PHENOLOGICAL BEHAVIOUR AND REPRODUCTIVE BIOLOGY OF *Salix tetrasperma* Roxb.

Thesis

by

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(F-2019-40-M)

submitted to

Dr. YASHWANT SINGH PARMAR UNIVERSITY OF HORTICULTURE AND FORESTRY
SOLAN (NAUNI) HP – 173 230 INDIA

in

partial fulfilment of the requirements for the degree

of

MASTER OF SCIENCE
(FORESTRY)
FOREST GENETIC RESOURCES

DEPARTMENT OF TREE IMPROVEMENT AND GENETIC RESOURCES
COLLEGE OF FORESTRY

2023

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CERTIFICATE-I

This is to certify that the thesis entitled **"Phenological behaviour and reproductive biology of *Salix tetrasperma* Roxb."** submitted in partial fulfillment of the requirements for the award of degree of **MASTER OF SCIENCE (FORESTRY)** in the discipline of **FOREST GENETIC RESOURCES** of Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP)-173 230 is a bonafide research work carried out by **Ms. Gauri Mahajan (F-2019-40-M)** daughter of Sh. Ravinder Mahajan under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigations have been fully acknowledged.

Place: Nauni, Solan
Dated

Dr. Jai Pal Sharma
Major Advisor

CERTIFICATE-II

This is to certify that the thesis entitled,"**Phenological behaviour and reproductive biology of *Salix tetrasperma* Roxb.**" submitted by **Ms. Gauri Mahajan** daughter of **Shri Ravinder Mahajan** to the Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP)-173230 India in partial fulfillment of the requirements for the degree of **Master of Science (Forestry)** in the discipline of **Forest Genetic Resources** has been approved by the student's advisory committee after the Viva-Voce Examination of the student in collaboration with an **External Examiner**.

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ACKNOWLEDGEMENT

Not for the sake of convention done but out of deep seated conviction, it gives me profound pleasure to express my heartfelt feeling for those, who helped me a lot in completing this work.

Firstly, I would like to express my sincere graceful filled thanks to ***"ALMIGHTY GOD"****, who has protected me so long and permitted me to undertake this journey. The journey is still continuing with many more destinations to cover.*

It is my privilege to express my deep sense of gratitude to my major advisor ***Dr. Jai Pal Sharma,*** *Associate Professor, Department of Tree Improvement and Genetic resources for his valuable guidance, timely suggestion, close counsel critical evaluation, everlasting patience, constant encouragement at every step of work and creating research ability and aptitude in me.*

It is my sole prerogative to place on record my indebtedness and everlasting gratitude to ***Professor & Head Dr. H P Sankhyan****, of Tree Improvement and Genetic resources and esteemed members of my advisory committee for their valuable suggestions and generous cooperation during the course of investigation and preparation of this manuscript.*

Iam grateful to Dr. Anita Kumari, Dr. Shikha Thakur, Dr. Pratima Vaidya, Dr. Nitin Sharma and Dr. Lalit Thakur for their kind suggestion.

I am grateful to my parents ***Sh.Ravinder Mahajan*** *who has been always a source of motivation behind all my achievements, my mother* ***Smt. Jyoti Mahajan*** *who has been a constant source of encouragement for me and my sisters Er. Ritika Mahajan and Dr. Shivam Mahajan for their moral support and motivation.*

No adequate words can be found to express my warmest thanks to all my seniors especially, Divya, Jyoti Dhiman, Mohit kundal, Rishabh Sood, Shilpa Sharma, Akshiptika Chandel and Aditi ma'am.

Genuine appreciation goes for my classmates and friends who kept me in an exalted state even during the moments of despondency and were always with me with supporting hand, Alisha sood, Aman Mahajan, Anchal, Akash Sharma, Abhishiek , Ankush, Akshay, Anu, Akanaksha, I.Z. Synnah, , Laqshika, Priyanka Sahoo, Pawan Singta, Raveena , Riya Thakur, Riya Pathania, Shweta, Sugandhi, Shivani, Shubham Sharma, Sumit , Tushal , Diksha Thakur and Deeksha for their cooperation, support and help rendered.

Acknowledgements are inherently endless & incomplete, and I request indulgence from many friendly & helpful people whom I could not name here, due to paucity of space.

Needless to say, all omissions and errors are mine.

Place: Nauni, Solan
Date : **(Gauri Mahajan)**

CONTENTS

LIST OF ABBREVIATIONS

amsl	:	Above mean sea level
ANOVA	:	Analysis of variance
%	:	Per cent
&	:	And
cm	:	Centimeter
CD	:	Critical difference
CV	:	Coefficient of Variation
° C	:	Degree centigrade
et al.	:	And others / co-workers
e.g.	:	examples
Etc.	:	etcetera
ESS	:	Error sum of square
GCV	:	Genotypic Coefficient of Variation
GSS	:	Genotype sum of square
H.P	:	Himachal Pradesh
i.e	:	that is
PCV	:	Phenotypic Coefficient of Variation
RSS	:	Replication sum of square
RBD	:	Randomized block design
SE	:	Standard error
Sp. or spp.	:	Species
viz	:	videlicet (namely)

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Chapter-1

INTRODUCTION

Forests are an integral part of country's economy and play an important role in socio-economic development of country by providing variety of goods like food, fuel, medicines, wild edible fruits, timber, non timber forest products (NTFPs), raw material for industrial processing as well as various ecosystem services. They are indispensable for nation's development and are huge resource in economic terms.

With the progressive development of science in forestry, the desire for learning about the reproductive and phenological behavior of the forest tree species has increased. Taxonomical phylogeny, pollination ecology and out-crossing mechanism are all based on floral morphology. In order to begin a successful breeding (hybridization) programme, investigations on many elements such as flowering duration, stigma receptivity, pollen anthesis, pollen germination, pollen viability and pollen storage are required. Hence, it becomes essential in pollination studies to quantify a variety of morphological traits of plant, inflorescences or flowers (Fenster, 1991).

Phenology deals with the growth of buds, leaf flushing, anthesis, fruiting and leaf fall in relation to seasons. The phenological investigations are crucial for a better knowledge of the ecological adaptations of plant components as well as for the protection of tree genetic resources and forestry management. In order to monitor plant growth and choose the best times for different cultural techniques including irrigation, fertilisation, harvest time, and crop protection, growers use phenological observations (Kikim and Yadava, 2001). It is observed that the study on reproductive biology has been carried out from several perspectives like phenology, floral biology, pollination study, seed germination etc. but the final phase in this process is successful establishment of seedlings and saplings in an environment where they can grow into reproductively mature trees. Phenology plays an important role in the design of successful *in situ* conservation management plans for any plant population particularly endangered plant population.

Reproductive biology is the study of flowers and their associated processes such as first opening of flower buds (anthesis), anther dehiscence, pollen viability, pollination pattern and stigma receptivity. The study of reproductive biology covers factors influencing fertility

and the success of reproduction, as well as the acquisition of reproductive maturity, floral initiation and the order in which flowers develop. In flowering plants or trees, the reproductive process starts with the formation of flower buds and continues until they mature to flower, develop fruit and produce mature seeds. Reproductive characteristics such as seed dispersal, germination capacity, flowering, seeds refers to a set of responses that allow a species to adapt to a particular environment. A highly diverse array of floral traits and reproductive systems have evolved, varying from obligated cross fertilization to obligated or promoted self-fertilization, with each strategy presenting selective advantages and disadvantages, in flowering plants (Takebayashi and Morrell, 2001). One of the primary factors of an organism's abundance, dispersion, and genetic diversity is its reproductive pattern. The reproductive biology of species is an important part of the breeding system in deciding the breeding procedure for genetic improvement of tree species. All aspects of sex expression in plants that influence the relative genetic contributions of individuals to the following generation within a species are included in breeding systems (Wyatt 1983). The investigation of breeding systems helps to express the study of evolution and population genetics (Richards, 1997). The conservation of threatened and endangered tree species also depends on knowledge of breeding systems (Sedgley and Griffin 1989). It is preferable to collect all the information on the species' diversity with relation to tree fruit, flower, seed, and even chromosomal level before implementing any tree improvement programme. Seasonal flowering timing, or flowering phenology, is significant for ecological and evolutionary reasons (Kalloo, 1991).

