



Research article

Phenology, pollination biology and breeding behavior of *Lasiurus indicus*: an important forage species in arid climate of the Thar desert

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Abstract

Variations in plant morphology and reproductive characteristics, along with the pollination mechanism, were studied in *Lasiurus indicus*, a key forage species in the arid conditions of the Thar Desert. The pollen viability and stigma receptivity were highest during anther indehiscence (82.40 and 96.33%) and dehiscence phases (79.54 and 92.71%). The pollination syndrome observed is anemophily. Breeding experiments revealed that *L. indicus* is a cross-fertilization species. The grain set (%) was highest in open pollination (100%), followed by geitonogamous (nearly 15%) and self-pollination (nearly 3%). The study will be helpful in a range of genetic investigations and improvement programs aimed at conserving and managing this distinctive plant species, particularly for rangeland management programs.

Keywords: Breeding behaviour, *Lasiurus indicus*, Phenology, Pollen, Rangeland, Stigma

Introduction

Sewan (*Lasiurus indicus* Henr.), a rhizomatous C₄ grass species commonly referred to as the 'king of desert grasses', has effectively adapted to the arid climate of the Thar Desert. This grass is distributed in India, Pakistan, Afghanistan, Iran, Iraq, Saudi Arabia, and certain African countries. In India, its presence is confined to the northwestern hot arid zone, particularly in the western regions of Rajasthan, encompassing Jaisalmer, Bikaner, and Barmer districts, where approximately 6.0 million hectares of open pasture land are dominated by *L. indicus* grass (Kumawat *et al.*, 2019). It is an erect, tufted and branched grass up to 2m tall with linear, acuminate, convolute, or flat leaves. Inflorescence is almost 8-14 cm long, erect, white, and possesses 13-23 spikelets. This species has sparse hair on leaves and bracts. The florets are both hermaphrodite and staminate types. Its seeds are covered with glumes and other appendages and the grains are yellowish to brown in color.

Sewan grassland, characterized by its perennial nature (capable of surviving up to 20 years), flourishes in areas receiving annual rainfall below 200 mm, particularly on sandy plains, low dunes, and hummocks; therefore, it can be utilized for rangeland rehabilitation (Goswami *et*

al., 2020a). Known for its high palatability and nutritional value, this grass serves as a valuable resource for livestock (Rani *et al.*, 2022). The process of reproduction plays an important role in ensuring the survival and succession of plants. Insights into reproductive biology, particularly pollination mechanisms and breeding systems, are fundamental for interpreting the phenological adaptations that support plant survival and extreme conditions (Rani and Sanyal, 2024). Despite its significance, the pollination biology and breeding behavior of this species have not been well documented until now. Understanding the reproductive biology of Sewan grass is crucial for the conservation and management of this distinctive grass species, especially in the context of rangeland management programs.

Materials and Methods

Plant materials and study sites: The plant material consists of approximately 150 plants of *L. indicus* of a released variety, CAZRI Sewan-1, planted at 2m inter row spacing, at the field research area, ICAR-CAZRI, Jodhpur, Rajasthan. Out of the total plant population, 30 plants were covered individually with different covering

materials (Malasiya cloth, cotton white khadi cloth and plastic clear sheet) before flowering during 2020-21 and 2021-22. Ten plants were used as controls, while the remaining plants were used for recording floral morpho-phenological observations under field conditions. Nearly 1000 single spikes covered with parchment paper were harvested at different durations (30th, 35th day and 40th day) of spike emergence for grain formation observation during both seasons. About 300 random spikes were tagged, out of which 150 were harvested at the 21st day of spike initiation, and the remaining 150 at 1-2 upper seed maturity for observing anthesis-related traits. Single plants of sewan grass were grown in isolation at different locations to observe pollination behavior. Data analysis was performed by using SPSS version 23.0 (IBM, USA) software (IBM Corp. 2023).

