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Exploring the Floral Biology of *Salix alba*: Insights into Reproductive Dynamics, Pollen Production and Pollen Morphology in Kashmir Himalayas

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study investigates the floral phenology, pollen production and pollen characteristics of *Salix alba* (white willow) over the years 2022 and 2023, highlighting inter-annual and individual tree variations. In 2022, male catkins began flowering between March 8 and March 10, while female catkins initiated between March 14 and March 22. Flowering for male catkins lasted until mid-May and for female catkins until late May. In 2023, male catkins started flowering from March 10 to March 14 and female catkins from March 17 to March 24, with similar flowering durations. Inflorescence diameters varied among individual trees, ranging from 4.4 mm to 7.7 mm for male catkins and 6.1 mm to 8.1 mm for female catkins across the two years. Pollen production exhibited significant variability with the highest recorded at 4,600,995,000 grains per tree and the lowest at 498,312,500 grains per tree. On average, trees produced approximately 2,164,836,667 pollen grains. Pollen viability ranged from 75% to 80%, with sizes between 16 µm and 26 µm. The pollen-ovule ratio also showed substantial differences, with the highest ratio observed at 7600:2 and the lowest at 2345:7. These findings underscore the considerable year-to-year and tree-to-tree variation in the reproductive traits of *Salix alba*, which may have implications for its breeding and conservation strategies.

Keywords: Salix alba; reproductive traits; floral phenology; pollen characteristics.

1. INTRODUCTION

In the rich tapestry of our natural world, *Salix alba,* commonly known as White Willow, emerges as a captivating subject of scientific inquiry within the genus *Salix* and family Salicaceae [1]. Originating from the Celtic words 'sal' meaning near and 'lis' signifying water, the genus *Salix* aptly reflects its preference for aquatic habitats, thriving along riverbanks and lakeshores [2,3].

Salix alba, among the 450 species in the genus *Salix*, holds significant ecological and economic importance globally, ranging from tropical to cold-temperate regions [4,5,6]. Widely distributed from Europe to Central Asia and North Africa, this species was introduced to the America in the 17th century, becoming naturalized across continents [7,8,9].

Historically, Salix cultivation traces back to ancient times, utilized by civilizations like the Romans for crafting baskets, furniture and medicinal remedies [10]. Modern cultivation and breeding programs have further diversified its applications, ranging from ornamental landscaping to biomass production for renewable energy [11].

In India, *Salix alba* finds a habitat in the Western Himalayas, thriving at elevations up to 2400 meters along streams and canal banks in Kashmir and Kulu valleys [12]. Introduced to Kashmir in 1927, this species has since been cultivated for various purposes, including soil conservation, fuel wood and fodder [13].

Salix alba exhibits remarkable diversity, encompassing varieties such as var. alba, var. vitellina (Golden Willow) and var. caerulea (Cricket-bat Willow), each serving distinct economic roles from ornamental use to specialized timber production [14]. Its timber, valued for its lightness, strength and shock resistance, is particularly sought after for applications like cricket bats and furniture [15].

The reproductive biology of *Salix alba* presents a compelling area of study, characterized by dioecious traits where male and female individuals bear separate catkins. These unisexual flowers play a pivotal role in its reproductive strategy, facilitated primarily by insect pollination, although wind pollination (ambophily) also contributes to its genetic diversity [16].

Floral biology studies on *Salix alba* encompass the phenology of its flowering events, pollen production, pollen viability and the morphological characteristics of its reproductive structures. These aspects are crucial for understanding its reproductive success, genetic variation, and adaptation strategies in changing environments [17].

By delving into the intricate details of its reproductive and floral biology, this research aims to unveil the mechanisms underlying *Salix alba's* ecological resilience and economic potential. Such insights not only enrich our understanding of this versatile species but also inform sustainable management practices and conservation efforts amid contemporary environmental challenges.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted in the Ganderbal district of Jammu and Kashmir, India. Ganderbal is situated in the central part of the Kashmir Valley and is known for its diverse flora, which includes a variety of tree species such as *Salix alba*. The region experiences a temperate climate with distinct seasonal variations, including cold winters and warm summers, which significantly influence the phenological events of plant species. The study site within Ganderbal were chosen to represent a range of microclimatic conditions to capture the variability in reproductive parameters of *Salix alba.* The specific GPS coordinates of the study area are approximately 34.2262° N latitude and 74.7787° E longitude.