The willows belonging to the genus *Salix* (Salicaceae) are one of the most important taxonomic entities of the world because of their great number, species and varieties. The genus *Salix* comprises of 350-500 species worldwide (Argus, 1997). The species is mostly found in the arctic region and north-temperate zone and only few species occur in the southern hemisphere covering tropical and sub-tropical zones. Asia is believed to be the centre of origin of genus *Salix* with around 275 species, including 189 endemic ones exists in China (Fang, 1987; Choudhary *et al.* 2011). Similarly, in India, about 31 species are found in temperate regions except *Salix tetrasperma* and *S. acmophylla*, which are found in tropical and sub-tropical riparian areas of the entire country (Sharma *et al.* 2011).

Salix tetrasperma (Indian Willow) is a medium sized tree found in wet and swampy places. Its bark is rough, with deep, vertical fissures and the young shoots leaves are silky.

The leaves are lance-like, or ovate-lance like, 8-15cm long, with minutely and regularly toothed margins. The male scented catkins and female catkins are borne on leafy branchlets. The capsules are long, stipulate, in groups of 3 to 4 (Troup, 1921). In sub-Himalayan tract and outer Himalayan ranges, it ascends up to about 1800 m elevation and in the Nilgiris up to 2,100 m elevation (Brandis, 1906). It is a frost hardy, light demander and resistant to drought, however good growth takes place in moist conditions. It coppices and pollards well. Seed germinates readily on getting optimum moisture and temperature. Germination is epigeous. Growth of seedlings in first season is somewhat slow but later on it becomes fast. It propagates easily by vegetative means. The species sheds leaves, exhibit flowering and fruiting at different time in different parts of the country. In North India, the tree is leafless in cold season, the leaves fall after the end of rainy season, the new leaves and catkins appear in February-March and the fruits ripen in April. In Maharashtra, the trees are in flowering in October – November. Due to the presence of a variety of bioactives, including those with antioxidant, antimicrobial, antipyretic, anti-inflammatory, laxative, analgesic and diuretic properties, the plant has medicinal value and can be used to treat a number of illnesses, including epilepsy, swellings, diabetes, piles, rheumatism, bladder stones, dysentery, etc (Luna, 2005). Indian willow is eco-friendly, multipurpose, fast growing species and widely used for plantation across the world, some of which have been cultivated for a variety of end uses *viz*., making of baskets, ropes, bats, hurdles, plywood, paper and pulp, etc (Verwijst, 2001; Kuzovkina *et al.* 2008).

Studying adaptation and genetic bases of adaptive traits is an important step especially for complex traits that have a polygenic origin and strongly influenced by the environment. In order to isolate local adaptation and evolutionary changes, biologists have developed in experimental fields (common garden) by means of selecting individuals from or procured from different populations and growing them in a common environment for a short period of time (Villemereuil *et al.,* 2016). Growth phases encompass time periods that are crucial to humans as well as to plants from a biological point of view. There are numerous methods for tracking the stages of plant growth, including both numerical and alphanumerical systems (Chmielewski, 2003; Zadoks *et al.,* 1974).

Since, not so much work has been done on phenological pattern and variation in the reproductive biology of *Salix* spp. and *Salix tetrasperma* in particular. The variability in

reproductive and phenological characters can be exploited in developing hybrids with superior *Salix* clones already introduced in the department of Tree Improvement and Genetic Resources of Dr. Yashwant Singh Parmar U.H.F, Nauni, Solan, H.P. (Sharma *et al.* 2019). Therefore the genotypes of *Salix tetrasperma* collected from different sites of North India will be studied for reproductive and phenological characters with the following objectives.

a) Objectives:

1) To study the floral biology of different genotypes of *Salix tetrasperma* Roxb.
2) To study the pollen viability and germination variation in different genotypes of *Salix tetrasperma* Roxb.

Chapter-2

REVIEW OF LITERATURE

Keeping in view the importance of the objectives taken for the investigation, the most relevant literature on *S. tetrasperma* and other species is being reviewed under the following heads:

2.1 Phenological and floral studies

2.2 Pollen studies

2.1 PHENOLOGICAL AND FLORAL STUDIES

Griffin (1980) observed flowering phenology in a stand of *Eucalyptus regnans* F. Muell at Narracan, Gippsland, Victoria (1977 and 1979) and noticed the beginning of flowering on February 15, 1977, and January 30, 1979, and all trees finished flowering after 90 and 100 days, respectively in both seasons with the median value for the number of trees flowering at the same time occurred 40 days following the start of the season. In 1979, he observed abundant flowering along with temporal isolation. He examined flowering in the crowns of three trees and revealed that the mean time between the anthesis of the earliest and last flowers in inflorescences was six days and fourteen days for flowers in branches.

Renner (1986) conducted a study on the flowering periods of *Bellucia dichotoma, B. grossularioides* and *B. acutata* by periodic observations from October 1980 to November I982 and April 1982 to November 1984 and found that these three species flowered for prolonged periods or consistently throughout the year and fresh flower buds were present around the start of the rainy season from about October to January; however, some flowers did flower over 11 months of the year. She observed that the flowers of the shrub species were three to four centimeters in diameter; while the flowers of the tree species ranged from six to eight centimeters in size and the petals expand horizontally by morning as the buds start to split apart in the previous evening. Depending on the species, the pollen is more or less a pale golden colour and has the same scent. The pollen of *Bellucia* were smooth and dry. They stick to the abdomens of the bees and just slightly to the rest of their bodies. Thus, pollen placement lacked specificity.

Boes and Strauss (1994) examined seasonal changes in the development of reproductive structures for *Populus trichocarpa* Torr. and Gray. They observed that the development of reproductive meristems begins early in the spring, before leaves emerge and until late spring the anatomy of male and female flowers was virtually undistinguishable and the gynoecium structure develop in about 2 weeks and then continue to enlarge through the summer and autumn until the trees become dormant. Stamen development begins in the centre of a disk-shaped meristematic region and proceeds centrifugally. Megasporogenesis and microsporogenesis take place late in the winter, approximately 2 weeks before anthesis. It was further noticed that *P. trichocarpa* was found to have a relatively large number of reproductive structures when compared to other *Populus* species.

Kigomo *et al*. (1994) conducted a study on the phenology and reproductive biology of the dioecious tree *Brachylaena huillensis* O. Hoffm in its native environment in Kenya. They found that the species produce fruits and flowers twice a year, between mid-April and the end of June and again between mid-November and early January. The flowering and fruiting periods are relatively short and well-defined although the frequency and amount of rainfall affect flowering. They further noticed that males generate more flower heads per panicle and the amount of fruit produced was enormous, but up to 80 per cent of fruit was vulnerable to insect attack before dissemination. They found that a single tree's flowering and fruiting season may only last a week or two, but for the entire population as a whole, it lasts for around six weeks, with individual trees beginning at somewhat different periods.

Setterfield and Williams (1996) studied the pattern of flowering and seed production in *Eucalyptus miniata* and *E. tetrodonta* in Tropical Savanna, Northern Australia. They observed that within eight months of the appearance of floral buds on the trees, the reproductive cycles of *E. miniata* and *E. tetrodonta* were completed. Their study has demonstrated that seed production and seed supply of *E. miniata* and *E. tetrodonta* varies substantially between years and that this pattern of the temporal variation is consistent between sites. As the ovule survival from bud initiation to seed fall did not vary significantly between years. Therefore, years of low seed production are due primarily to low densities of floral bud initiation per tree canopy and low numbers of fecund trees per stand.