Phenological traits: Plant phenological traits like germination, first leaf emergence, flowering and fruiting period were recorded. Different floral quantitative traits, viz., days of anthesis, spike length, number of spikelets/inflorescence, number of pollens/anther, number of florets/spike, stamen length and pistil length were monitored and recorded with applied standard deviations in the field directly using a hand lens. Anthesis (time of commencement, peak and termination in flowering) was observed as per Dafni (1992). Floral morphological stages were examined, starting from the single spike to the ovule (under Olympus SZX9 Stereo-zoom microscope and dissecting microscope). To determine the different pheno-events and anthesis in *L. indicus*, 5 inflorescences of 20 randomly selected individual plants were tagged and observed regularly until inflorescence maturation for two consecutive years.

Pollination mechanism: Outcrossing index (OCI) as per Dafni (1992) and pollen-to-ovule (P/O) ratio as per the criteria of Cruden (1977) were calculated on the basis of floral morphological traits. Pollen count was determined as per Dafni (1992). Average number of pollen grains/floret = Pollen count/anther × Number of anthers/floret. The number of pollen grains/inflorescence was estimated by multiplying the number of pollen grains/floret by the number of florets/inflorescence, respectively. *In-vitro* pollen longevity was evaluated at 0, 1, 2 and 3 hours after anther dehiscence on wet and dry blotting papers from 10 anthers. Ten microscopic fields at 10X were used to observe and count the number of viable and non-viable pollen grains. To check anemophily, pollen dispersion was determined by hanging slide experiments at varying distances from plants (Mulugeta *et al.*, 1994). However, to estimate the pollen dispersion from different parts (upper, middle and lower) of an individual spike, pollen traps were constructed just surrounding the inflorescences and pollen grains were observed. Temperature and relative

humidity were recorded during the pollen dispersion periods.

To observe the dynamic changes in pollen viability (%) using 2% acetocarmine solution (Shivanna and Tandon, 2014), pollen grains were collected every hour from 6 am to 6 pm for observation under a Leica DM 3000 compound microscope. Pollen viability (%) was calculated as the number of viable pollen grains/total number of pollen grains × 100. Stigmas collected at different time intervals (from 6 am to 6 pm) were immersed in 3% hydrogen peroxide solution on a cavity glass slide and continuously examined for bubble formation and change of reaction solution color (Dafni, 1992) under an Olympus SZX9 stereo-zoom microscope (at 12.5X). Stigma receptivity (%) was measured as per Tong (2022).

Breeding behaviour of *L. indicus*: Pollination experiments with four different types of treatments (spontaneous self-pollination test, geitonogamous pollination test, open pollination test and isolation pollination test) were performed to determine the breeding behavior and grain setting (%) was considered as the end product of effective pollination. Grain setting (%) = Total number of grains in inflorescences that set grains/total inflorescences harvested.

Results and Discussion

Phenology and floral traits of *L. indicus*: Data were recorded on different morpho-phenological characters. Wide range of variation was observed for days of anthesis (3–19), spike length (7.7–13.8 cm), number of tillers/plant (46–262), number of spikelets/inflorescence (13–29), number of florets/spike (65–145), number of pollens/anther (27–60), *etc.* (Table 1). The number of hermaphrodite (14016–71288) and staminate (21024–106932) flowers in an inflorescence was found to be highly variable. Floral morphological observations were recorded, starting from the single spike to the ovule (Fig 1a-1f). The flowering period of a spike lasted for 7-21 days and two flower types (bisexual and staminate) with zygomorphic symmetry were found. First-time spike initiation happened up to February in some plants. The morpho-phenological observations, including traits for morphology, color and fragrance, indicate congruent adaptations of flowers for different pollination mediators (Fenster *et al.*, 2004; Conner and Sterling, 1995).