The reproductive parameters of *Salix alba* were studied randomly across the Ganderbal district. Six young, sexually mature trees were selected, with four major branches marked in each of the cardinal directions (North, South, East and West) using metal tags for consistent observation.

Floral phenology was recorded by observing the initiation, duration and termination of flowering, type of inflorescence and inflorescence diameter using a digital Vernier caliper. These observations were made visually on permanently marked trees, noting the timing of each parameter during both vegetative and reproductive phases.

Pollen production was assessed by determining the number of pollen grains per anther using a microscope, as per Dafni [18] and Kearns and Inouye [19]. Pollen production per flower was calculated by multiplying the number of pollen grains per anther by the number of anthers per flower. This was extended to per inflorescence by multiplying the average number of flowers per inflorescence with the average number of pollen grains per flower. Total pollen production per tree was estimated using the formula: $\sum TP = \sum N \times F \times A \times P$, where $\sum TP$ represents total pollen grains per tree, $\sum N$ is the number of inflorescences per tree, F is the average number of flowers per inflorescence, A is the average number of anthers per flower, and P is the average number of pollen grains per anther [20].

Pollen-ovule ratios were determined by dividing the number of pollen grains per flower by the number of ovules per flower, following [21]. Pollen viability was estimated in vitro using Acetocarmine staining methods [22] and pollen size was measured with a stage micrometer as described by Ascari et al. [23].

3. RESULTS

The study observed significant variations in the floral phenology, pollen production and pollen characteristics of *Salix alba* across different years and individual trees.

3.1 Floral Phenology

For the year 2022, it was observed that the flowering initiation for male catkins ranged from 8th March to 10th March and for female catkins from 14th March to 22nd March. The flowering duration for male catkins was from 8th March to 14th May and for female catkins from 14th March to 25th May. Termination of flowering occurred between 10th May and 14th May for male catkins and between 20th May and 25th May for female catkins. Inflorescence diameter varied from 4.4 mm to 7.7 mm for male catkins and from 6.1 mm to 8.1 mm for female catkins.

For the year 2023, it was observed that the flowering initiation for male catkins ranged from 10th March to 14th March and for female catkins from 17th March to 24th March. The duration of flowering for male catkins was from 10th March to 12th May and for female catkins from 17th March to 26th May. Termination of flowering occurred between 9th May and 12th May for male catkins and between 22nd May and 26th May for female catkins. Inflorescence diameter ranged from 4.4 mm to 7.2 mm for male catkins and from 6.1 mm to 7.6 mm for female catkins.

3.2 Pollen Production

The highest pollen production was observed in T5 with 4,600,995,000 pollen grains, while the lowest was in T6 with 498,312,500 pollen grains. The average pollen production was 2,164,836,667 pollen grains per tree, indicating substantial variability.

3.3 Pollen Viability, Size, and Pollen-Ovule Ratio

- Pollen viability ranged from 75% (T5) to 80% (T2). Pollen size varied from 16 µm (T1) to 26 µm (T6). The pollen-ovule ratio also showed significant variation, with the highest pollen count per ovule observed in T1 (7600:2) and the lowest in T6 (2345.

4. DISCUSSION

The study on the reproductive parameters of *Salix alba* in the Ganderbal district revealed significant variations in floral phenology, pollen production and pollen characteristics. These findings provide valuable insights into the reproductive strategies and potential environmental adaptations of *Salix alba.*

The observed variability in flowering initiation, duration and termination corresponds with previous research indicating the strong influence of local climatic conditions on floral phenology [24]. The earlier onset and prolonged flowering in 2022 compared to 2023 likely reflect annual climatic fluctuations, which can affect pollination dynamics and seed production [25].