Fenner (1998) investigated the phenology of leafing, blooming, and fruit production in a variety of species and populations. He noticed that within the constraints imposed by phylogenetics, the phenological patterns (timing, frequency, duration, degree of synchrony,

and so on) of each phase were most likely the result of a compromise between a variety of selective pressures such as seasonal climatic changes, resource availability, pollinator, predator, and seed dispersal. According to his study, the need for long-term recording was emphasized, especially in species that produce irregular fruit and came to the conclusion that understanding plant phenology is critical to understanding community function and diversity.

Nagarajan *et al*. (1998) studied the reproductive biology of *Tamarindus indica* L. and observed that the flowers were bisexual, herkogamous, cream yellow in colour, with five sepals, five petals, with the odd petal with three fused stamens and filaments curled toward the base of the ovary, a simple style, an a superior ovary containing 12 to 14 ovules. Numerous uniseriate hairs and a lot of nectar were present at the ovary base. They further noticed that on only alternate days, flowers in an inflorescence bloomed and between clones, there were considerable differences in floral production. They observed that more flowers were produced by clones having longer vegetative terminal branches. The clones' styles and ovary sizes differed between clones. Every year, terminal vegetative shoots were generated, but they didn't develop flowers until the next immediate flowering season drooping. Maximum foliage was produced by clones having longer terminal shoots.

Chavan *et al.* (1999) investigated blooming phenology, floral biology, and phenotypic diversity in floral features in *Tamarindus indica* L, and observed the plant flowered from the later week of March through the first two weeks of June, between 9-11 a.m. when dehiscence occurred. Only two trees were found to bloom more than one floral bud every day. The majority of trees bloom one floral bud every alternate day, while 33 per cent of trees bloom at least one floral bud per day on a regular basis.

Williams *et al*. (1999) studied interspecific and interannual variation in the reproductive phenology of 50 common species of trees and shrubs from a mesic savanna near Darwin, northern Australia between September 1992 and February 1995. They found that the most of species flowered around at the same time each year and the mid to late dry season (July–August) and the transitions between the dry and rainy seasons were the peak flowering times (October–November). During the dry season, the two major trees, *Eucalyptus miniata* and *E. tetrodonta*, flowered. At the end of the wet season (February/March), flowering and fruiting were less prevalent, but two species *E. porrecta* and *Terminalia ferdinandianas* flowered at this time.

Maloof (2000) conducted a study of flower longevity in *Corydalis caseana* and observed that the flowers stayed open and healthy for around 4 days, follows the mixed mating system, were self-fertile, generate fewer seeds per fruit (a mean of 2.9 compared with a mean of 4.7) and a potential for inbreeding depression. The flowers on the terminal raceme were found to bloom first, from the bottom to the top (acropetally). On enormous, terminal racemes, the flowers at the bottom may be setting fruit while those at the top are still in the bud. The flowering started between June 14 and June 20 the stigmas are responsive and the pollen is viable for at least the first four days when the flowers are open.

Ng and Corlett (2000) studied the comparative reproductive biology of six *Rhododendron* species of Hong Kong: *Rhododendron farrerae*, *R. simsii*, *R. moulmainense*, *R. championiae*, *R. hongkongense*, and *R. simiarum*. They found that there were differences in the duration and timing of the flowering period between species and locations.

Pop *et al.* (2000) studied the factors controlling bud break in two arctic deciduous shrub species, *Salix pulchra* and *Betula nana*, using field observations and growth chamber studies. They observed that the two species responded similarly in terms of bud break timing and response to air temperature in both field and controlled environments. They noticed that in the field, the timing of bud break was strongly influenced by air temperatures once snowmelt had occurred. Growth chamber studies showed that a period of chilling is required before buds break in response to warming. They further observed that in contrast to chilling treatment, warm spring temperatures determine the timing of bud break. They concluded that changes in bud break timing of the deciduous shrubs would likely have important consequences on the relative abundance of shrubs in future communities and consequently ecosystem processes.

Chauhan and Singh (2001) observed that the anthesis of *Terminalia arjuna* began in April and ended in July, with flowers opening between 5:30 and 7:30 a.m. Leaf fall began in October and lasted until April, with the most significant amount of the leaf fall in January.

Ratnaningrum *et al*. (2001) carried out a study on phenology and reproductive biology in the three-and-a-half-year-old *Eucalyptus pellita* plantation in Yogyakarta, Indonesia andobserved that *E. pellita* took 302 days to complete the development of its reproductive organs, from the beginning of the floral phase through the ripening of the fruit and seed dissemination. They recorded 145 days for the anthesis and characterized as a protandry

dichogamy type depending on when the sexual organs reach maturity. Just before anthesis, anther dehiscence begins, and three days later, stigma achieves its maximum receptivity. They further noticed that floral initiation started in January with peak flowering occurred in March-April when the wet season gave way to the dry season, while fruit maturity and seed distribution took place in September.

Yu, Q*et al*. (2001) studied phenological traits of four aspen hybrid clones (*Populus tremula* × *Populus tremuloides*) and one local *P. tremula* seedling source. They observed shorter mean times for the onset of each stage of growth differences in phenological and growth parameters between hybrid clones and *P. tremula*. The hybrids' mean times for the onset of each stage of growth were shorter than those of *P. tremula*: for the emergence of bud, it required 9 days, for leaves to unfurl required 7 days, and 7 days for full-sized leaves.

Pellis *et al.* (2004) examined the leaf phenology of poplars (*Populus spp*.) clones differing in their latitude of origin (45^{o}30′N to 51^{o}N), including spring phenology, length of the growth period and end-of-year phenology, over several years of different rotations. They observed that the clones from 45^{o}30′N to 49^{o}N reached bud burst almost every year earlier than clones from 50^{o}N to 51^{o}N. Some clones experienced late bud burst and early leaf fall as a result of increased rust incidence throughout the course of the observational years. High coefficients of variation between years for these clones reflect the variability in leaf phenology.

Tandon *et al*. (2003) studied phenology of *Butea monosperma* and observed that in the first year, 27 trees flowered, but in the second only 18 trees and the flowering trees started shedding their leaves in December and were leafless by January and those which did not show flowering, retained the leaves. Their study indicated that although species shows regular flowering, all trees do not flower every year. They found that flowers were papilionaceous with wet papillate stigma, hollow style, enormous, vivid orange-red in colour, abundantly nectar-filled, and exhibit diurnal anthesis, which is indicative of bird pollination. At the end of February, inflorescence primordia began to form in the leaf axils. The dark and velvety flower buds started to open in the first or second week of March. The first week of April marked the peak flowering period in both years, with the trees staying in bloom for 6–8 weeks. In April or May, leaf primordia began to form, and by May or June, leaves had grown to their maximum size.

Berry and Gorchov (2004) found that males and females within the population of *Chamaedorea radicalis* flowered during the same time period from May to June 2001 at Tamaulipas, Mexico. The prolonged flowering cycle of the males, enabled one male to be a source of pollen to receptive females for as long as a month and assisted in the successful wind pollination of this palm. They observed that the female plant's size affected how many flowers and fruits it produced, but had no strong correlation with fruit production. They further noticed that in the study area, fruit production was not influenced by local sex distribution, male density, or male proximity, indicating that the availability of pollen didn't limit female reproductive success.

Chauhan *et al*. (2004) observed the leafless period of *Dalbergia sissoo* from December to January. They noticed an increase in temperature affects the flowering from March to April. From bud initiation to flower blooming, the entire process took fifteen to twenty days, and seven to eight months from bud initiation to fully ripened pods. They observed that the anthesis lasted from 10:00 a.m. until 2:00 p.m. In the bud stage, shortly before the flower opened, the anthers dehisced in the morning hours.

