laggard formation (Arabi et al. 2022). The frequency of abnormal PMCs decreased as the cells progressed during meiosis, as seen by a comparison of the stages during the course of meiosis. A recovery mechanism that may successfully combat the anomalous behaviour of PMCs and restores fidelity during division with cell cycle advancement (Grewal and Rani 2022). The meiotic aberrations present in the target species can lead to abnormal microsporogenesis without micronuclei. This type of atypical meiotic behaviour results in sterile pollen grains, which lowers pollen viability, seed and fruit set as seen during the present study.

The intrinsic factors (meiotic abnormalities) associated with the species is a constrain in its reproductive process. Therefore, these factors can lead to loss of reproductive efficiency with low seed and fruit formation and reduction in the pollen fertility of the species (Rashid et al. 2022a; Rashid et al. 2022a). It is evident from the current study that designing of effective future conservation programs of the target species should also consider the intrinsic factors that hold capacity to reduce the population of the species in nature.

AUTHOR CONTRIBUTIONS

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