Pollination mechanism: Taking into consideration the average inflorescence diameter, temporal separation in anther dehiscence and stigma receptivity and positioning level of anthers and stigma in a floret, the outcrossing index (OCI) calculated was 2 indicating the breeding system of *L. indicus* was facultative autogamy. OCI is a general indication of the breeding system and not the final proof for it. The number of pollen grains and ovules

Table 1. Floral morphology of *L. indicus*

Characters	Sample size	Range	Mean	SD
No. of days to first flowering	100	54-213	112.40	41.43
Lifespan of an individual inflorescence	100	15-59	32.17	8.80
No. of inflorescences/plant	90	73-315	182.30	64.34
Peduncle length (cm)	100	3.6-20.3	12.42	3.06
Inflorescence length (cm)	100	7.7-13.8	10.06	1.18
No. of spikelets/inflorescence	100	13-29	21.49	3.22
Length of spikelets (cm)	100	0.9-1.2	1.04	0.09
No. of florets/inflorescence	100	65-145	107	16
No. of florets/plant	100	7008-35644	19796	7689
No. of Pollens/anther*	100	27-60	40	6
No. of Pollens/inflorescence*	100	7862-15504	11453	2150
Stamen length (mm)	50	0.3-0.4	0.31	0.03
Pistil length (mm)	50	0.2-0.3	0.22	0.04

*Average of 10 microscopic fields at 10X magnification

were counted to calculate the P/O ratio, which comes out to be 121.82 ± 18.21 , respectively, which also indicate towards breeding system with facultative autogamy. Additionally, our findings from different pollination treatments indicated that there is outcrossing in *L. indicus* (Table 2).

Pollen count per anther, floret and per inflorescence is 40.61 ± 6.07 (with a range of 27-60), 121.82 ± 18.21 (range of 81-179) and 11453.5 ± 2150.02 (range of 7862-15504), $n=100$. Pollens were counted for the purpose of estimating the pollen-ovule ratio, pollination mechanism, pollination efficiency and gene flow studies. Insignificant differences in the values of pollen longevity were observed on wet and dry blotting papers during initial hours (after 0 and 1 hour) of anther dehiscence while in the later durations (after 2 and 3 hours), this difference was quite significant. The pollen longevity values on wet and dry blotting papers were 81.06% and 76.51% (at 0 hr), 78.94% and 73.41% (at 1 hr), 76.78% and 66.69% (at 2 hrs) and 70.83% and 53.46% (at 3 hrs) after anther dehiscence.

Hanging slide experiments showed ample amounts of pollen grains on the slides hanging at different distances, making outcrossing indispensable in *L. indicus*. The pollen count was found more during 10 am (806 and 1071 pollen grains/cm²) and 11 am (754 and 1036 pollen grains/cm²) at 4 m and 8 m distances and thereafter a gradual decline in the pollen count was observed. Similarly, with the modified method for pollen count estimation (Fig 2a-2b), slides placed in funnels at different heights of spikes exhibited maximum pollen score from the middle part of the spikes (545 pollen grains/cm²) while the minimum pollen score from the lower part (373 pollen grains/cm²). Environmental factors such as temperature

and relative humidity influenced anther dehiscence, which is observed more during higher temperature levels.

The viability of pollen and the receptivity of the stigma are essential factors that play a crucial role in enabling the successful initiation of the interaction between pollen and the pistil (Kumari *et al.*, 2021). Enhanced stigma receptivity on the pistil plays a pivotal role in the processes of pollination, fertilization, and the overall reproductive success of plants, ensuring the development of grain (Castro *et al.*, 2008).

In the current experiment, pollen viability was found to vary during different day hours (Fig 3a). The highest pollen viability (nearly 80%) was observed during 10 am-12 noon, when pollen grain was released at the anther indehiscence stage, revealing that *L. indicus* sustains a significant degree of sexual fertility during this phase. Thereafter, the pollen viability decreased dramatically to a minimum of 11.17%. Almost a similar trend of variation was observed for stigma receptivity, which was at its peak at 10 am (100%) during the anther indehiscence stage and declined thereafter (Fig 4a), reaching a minimum of 29.21% in a mature spike.