Lennartsson and Ogren (2004) investigated the differences in temperature requirements for budburst among *Salix* clones used for biomass production covering a wide range of geographical origins. They observed that the total temperature needed for budburst varied significantly among clones, with values ranging from 110 to 191^{o}C and that was only weak linkage with the geographical origin of the clones. They noticed that all clones according to freeze tests, until at least a couple of weeks before budburst, could tolerate temperatures $\leq 15^{o}$C. The findings suggested that the clonal variation in susceptibility to spring frosts was related to the clonal variation at the timing of budburst, largely determined by the temperature sum requirement.

Pande *et al.* (2004) evaluated the phenology of neem (*Azadirachta indica*) from several agro-climatic zones. They observed that the leaf fall season was from February to March, except in the Narmada Valley, where it lasted up to April. The leaf emergence began in February and continued until April in various agro-climatic zones. The vegetative period lasted seven months (August to January) in all climatic zones except in the Vainganaga valley, where it only lasted six months. Flowering began in February in all climatic zones except the Narmada Valley, where it began in March and fruiting began in March-April.

Singhal *et al.* (2005) investigated the floral biology, pollination mechanisms, and breeding systems of *Bauhinia variegata* and *Bauhinia candida*, respectively. They observed that flowers bloomed in both species in February-March, according to the researchers. It was further observed that flower opening, anther dehiscence, and stigmatic receptivity all occurred at the same time in variety *variegata* and 05:00-09:00 in variety *candida*. The anthers in the bloom all dehisce at the same time.

Nagarajan *et al.* (2006) conducted studies on phenology, floral biology and seed production in two provenance trials and clonal hedge orchard of *Casuarina equisetifolia.* They observed that flowering occurs two times a year coinciding with the South West and North East monsoons. It was observed that *C. equisetifolia* exhibits strong anemophilous adaptations such as very high pollen output, reduced flowers with the large stigmatic area and with light weighing winged fruits. They recorded pollen viability up to 99 per cent, which can be stored in 4°C up to three months with no loss in fertility.

Wani and Chauhan (2008) studied the floral biology and stigma receptivity of *Bauhinia variegata* and found that the species began flowering in March-April, with flowering times varying from 26 to 49 days. The average time it took for flower buds to develop was found to be between 20.88 hours and 24.92 days, with maximum anthesis and anther dehiscence occurring between 7:30 and 8:30 a.m.

Borges *et al*. (2009) carried out a study on the reproductive biology of *Caesalpinia echinata* and found that flowering took place primarily in the dry season and that seed distribution peaked at the start of the rainy season and diurnal anthesis lasts for one day. They observed that zygomorphic yellow flowers possess a pleasant aroma. Average measurements for nectar volume and sugar content were 29±1.0 µL and 29.5±9.4%, respectively. They further noticed that the pollen:ovule ratio was 5631.2, the ovary had 1-4 ovules (2.35±0.58), and the pollen viability was high (95.9±4.8%). They found that *C. echinata* exhibits late-acting self-incompatibility as per the results of the controlled pollinations and examination of pollen tube growth.

Fidalgo and Kleinert (2009) examined the floral phenology and reproductive biology of six sympatric Myrtaceae species' between September 1999 and April 2002 in the coastal forest of Ubatuba, Brazil (44°48'W, 23°22'S). They found that beginning with the changeover from the driest to the most humid season (Sept/Oct), flowering continued throughout March.

They further noticed that the species regularly bloomed, in the same manner, each year but the beginning, peak, finish, and overlap of flowering varied from year to year.

Weih (2009) examined six commercial willow (*Salix* spp.) varieties in order to investigate the effect of genotype and environment on spring and autumn phenology and the relationships between phenology, shoot growth and leaf nitrogen translocation. It was observed that bud–burst date varied by 19 and 39 days in the two years and leaf unfolding duration varied by 2.5 weeks and completion of leaf abscission (>90% of leaf shed) by more than 3 weeks between genotype and treatments. He observed that delayed growth cessation and leaf abscission were generally associated with greater biomass production. He concluded that the timing of bud-burst and leaf abscission is more important for willow biomass production than growth cessation.

Jadeja and Nakar (2010) studied ten woody tree species from eight different families in the Girnar Reserve Forest, near Junagarh in Gujarat. They noticed that during the year, the phenological behaviour of all ten species was identical. Leaf fall occurred in most species in January and February to March, with new leaves arriving before the monsoon. In the month of December, five species had their highest fruiting activity. It was further noticed that fruit dehiscence was completed in two species before pre-monsoon showers in June, although fruit dehiscence was incomplete in eight species. *Derris indica* had a maximum flowering time of 246 days for fruit and a maximum flowering time of 44 weeks in the year, while *Tectona grandis* had a maximum flowering time of 146 days.

Margaret and Kuzovkina (2010) studied the phenological growth stages of four different *Salix* species and observed that the bud covered by closed scales stage was achieved after leaf fall and *Salix gracilistyla* bud scales appeared glossy red. They found that as the growing season progresses there is a change in the colour of the soft tissues of the annual stem. *Salix gracilistyla* when exposed to sunlight becomes purple/ tan and a change in bark colouration may be due to anthocyanin content. It was noticed that generative bud scales change colour from green to reddish, brownish or yellowish depending upon the species. In *Salix gracilistyla,* on exposure to sunlight generative bud scale appear to blush on the stem. It was further noticed that during the generative bud swell stage, colour may change, surface (glabrous to pubescent) which indicates a progression from dormancy to flower development.

Chaudhary *et al*. (2011) studied the phenology and reproductive biology of nine important willows (*Salix* spp.) procured from different countries and concluded that *Salix tetrasperma, S. jessonensis, S. gracillistyla* and *S. acmophylla* were among early flowering species, whereas, *S. udensis* flowered in a staggered manner from last week of January to last week of March. *S. nigra* flowered between the last week of March and 1st week of April and was last in flowering among all the species. The male flower buds burst earlier than the vegetative bud, whereas female flower buds burst after the leaves started to emerge in all species.

Azad (2012) observed the bud burst spring phenology of poplar (*Populus tremula* and *P. tremuloides*) from eight distinct provenances, originating from Europe and the United States during March and April 2009. He identified the phenological stages of the seedlings using a six-stage subjective grading system of bud burst phenology, with each plant being monitored twice a week. The aim of the study was to anticipate phenotypic variance among poplar trees grown in similar settings that originated between 42° and 60° N latitude. He observed that seedlings from provenance 3 (42.35° N latitude) started and finished flushing substantially earlier than those from other provenances, whereas seedlings from provenance 5 (54.29° N latitude) started flushing quite late and just a few plants reached top scoring at the conclusion of the trial period. He concluded from his study that within provenances, the association between score and flushing periods was quite strong, despite the fact that the flushing pattern varied.

Bajpai *et al.* (2012) found that flowering began in the third week of February and lasted until the second week of March in *Syzygium cuminii* L. They noticed that the number of panicles per branch ranged from 5 to 13, there were 19 to 73 buds per panicle in various orientations of the canopy and there was a strong correlation between panicle size and the number of flowering buds. They observed that the entire flowering phase, which culminated in fruit set and subsequent ripening, lasted for 119–126 days with a lengthy phase of flower bud initiation that lasted for 45–50 days. The natural pollen transmission was effective, and fruit production after open pollination was relatively high.

Bajpai *et al.* (2015) evaluated the phenological features of two *Shorea robusta* and *Ficus hispida* species found in the Katarniaghat Wildlife Sanctuary (KWS), a tropical moist deciduous forest near the Indo-Nepal border in Northern India. They recorded the phenophases such as leaf bud initiation, leaf emergence, young leaf formation, leaf fall,

flower bud growth, flowers, and fruit production, on a monthly basis. They further recorded the number of leaf buds, juvenile leaves, mature leaves, and total leaves. Leaf bud bursting and flowering began in both species in the post-winter months (March-April), with maximum leaf fall occurring in the post-monsoon season (November-February).