Fig. 1. Male catkin of Salix alba

Fig. 2. Female catkin of Salix

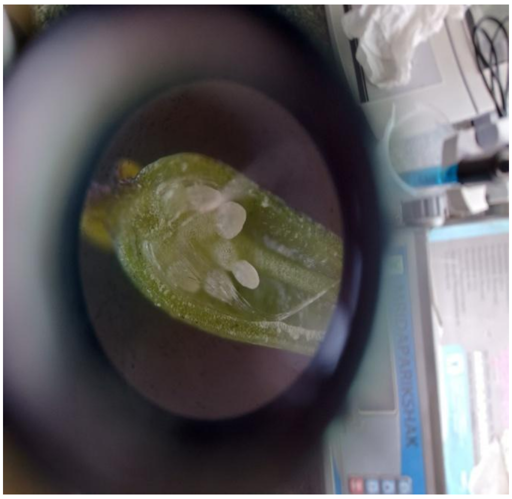

Fig. 3. Ovules of Salix alba

Fig. 4. Pollens of Salix alba

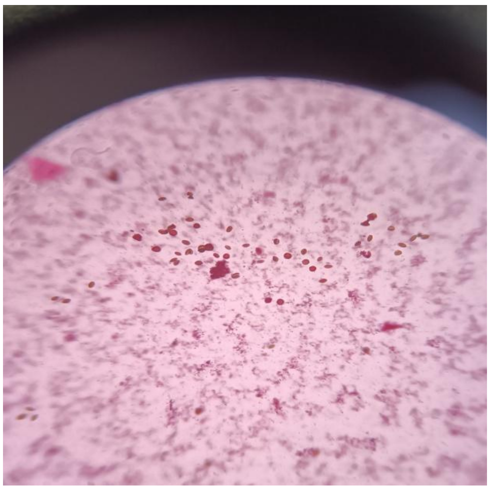

Fig. 5. Pollen viability of Salix alba

Table 1. Floral Phenology of *Salix alba* (2022)

Tree No.	Flowering Initiation (Male Catkins)	Flowering Initiation (Female Catkins)	Duration of Flowering (Male Catkins)	Duration of Flowering (Female Catkins)	Termination of Flowering (Male Catkins)	Termination of Flowering (Female Catkins)	Type of Inflorescence	Inflorescence Diameter (Male Catkins)	Inflorescence Diameter (Female Catkins)
T1	8th March	18th March	8th March to 10th May	18th March to 25th May	10th May	25th May	Catkin or Ament	5.8	6.4
T2	9th March	15th March	9th March to 11th May	15th March to 24th May	12th May	24th May	Catkin or Ament	7.7	6.1
T3	8th March	20th March	8th March to 12th May	20th March to 22nd May	11th May	22nd May	Catkin or Ament	4.4	8.1
T4	9th March	19th March	9th March to 13th May	19th March to 23rd May	13th May	23rd May	Catkin or Ament	5.1	7.3
T5	8th March	14th March	8th March to 14th May	14th March to 20th May	14th May	20th May	Catkin or Ament	5.8	7.5
T6	10th March	22nd March	10th March to 11th May	22nd March to 22nd May	11th May	22nd May	Catkin or Ament	6.2	7.9

Table 2. Floral Phenology of *Salix alba* (2023)

Tree No.	Flowering Initiation (Male Catkins)	Flowering Initiation (Female Catkins)	Duration of Flowering (Male Catkins)	Duration of Flowering (Female Catkins)	Termination of Flowering (Male Catkins)	Termination of Flowering (Female Catkins)	Type of Inflorescence	Inflorescence Diameter (Male Catkins)	Inflorescence Diameter (Female Catkins)
T1	10th March	19th March	10th March to 9th May	19th March to 26th May	9th May	26th May	Catkin or Ament	5.6	6.3
T2	11th March	17th March	11th March to 10th May	17th March to 25th May	10th May	25th May	Catkin or Ament	7.2	6.1
T3	12th March	23rd March	12th March to 10th May	23rd March to 25th May	10th May	25th May	Catkin or Ament	4.4	7.6
T4	13th March	21st March	13th March to 12th May	21st March to 23rd May	12th May	23rd May	Catkin or Ament	5.0	7.2
T5	12th March	18th March	12th March to 12th May	18th March to 22nd May	12th May	22nd May	Catkin or Ament	5.5	7.1
T6	14th March	24th March	14th March to 9th May	24th March to 24th May	9th May	24th May	Catkin or Ament	6.1	7.5