Breeding behavior of *L. indicus*: A wide range of variation was recorded for spike and grain formation in plants under different covering materials. In white Khadi cloth-covered plants, there was good plant growth and spikes formed in all plants, but grains formed only in a few plants. White khadi cloth-covered plants were harvested during different time intervals, starting from September to March. In Malaysia, cloth-covered structures, good plant growth was observed, but no spike

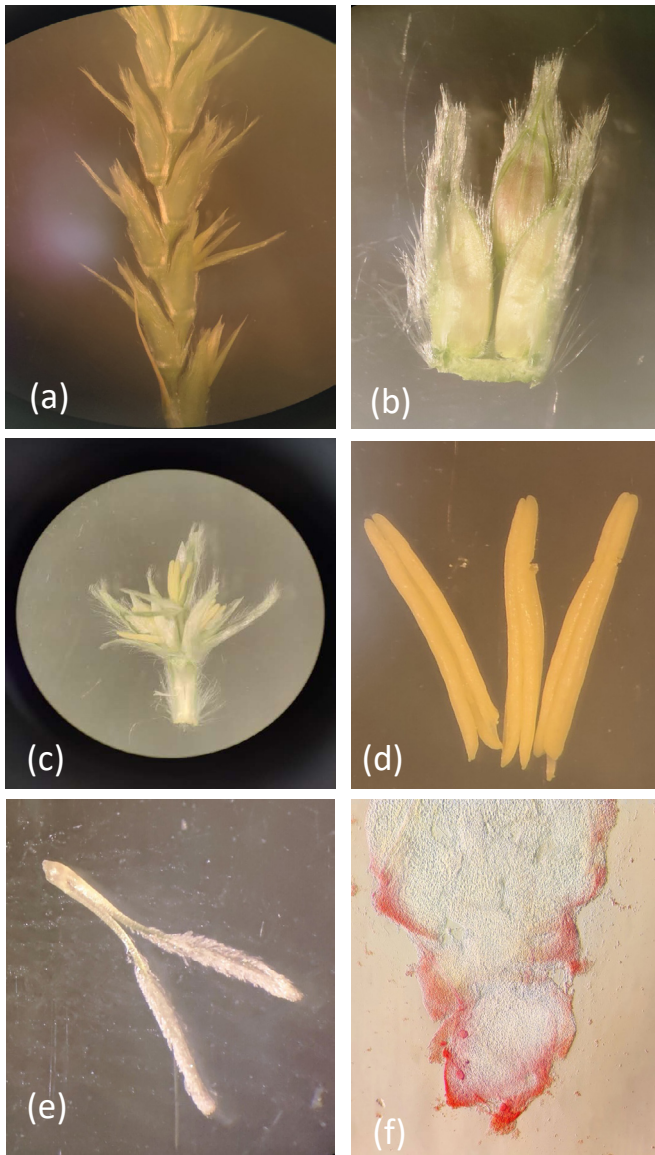


Fig 1. (a) Spike, (b) closed spikelet, (c) opened spikelet, (d) androecium, (e) gynoecium and (f) ovule

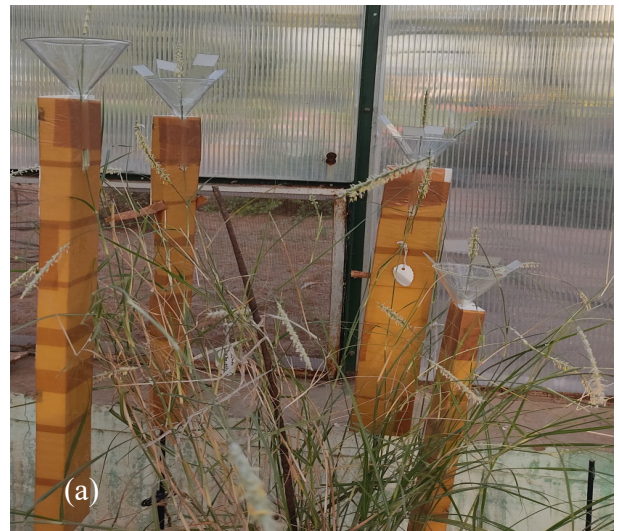


Fig 2. (a) Modified method of pollen count at different heights of spikes (b) individual funnel showing the arrangement of slides placed into it