Neto (2013) studied the floral biology and breeding system of *Bauhinia forficata* and found that the plant displayed floral characteristics that were indicative of sphingophilous condition. He observed that the anthesis began at dusk, and pollen and nectar production took place at 8:00 and 10:00 p.m., respectively. It was further noticed that the flower became protoandrous and the stigma receptive around 11:00 p.m.

Johar *et al.* (2015) observed that that moderate defoliation began in the fourth week of November and persisted through the end of February in *Melia composita*, with the majority occurring from January 13–28. They recorded that after all the leaves had fallen off, leaf primordial began to emerge and a week after the emergence of a new leaf, the panicle with its little projecting buds became visible. They further observed that it took the flower buds 18–28 days to flower and 08:00 and 09:00 hours was the optimum time to record the observation as more than 80 per cent of flower buds opened during this time. There was a time period of 243-264 days from the start of the panicle up to the maturity of the fruit.

Shivaprasad *et al*. (2015) conducted a study on the phenology and reproductive biology of *Cinnamomum sulphuratum* and found that the leaf initiation began in the first week of October in 2011, the second and third weeks of October in 2012, and 2013. In 2011 and 2012, flowering began in the first week of December, whereas in 2013, flowering began the last week of November. In 2012 and 2013, fruit initiation began in the third week of March. Within a week's difference, phenological events were comparable in each of the three years that followed, from 2011 to 2013. Flowers of *C. sulphuratum* had peduncles that were greenish-white in colour, bracteate, actinomorphic, trimerous, perigynous, six perianths in two whorls of three each, and free stamens. The inflorescence is an axillary panicle that took 13±1.41 days to flower and has 62.48±7.01 floral buds. Hermaphrodite *C. sulphuratum* flowers grow in axillary panicles and average 12.37 ±2.40 cm in length. On average, there are 62.48 ± 7.01 flowers per inflorescence, and each flower has an average of 12 anthers arranged in two whorls.

Prasanna *et al*. (2015) studied the reproductive biology of *Canarium strictum* in the Western Ghats, Karnataka and found that in the second week of February, there was a zenith of leaf fall, and in the third week of the same month, there was the emergence of new leaves. As they matured during the second week of March, the leaves' initial colour of dark brownish-red had changed to yellowish green and fresh leaf maturation was followed by the beginning of flowering in the third week of March, and fruit setting began in the last week of March. Flowers on axillary, indeterminate raceme panicles were tiny, actinomorphic.

Elferjani *et al.* (2016) investigated the plasticity of four hybrid poplar (*Populus* spp.) clones established in 2005 along a latitudinal gradient in northwestern Quebec, Canada. They observed that the growing season duration between the southernmost to the northernmost sites ranged from 21 to 32 days, and was positively correlated to stem volume and negatively correlated to bud burst and bud set duration. Leaf net photosynthesis decreased or did not change northwards except for the most productive clone. Maximum rates of carboxylation and photosynthesis electron transfer decreased northwards for three of the four clones, suggesting that the photosynthesis of trees did not acclimate to lower temperatures from south to north. In the boreal zone, bud burst and leaf unfolding start earlier at lower latitudes where temperatures are warmer in early spring, marking the beginning of photosynthetic activity and biomass accumulation.

Lutter *et al.* (2016) observed that geographic origin did not affect the spring phenology of hybrid aspen. However, hybrids with *Populus tremula* parents of northern origin, with bud-burst occurring some days later, were able to unfold and develop full-sized leaves faster than genotypes with early bud-burst. The genotypes of southern origin (55° 53 to 57° 31’N) had a period from bud-burst to defoliation 27 days longer than that of genotypes of northern origin (60° 22’ N). They further observed that hybrid aspen genotypes from 55° to 57°N responded well to northward transfer, having a longer leafy period and greater height increment than southward transferred genotypes. They concluded that Northward-transferred genotypes were apparently better adapted to climate change of the growing season at higher latitudes.

Smitha and Thondaiman (2016) examined the reproductive biology of *Saraca asoca* (Roxb.) from the end of December to the beginning of May; the species have fragrant flowers in paniculate-corymbose inflorescences, with peak flowering in February and March. The anther dehiscence, stigma receptivity, and insect activity coincided with anthesis. Within two

hours of anthesis, pollen viability was at its peak and then declined until no pollen remained viable after six hours. At the time of anthesis, the stigma was responsive, and it lasted for 24 hours.

Vanbeveren *et al.* (2016) carried out a study on phenology in 12 poplars (*Populus*) genotypes and observed that the beginning of autumn senescence following coppicing was the same as it had been the year before. It was observed that the duration of the growing season in terms of leaf area was shorter and the leaf area index was higher in the year following coppicing compared to the year before. In the year following coppicing, the highest seasonal LAI value was twice as high as it was in the year prior to coppicing due to the change from single- to multi-stem coppiced stools: Up to 46 shoots per stool appeared following coppicing.

Ballian and Sito (2017) conducted a study on the growth and phenology of fourteen European provenances of Scots pine at the international experimental plot at Zepce, Bosnia. They analysed phenological development that there is variability between all provenances. They observed that the phenophase of early signs of bud opening and subsequent elongation first appeared on 18 April and last appeared on 29 May, in Romania provenance. The earliest appearance of this phenophase in the remaining nine provenances was on April 22. This phenophase lasted an average of 29 days before disappearing on May 24. They concluded that the phenological characteristics of bud burst and needle formation indicate that these observations need to be continued in order to evaluate the success and genetic variability of the provenances.

Shivaprasad *et al*. (2017) observed leaf initiation and new leaf emergence in second and last week of December, respectively in *Dipterocarpus indicus* having light green, while the stipule's colour remained pink/red in Western ghats, India. They noticed flower bud initiation in the beginning of third week of December and continuing through the end of January 2013 and 2014. The flowers were produced on prominent, drooping axillary racemes, complete, actinomorphic and hermaphrodite and a pink stripe was present in the centre of the five white petals of the corolla with twisted aestivation. The peak floral anthesis was seen between 8:00 and 10:00 a.m.

Cehulic *et al.* (2019) studied that the drought treatment had a substantial impact on the leaf phenology of plants from most of the tested provenances (p 0.001) of *Quercus robur*.

Drought caused flushing phenology to transfer over, resulting in delayed bud burst (from 0.6 to 2.4 days) in the second year and advanced bud burst (from 0.1 to 6.3 days) in the third year. As a result of variances in the time range when plants experienced water deficiencies, opposing shifts in flushing phenology may be caused. Autumn leaf phenology was unmistakably delayed following drought treatments for all tested provenances, in contrast to flushing (from 2.1 to 25.8 days). Differences in late frost sensitivity were primarily due to flushing phenology differences between provenances. The drought treatment, on the other hand, increased the plants' vulnerability to frost (the rate of frost-injured plants per provenance increased from 3 per cent to 78 percent).

Msukwa *et al.* (2019) assessed the phenology of 22 genotypes of *Sclerocarya birrea* (A. Rich.) Hochst. and found highly significant (P0.001) differences between provenances in all of the phenological traits. They observed flowering overlaps and synchrony between provenances and sexes with males flowering earlier than females and early and late flowering genotypes have evolved as subpopulations. They recorded that fruit requires 76±2 to 192±15 days to mature between August and January, whereas the late flowering genotypes flowered and have fruit from September to May. They further observed that between leaf flush, bud set, and flowering, there was a very strong positive connection ranging from r=0.81 to r=0.78.

Hidayat and Suhendri (2020) investigated the impact of microclimate on the flowering and fruiting cycle of *Acer laurinum* trees planted in 1986 at Cibodas Botanical Garden in Cianjur, West Java. They used a rigorous quantitative analysis based on exploratory inventory observations of flowering and fruiting from early January to April and from September to December at the end of the year, as well as high-period precipitation and found that the phenology of flowering and fruiting was not considerably impacted by the dynamic changes in the other micro-climate units.