Table 3. Pollen Production in *Salix alba*

Tree No.	*No. of Anthers per Catkin*	*No. of Pollens per Anther*	*No. of Pollens per Catkin*	*No. of Catkins per Tree*	*No. of Pollens per Tree*
T1	*96*	*7600*	*729600*	*2000*	*1,459,200,000*
T2	*72*	*5050*	*363600*	*5000*	*1,818,000,000*
T3	*88*	*6600*	*580800*	*6000*	*3,484,800,000*
T4	*78*	*7090*	*553020*	*3000*	*1,659,060,000*
T5	*87*	*7555*	*657285*	*7000*	*4,600,995,000*
T6	*85*	*2345*	*199325*	*2500*	*498,312,500*
Average	***84.33***	***6040***	***509373.33***	***4250***	***2,164,836,667***

Table 4. Pollen viability, size and pollen-ovule ratio in *Salix alba*

Parameter Tree No.	**Pollen viability (percent)**	**Pollen size (micrometer)**	**Pollen Ovule ratio**	
			Pollen	**Ovule**
T_1	76	16	7600	2
T_2	80	21	5050	3
T_3	79	23	6600	5
T_4	79	22	7090	8
T_5	75	25	7555	6
T_6	78	26	2345	7

Pollen production among *Salix alba* trees exhibited substantial variability, consistent with findings in other plant species [20]. This diversity in pollen production may stem from genetic differences, tree health and varying environmental factors. Trees with higher pollen production, such as T5, suggest a potential advantage in reproductive success, crucial for maintaining population viability across different environmental conditions.

The wide range of pollen-ovule ratios observed in this study aligns with Cruden's theory [26] that this ratio serves as an indicator of a plant's breeding system. Higher ratios in certain trees indicate an adaptation to optimize reproductive success under conditions of limited pollinator availability [26]. This finding is consistent with recent studies on other plant species [27].

High pollen viability rates (75%-80%) observed in *Salix alba* indicate strong potential for successful fertilization, supported by reliable staining techniques [22]. Variations in pollen size, influenced by environmental factors and genetic diversity, may affect pollen dispersal and germination success [23]. *Salix alba's* ambophilous pollination strategy, involving both insect and wind pollination mechanisms, underscores its reproductive flexibility and adaptability to diverse environmental conditions, as noted by Saunders [16].

The findings of present study are also in conformity with the findings of Rafeeq et al., [28] Mir et al., [29] and Wagay et al., [27]. The findings from this study contribute to a deeper understanding of Salix alba's reproductive ecology and adaptation strategies. The variability in floral phenology, pollen production, and pollen characteristics highlights the species' resilience and potential responses to changing environmental conditions. These insights are crucial for conservation efforts and sustainable management practices aimed at preserving *Salix alba* populations in varying ecosystems.

5. CONCLUSION

This study provides valuable insights into the floral phenology, pollen production, and characteristics of *Salix alba.* Over two years, significant variations were observed in flowering timing, duration, and pollen traits among individual trees. Floral phenology assessments revealed diverse patterns in the initiation, duration, and cessation of flowering events. Male catkins typically initiated flowering earlier than females, reflecting the species' adaptive response to environmental cues. Pollen production varied considerably among trees, with tree T5 showing the highest yield at approximately 4.6 billion pollen grains and tree T6 the lowest at 498.3 million grains per tree. This diversity underscores genetic variability and its impact on reproductive success. Analysis of pollen viability, size, and the pollen-ovule ratio highlighted differences in reproductive potential across trees. Pollen viability ranged from 75% to 80%, while pollen sizes varied from 16 µm to 26 µm. These findings underscore the species reproductive strategies and adaptive flexibility.

Understanding these dynamics is crucial for conservation and management strategies, particularly in the context of environmental changes. Future research could delve deeper into the genetic underpinnings of these variations, offering further insights into plant reproductive biology and ecosystem resilience.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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