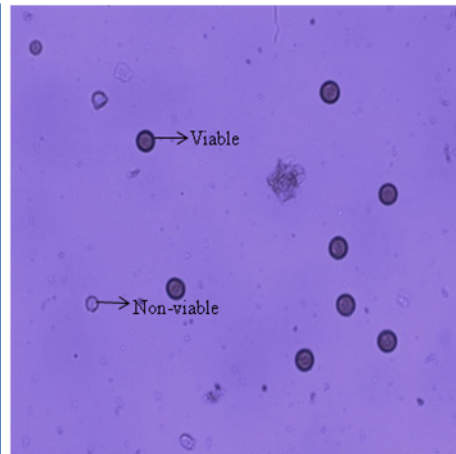
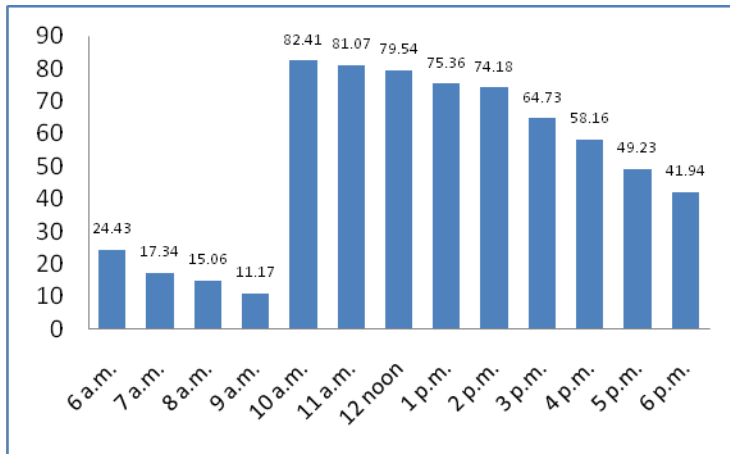


Fig 3. (a) Pollen viability (%) at different hours after anthesis, (b) viable and non-viable pollens

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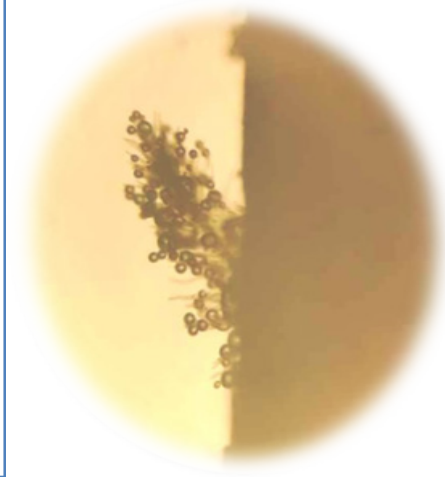
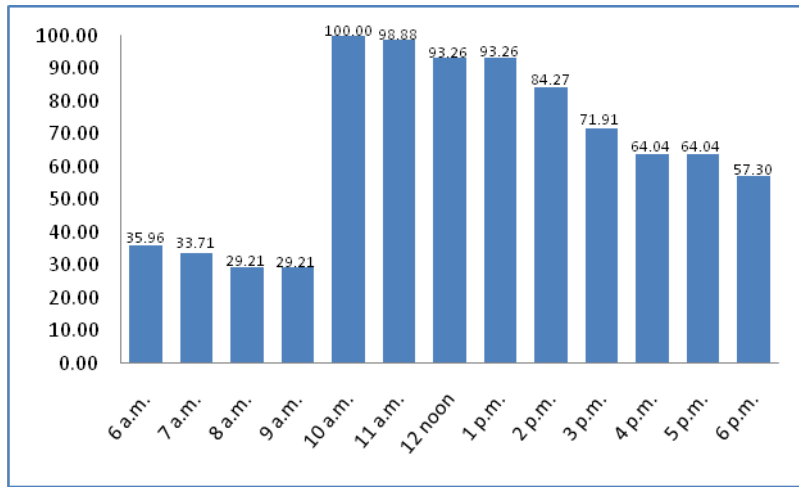


Fig 4. (a) Stigma receptivity (%) at different hours after anthesis (b) bubble eruption from receptive stigma