Sasidharan *et al*. (2020) carried out a study on the floral biology of *Dalbergia latifolia* and *D. sissoides* and found that the two species showed a large amount of variety in their phenological characteristics as *D. latifolia* flowered in August or September and began to have fruit in October and in case of *D. sissoides,* flowering began in the second week of March and lasted up to the end of the month and have produced fruits in April. They further noticed that both species' flowers produced an abundance of nectar with high sugar content and sticky pollen grains, which were the indications of adaptations for insect and bird pollination.

Adler (2021) studied growth and phenology traits across six climatically diverse locations in Sweden and the Baltics to calculate broad-sense heritability and genotype-by-environment (GE) interactions. They investigated the possibility that the early growth of particular poplar clones in Northern Europe is significantly influenced by both bud burst and bud set. For that reason, reference clones of *Populus trichocarpa* that had been adapted to the climate of Central Europe were compared with provenance hybrids that had been suited to the environment of Northern Europe. He found that phenology traits had heritabilities ranging from 0.31 to 0.91. By examining G–E interactions, he came to the conclusion that phenology was a key selection factor after growth for selecting clones.

Diatta *et al.* (2022) found that the development of leaves in *Acacia senegal* trees began prior to the commencement of the rainy season, with blooming and fruiting happening during the rainy season and concluded that leaf development was not triggered by rain but by phenology in *A. Senegal* is genetically controlled, as significant changes in populations and ploidy levels were seen when cultivated at the same location. They found that early leaf-flushing trees had a longer growing period and performed better than later leaf-flushing trees in terms of growth at the tested site. They also noticed that differences in phenology across trees appear to be linked to climate differences at their origin sites since the timing of leaf development in the common garden and the timing of the rainy season at the origin site were significantly correlated for diploid trees (not for tetraploids). They further observed that diploid trees from places with a late rainy season had formed leaves the earliest in the year.

Hernandez-Maximo *et al.* (2022) noticed significant genetic diversity in leaf phenology features, both within and within populations of *Cedrela odorata* provenances. The genetic variation in leaf-out traits was higher than in leaf-fall features. The population differentiation (Qst values) for leaf-out was 2–6 times higher than those for leaf-fall, indicating a moderate to significant genetic diversity among *Cedrela odorata* populations. Leaf fall was predominantly influenced by mean annual temperature, whereas leaf out and the length of the leafless phase were influenced by yearly precipitation and the aridity index of the origin site. They recorded that leaf phenology features indicated moderate to high genetic control (h2i = 0.12–0.67). They concluded that early flushing genotypes can be selected within populations to promote adaptability and growth due to genetic relationships between leaf phenology and growth attributes, but caution should be exercised when selecting between populations.

Orlandi *et al.* (2021) observed different phenological behaviour in three *Salix* species i.e. *Salix acutifolia*, *S. smithiana* and *S. viminalis*. *S. acutifolia* manifested no trend for spring and autumnal phases, *S. viminalis* presented low significant trends while *S. smithiana* was that with the more evident tendencies for all the considered vegetative phases during the study period. The reproductive phase for any *Salix* species during the study period was not influenced by the different meteorological variables and suggesting that photoperiod in this case may play an important role. The more evident phenological trends were represented for two *Salix* species by the advance of the leaf development during spring and by the progressive delay of the senescence during the last part of the summer. They noticed that the fallen leaves phase was recorded an average of two weeks later during the last years of the study period.

Singh *et al.* (2021) investigated the phenology and reproductive biology of ten trees of *Prosopis cineraria* (L.) Druce and concluded that defoliation started in the month of November and persisted through the end of January. They observed that complete defoliation occurred prior to the beginning of fresh vegetative growth, and new leaves only began to emerge after all of the old leaves had fallen off. They further observed that towards the end of February, the fresh leaves began to emerge, initiation of the panicle began in the first week of March in the trees that were chosen at random, and it lasted until the second of April when the flower buds began to open, from the first to the third week of April. They noticed that on the same tree, fresh flowers were grown at various periods due to the asynchronous flowering pattern and from the second week of April until the third week of May, the trees were in full flowering. They further noticed that flowering's peak duration ranged from 13 to 24 days and by the first week of June, flowering was over. Flowering lasted from 27 and 49 days. At the end of April, normal fruit setting was seen on all the labelled trees. They monitored pod maturity from May 23 to June 4.

Panda *et al*. (2021) conducted a study on the impact of climatic patterns on phenophase and growth of multi-purpose trees of north western mid-Himalayan ecosystem. They found that new leaf buds appeared on *Celtis australis*, *Melia. azedarach*, and *Toona. ciliata* by 15th February, which was followed by bud bursting in the 4th week of February and leaf flushing (dark green leaves for *M. azedarach*; small reddish-brown leaves for *T. ciliata*) in the first week of March. *M. azedarach* trees held onto their leaves until the final week of October and leaf fall happened in the final week of November. Similar to other species, *T.*

ciliata entered senescence from 20th September to 15th November. From December through January, neither *T. ciliata* nor *M. azedarach* produced any leaves. *C. australis* were leafless from the last week of December and throughout the month of January. In the last week of February, there was a development of new leaf buds on *Sapindus mukorossi* and *Robinia pseudoacacia*, which was followed by bud bursting (1st–5thMarch) and the emergence of fresh green leaflets (15th-20thMarch). *S. mukorossi'*s leaves matured between November 5 and November 15, and they dropped off in December. From the first week of September through the end of September, *R. pseudoacacia* trees lost their leaves, and from October through February, the trees were leafless.

2.2 POLLEN STUDIES

Boden (1958) studied the handling and storage of pollen in *Eucalyptus* breeding. He observed considerable variation in both pollen production and germination and pollen tube growth despite of adverse weather conditions. It was observed that at low temperatures the rate of germination and pollen tube growth was slow. However, pollen tubes which have commenced to grow, responded to temperature increase and in cold regions there was a diurnal fluctuation in tube length extension, resulting in successful fertilization.

Ahlgren and Ahlgren (1978) performed experiments to study the viability and fertility of vacuum-dried pollen of 5 – needle pine species. They observed that in *in-vitro* tests, pollen of all species (eastern white pine, Korean pine, Balkan pine, Swiss stone pine and Himalayan pine) remained viable for 5 or more years. Eastern white pine pollen remained viable for 8 years and Swiss stone pine for 12 years or more.

Rodriguez and Barrow (1986) evaluated several methods of storing cotton pollen (*Gossypium hirsutum* L.). They observed that storage under ultra-low temperatures in liquid nitrogen or at 5° C was not successful. No storage method maintained pollen fertility for more than 72 hours. Cotton pollen did maintain adequate fertility for up to 24 hours at 10°C and 15°C, at both low and high humidity when the pollen was stored in the detached flowers. It was further noticed that minimally acceptable pollen fertility was maintained in flowers stored at 15°C at 100% R.H. for 72 hours.

Nagarajan *et* al. (1998) studied the pollen sterility and viability in clones of *Tamarindus indica* L. and found that all clones were determined to have very low pollen

sterility. Pollen viability was 88 per cent up to 3 days under natural conditions (37 to 40 °C), but 97 per cent up to 100 days when kept at 4 °C. There were two different sizes of pollen generated (40 µm to 42 µm and 22 µm to 25 µm), with the number of smaller pollens increasing during late flowering seasons maybe as a result of inadequate nourishment

Ng and Corlett (2000) conducted a study of pollen grains of six rhododendron species that grow wild in Hong Kong: *Rhododendron farrerae, R. simsii, R. moulmainense, R. championiae, R. hongkongense* and *R.simiarum*. They found sticky viscin threads and pollen tetrads. Vibrations induced by visitors moving around the flowers and strong winds might readily release pollen-loaded viscin from the anthers. They further noticed that as pollen tetrads were occasionally seen inside unopened flowers, the anthers must mature before the flowers open.

Kopp *et al*. (2002) conducted two experiments to test the effectiveness of various organic solvents for willow (*Salix tetrasperma*) pollen collection and observed that toluene and carbon tetrachloride were effective for pollen extraction, with average pollen germination percentages that were >15%, but both chemicals reduced pollen viability by 10-20% compared with untreated control based on in vitro germination tests. Toluene is the preferred solvent for willow pollen extractions because it is as effective as carbon tetrachloride and is not a known carcinogen as well as less expensive. Pollen extracted with carbon tetrachloride or toluene was successfully used in controlled pollination, and >100 new families were produced with this technique. Pollen viability remained high after 18 months of storage at -20°C.

Tandon *et al*. (2003) studied pollen morphology and pollen viability and found that the exine was reticulate, and pollen grains were trizonocolporate. They further noticed that a flower contained, on average, 52775.49 ± 1698.03 pollen grains (n = 20) and the anther from the free stamen had a significantly smaller number (4334.83 ± 329.22), compared to the anther from the nine fused stamens, which each bore 5382 ± 340.76 pollen grains. Slightly more than 37 per cent of pollen grains were found infertile. They found that at the time of anther dehiscence, about 63 per cent of pollen grains were alive but viability decreased to around 45 per cent after 24 hours and to 30 per cent after 48 hours, at room temperature.

Sunnichan *et al*. (2005) studied the phenology, floral biology and pollination ecology of *Boswellia serrata* Roxb. in a forest near Ghatti Gwalior, Madhya Pradesh and found that

when viewed from the equator, pollen grains range from spherical to oval and had a trizonocolporate composition and a somewhat rugulose exine. The average pollen grain diameter for large anthers was 68.5 ± 2.0 µm, while the average pollen grain diameter for smaller anthers is 60.5 ± 1.7 µm. A flower produces 10044 ± 1259 pollen grains in total. The number of pollen grains produced by each large anther from the outer whorl was 1624 ± 286, whereas the number of pollen grains produced by each small anther from the inner whorl was 474.5 ± 119. The ratio of pollen to ovules was 3348:1.Based on results from acetocarmine staining, a significant number of pollen grains (84.26 ± 4.5) from both the large and small anthers were found viable. They further noticed that in both kinds of anthers, the viability of pollen grains as assessed by an FDA test was also high (85 ± 4.89). The pollen sample kept in a lab steadily lost viability and had 0% viability after seven days.

Verma *et al*. (2011) investigated floral biology and pollen handling of *Grewia optiva* and they observed that flowering buds started appearing along with the appearance of new leaves at end of March. The dehiscence of anther took place prior to anthesis and the process of dehiscence took place within 10-20 minutes. They found that pollen grains were dark yellow in colour and the size of pollen varied from 43 to 53.1 microns in size in different genotypes. They further recorded the percentage of fertile pollen grains of the individual plant and the average viability percentage varied from 77.19 to 80.30 per cent. The maximum receptivity of the stigma was observed when the stigma becomes shiny, sticky and has some viscous liquid on the surface but the receptivity remains for a very short period.

Choudhary and Singh (2013) carried out a pollen study of *Salix* species and for that purpose pollen grains of different species/ clone were collected in bulk from January to March 2009-2010, both by solvent and by direct extraction from catkins. They recorded the size of pollen ranged between 40.49µ in clone 131/25 to 20.20µ in *S. babylonica. In- vitro* pollen germination percentages of directly collected controls viz., *S. tetrasperma* (control 1) and *S. alba* (control 2) were maximum of 54.28 per cent, 59.60 per cent and 63.58 per cent, 62.88 per cent in 2009 and 2010 respectively as compared to pollen extracted with toluene. The average pollen germination percentage was more in the year 2010 as compared to the average pollen germination in 2009. Similarly, pollen viability percentages of pollen extracted with toluene were lower than both of the directly collected controls in 2009 and 2010.

Shivaprasad *et al*. (2015) conducted a study on pollen grains in *Cinnamomum sulphuratum* and found that there were up to 12 anthers per bloom. In 2011, 2012, and 2013, the amount of pollen produced and pollen viability were 7536, 7753, 8470 and 82.60, 80.69, and 87.73%, respectively. In Brew Baker media, the percentage of pollen germination was seen to be 60.97±13.91. The ratio of pollen to ovules was 1256 in 2011, 1292 in 2012, and 1411 in 2013.

Prasanna *et al*. (2015) conducted a study on the pollination ecology of *Canarium strictum* in the Agumbe forest range of Western Ghats, Karnataka and observed that the average pollen count from male flowers with functional pollen was 29904 counts per flower and the average pollen-ovule ratio was 4984. They also observed that pollen germinated *in-vitro* at a mean percentage of 55.82. With TTC and fluorescence tests, the average pollen viability was 90.82 and 93.13 per cent respectively.

Usmani *et al*. (2016) carried out studies on phenology and reproductive biology in 15 populations of *Rauvolfia serpentina* (L.) Benth. ex Kurz (2n = 22). They observed that the stigma was most receptive one day before anthesis, between 08:45 and 09:15 a.m. They further found that per flower, there were four ovules, 225± 20 pollen grains per anther, and 1125± 100 pollen grains overall. There were two distinct sizes of pollen grains (82.5±5 and 52.3 ±5 lm), and their viability ranged from 85 to 95 per cent. They observed shiny, sticky, fresh and attractive stigmas to be receptive while dull, dried, faded and brownish stigmas were considered non-receptive.

Renjumol and Radhamany (2017) studied the phenology, pollination, and breeding system of *Acacia nobilis* in Thiruvananthapuram district and observed that it produced a considerable amount of pollen, with only 29±5 per cent viability in an FDA test and 35 per cent in vitro germination in B&K media, with only 25 per cent germinating on the stigmatic surface. The open-pollinated plant produced the most flowers and fruit.

Shivaprasad *et al*. (2017) found that pollens of *Dipterocarpus indicus* Bedd. were elliptic or circular in form, monad with radial symmetry, and 3-colpate. The average number of pollen grains per anther, which ranged from 520 to 1200, was 742 grains, and there were 22260±5426.27 grains per flower in 2011–2012 while it ranged 200 to 960 with an average of 548 pollens per anther in 2012–2013, and 16440±6108.22 pollens per flower in 2013–2014. In Brew baker media, the percentage of pollen germination was 52.64±13.41% (Mean± S.D).

In 2011 and 2013, the average pollen viability was 62.94% and 57.68% respectively. The pollen:ovule ratio in 2011, was 3710, whereas in 2013 it was 2740.

Chanda *et al.* (2019) conducted a study on pollen morphology at the Bangladesh Agricultural University, Mymensingh, Bangladesh and found that pollen grains were monad, tricolpate, prolate and *Sesbania bispinosa* pollen grains have the thickest exine (2.470.47 m) and the highest P/E ratio (1.81), while *S. cannabina* pollen grains had the thinnest exine (1.160.21 m and 1.57, respectively). *S. cannabina* had the highest pod setting (50.35%), and *S. sesban* ratoon had the lowest (21.68%). They further noticed that *S. bispinosa* had the highest seed setting (91.06%) while *S. cannabina* had the lowest (87.78%).

Kadri *et al.* (2021) conducted a study to evaluate the impact of pollen storage temperature and duration, pollination time following spathe cracking and the hour of daytime on pollen viability, germinability, fruit set and yield of date palm cultivar. It was observed that in *vitro* tests, fresh pollen showed the maximum viability (96.3%) and germination (85%) but it decreased thereafter upon the storage temperature (28, 4 and -30°C) and duration (3, 6, 9 and 12 months). In this respect, pollen stored at -30° C retained the highest viability and germinability followed by those stored at 4 and then 28°C.