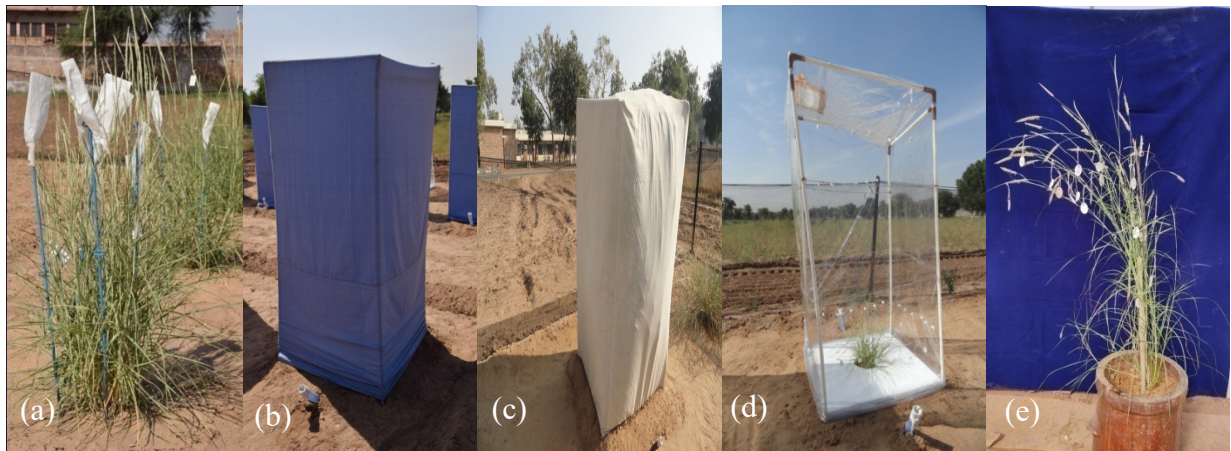


Fig 5. (a) Bagged individual inflorescences using parchment paper bag, (b) individual *L. sindicus* plants covered with malasiya cloth, (c) cotton white khadi cloth, (d) plastic clear sheet and (e) single uncovered plant in isolation

formed in any plant. In polythene structures, there was poor to medium plant growth, very few spikes formed in 4 plants, but no grain formed (Fig 5).

Individually bagged spikes (considered for self-pollination) harvested during different time intervals starting from August to February showed the highest grain formation (%) during the months of December (10.95%) and January (12.08%). Different values of grain formation (%) varying from 3.03 to 3.83% for individual inflorescences covered with parchment paper bags (spontaneous self-pollination), 13.85 to 17.77% for individual plants bagged with khadi cloth (geitonogamous pollination), 100% for tagged open inflorescences without any manipulation (open pollination) and 10.64 to 11.07% for individual plants grown in isolation at different locations (isolation pollination) were observed (Table 2).

Thus, the findings indicated that grain setting (%) in *L. sindicus* from open pollination, geitonogamous pollination, and isolation pollination exceeded that of self-pollination. Moreover, the analysis of variance (ANOVA) indicated that among these four pollination treatments, the proportion of spikes that set grains ($p < 0.001$) and grain formation (%) ($p < 0.001$) in open pollination varied significantly from the rest of the treatments and indicated for outcrossing behavior of *L. sindicus*.

Gaining insight into the breeding system of a species is beneficial for revealing evolutionary traits and life history influenced by different factors (Li *et al.*, 2018). To the best of our understanding, this marks the initial documentation of breeding behaviour in *L. sindicus*, consequently, comparing these findings with existing literature proved to be quite challenging.

Table 2. Grain formation (%) in different systems of *L. indicus*

S. No.	Type of system	Year	Total spikes harvested	No. of spikes set grains	Total no. of seeds in spikes which set grains	Total no. of grains in spikes which set grains	Seed: grain ratio	Grain formation (%)
1	Individual bagged spikes	1 st	957	22	294	29	1: 0.09	3.03
		2 nd	1330	25	391	51	1: 0.13	3.83
2	Spikes in individual covered plants	1 st	135	12	168	24	1: 0.14	17.77
		2 nd	325	83	1297	45	1: 0.03	13.85
3	Uncovered spikes	1 st	300	300	-	-	-	100
		2 nd	300	300	-	-	-	100
4	Plants in isolation	1 st	271	30	524	30	1: 0.05	11.07
		2 nd	235	29	571	25	1: 0.04	10.64

Conclusion

This first-ever study delved into the phenology, floral biology, pollination biology, and breeding behavior of *L. indicus*. This particular species displayed a range of floral syndromes tailored for wind-mediated cross-pollination, which limited the production of selfed seeds, potentially ensuring reproductive success and population sustainability. These adaptations facilitated successful reproduction even in the challenging environment of western Rajasthan. The substantial seed production indicated the ability of this species to maintain its progeny in natural populations. This valuable information about *L. indicus* not only holds critical implications for its management and conservation but also offers insights applicable to pollination ecology and breeding system studies in various other grass species.

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References

- Castro, S., P. Silveira and L. Navarro. 2008. Effect of pollination on floral longevity and costs of delaying fertilization in the outcrossing *Polygala vayredae* Costa (Polygalaceae). *Annals of Botany* 102: 1043-1048.
- Conner, J. K. and A. Sterling. 1995. Testing hypotheses of functional relationships: a comparative survey of correlation patterns among floral traits in 5 insect-pollinated plants. *American Journal of Botany* 82(11): 1399-1406.
- Cruden, R. W. 1977. Pollen ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution* 31(1): 32-46.
- Dafni, A. 1992. *Pollination Ecology: A Practical Approach*. Oxford University Press, New York.
- Fenster, C. B., W. S. Armbruster, P. Wilson, M. R. Dudash and J. D. Thomson. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics* 35: 375-403.
- Goswami, B., R. Rankawat and B. R. Gadi. 2020a. Physiological and antioxidative responses associated with drought tolerance of *Lasiurus indicus* Henr. endemic to Thar desert. *Brazilian Journal of Botany* 43(4): 761-73.
- IBM Corp. 2023. *IBM SPSS Statistics for Windows* (Version 29.0) [Software]. IBM Corp.
- Kumari, P., A. Khajuria, I. A. Wani, S. Khan and S. Verma. 2021. Effect of floral size reduction on pollination and reproductive efficiency of female flowers of *Valeriana wallichii*, a threatened medicinal plant. *National Academy Science Letters* 44: 75-79.
- Kumawat, R. N., A. K. Misra and M. Louhaichi. 2019. Managing rangelands: Promoting sustainable forage-grass species: *Lasiurus indicus* Henrard: A promising, drought-tolerant, tussocky perennial grass suitable for pasture development in desert areas. International Centre for Agricultural Research in the Dry Areas (ICARDA). <https://hdl.handle.net/20.500.11766/8491>.
- Li, T., X. Liu, Z. Li, H. Ma, Y. Wan, X. Liu and L. Fu. 2018. Study on reproductive biology of *Rhododendron longipedicellatum*: A newly discovered and special threatened plant surviving in limestone habitat in southeast Yunnan, China. *Frontiers in Plant Science* 9: 33.
- Mulugeta, D., B.D. Maxwell, P.K. Fay and W.E. Dyer. 1994. *Kochia* (*Kochia scoparia*) pollen dispersion, viability, and germination. *Weed Science* 42: 548-552.
- Rani, R., J. P. Singh, A. Sanyal, M. P. Rajora and A. Trivedi. 2022. *Lasiurus indicus* Henr., a key perennial fodder

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- grass for desert ecosystem. *Arid Land Research and Management* 37(1): 1-19.
- Rani, R. and A. Sanyal. 2024. Reproductive biology of *Lasiurus indicus*: a vital perennial fodder grass for arid ecosystem. *BMC Plant Biology* 24:1159. <https://doi.org/10.1186/s12870-024-05803-0>.
- Shivanna, K. R. and R. Tandon. 2014. Reproductive ecology of flowering plants: A manual. New Delhi: Springer. pp. 107-123.
- Tong, L., F. W. Lei, Y. M. Wu, X. L. Shen, X. F. Xia, D. H. Zhang, X. Y. Mu and Z. X. Zhang. 2022. Multiple reproductive strategies of a spring ephemeral plant, *Fritillaria maximowiczii*, enable its adaptation to harsh environments. *Plant Species Biology* 37: 38-51.