Chapter-3

MATERIALS AND METHODS

The present investigation entitled "**Phenological behaviour and reproductive biology of *Salix tetrasperma* Roxb.**" was carried out in the experimental field and laboratory of the Department of Tree Improvement and Genetic Resources, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) during 2021-2023. The experimental details relating to the experimental site, material used and methodology adopted for the study is described under the following headings:

3.1 EXPERIMENTAL SITE

3.1.1 Location

The experimental site is located at 1200 m above mean sea level in north-western Himalayas and between 30˚51' N latitude and 76˚11' E longitude. The annual rainfall ranges between 800-1300mm with maximum downpours during the monsoon season. The genotypes collected from different regions or sites were planted at the experimental area, i.e Naganji nursery of Department of Tree Improvement and Genetic resources for the study in the year 2021-23. The experiment i.e *in-vitro* pollen studies and germination were carried out in the laboratory of the Department of Tree improvement and Genetic resources. The details regarding the latitude, longitude and altitude of the sites from where the genotypes were collected (Sharma *et al.,* 2021) are given in Table 1 and Figure 1.

3.2 EXPERIMENTAL PROCEDURE

3.2.1 Phenological characters

3.2.1.1 Vegetative bud swell

In order to study the time and duration of leaf bud swell, three genotypes per site were taken into consideration. Among selected genotypes, three branches in different directions were selected, the date of the first and last bud swell was recorded and days were counted.

Table 3.1. Latitude, longitude and altitude of the collection sites of *Salix tetrasperma* genotypes.

Sr. No.	Site	State	Latitude (°N)	Longitude (°E)	Altitude (m amsl*)
1	Devamanal	Himachal Pradesh	30.912	77.522	1726.00
2	Jakholi	Uttrakhand	30.519	78.973	1405.50
3	Rampur	Himachal Pradesh	31.592	77.624	942.50
4	Rupnagar	Punjab	31.021	76.650	256.50
5	Tandi	Punjab	31.059	76.228	298.00
6	Suhanpur	Punjab	31.549	75.444	176.50
7	Hamirpur	Himachal Pradesh	31.830	76.585	649.00
8	Namhol	Himachal Pradesh	31.472	76.919	1094.00
9	Bhunter	Himachal Pradesh	32.485	77.196	1108.00
10	Chinani	Jammu & Kashmir	33.144	75.513	1116.50
11	Jammu	Jammu & Kashmir	32.894	74.905	287.50
12	Deothi	Himachal Pradesh	31.135	77.141	1424.00
13	Balh	Himachal Pradesh	31.568	77.063	1153.50
14	Chowari	Himachal Pradesh	32.668	76.059	987.00
15	Chamba	Himachal Pradesh	32.673	76.233	896.00

*amsl = Above mean sea level

3.2.1.2 Vegetative bud burst

The time and duration of leaf bud burst were noted down in all the selected branches in each genotype of each site and days were counted.

3.2.1.3 Leafing / Leafy days

To study leafing/ Leafy days, the date of leaf initiation was recorded and observation continued till the last leaf burst, leaf attained maximum size and duration up to which the genotype retained the leaves and the days were counted.

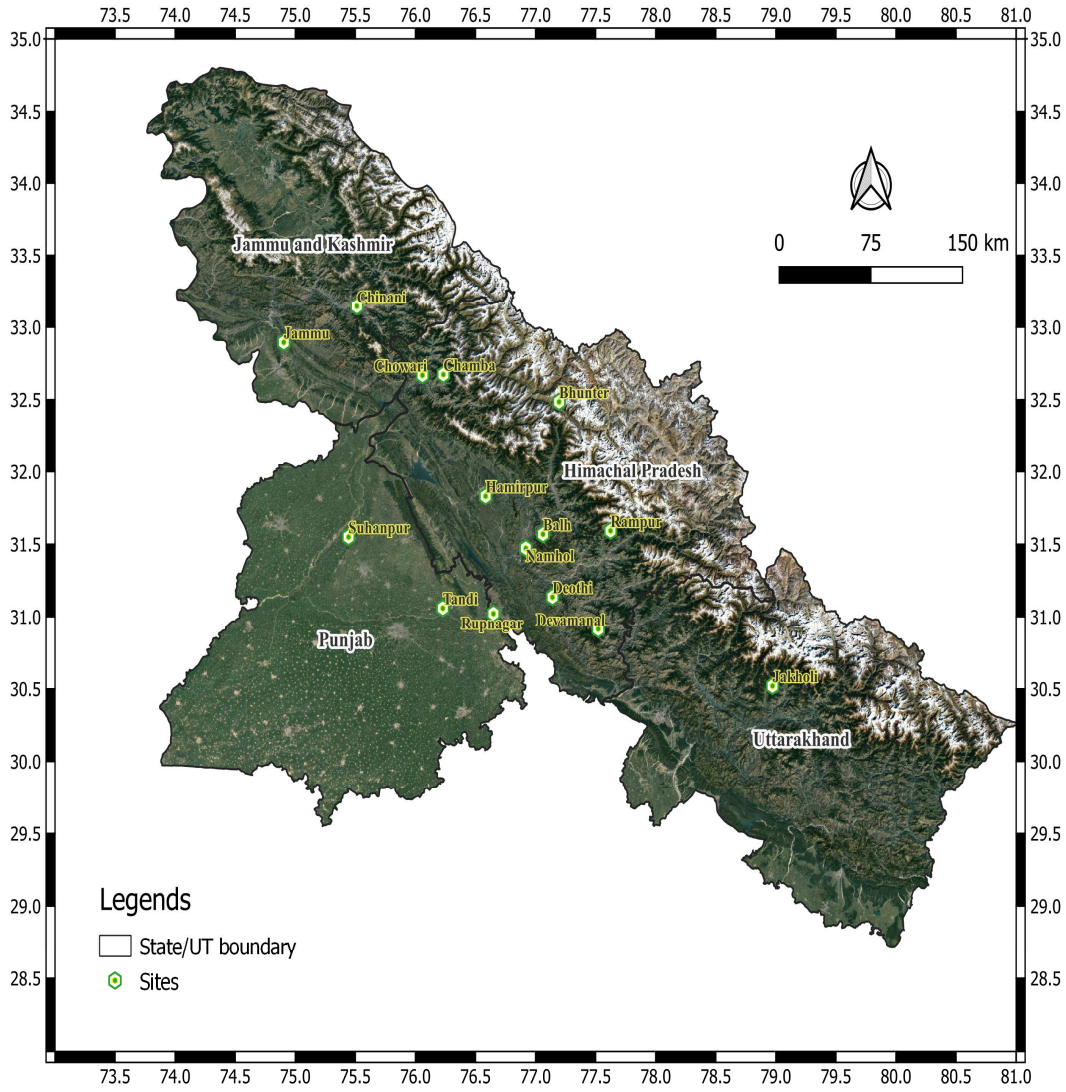


Figure 1. Locations of collections sites of genotypes of *Salix tetrasperma*

3.2.1.4 Leaf Shedding (days)

The days were counted from first leaf fall to 50 per cent, 80 per cent, complete leaf shedding, 50 to 80 per cent, 80 to complete leaf fall and 50 per cent to complete leaf shedding. Complete leaf shedding was considered when all or about 2-3 leaves were retained on the branch.

3.2.2 Reproductive characters

3.2.2.1 Floral bud swell

The time and duration of bud swell were recorded by selecting three branches per genotype and the date of first and last flower bud swell was recorded on these branches.

3.2.2.2 Floral bud burst

To study the time and duration of floral bud burst, three branches in different directions of each genotype were selected and marked and the first and last flower bud burst date was recorded.

3.2.2.3 Catkin development

During the development of flower bud or catkin many morphological changes like change in their shape and size took place and were observed from the time of their emergence to the anthesis stage. So on the basis of their distinguishable characteristics; they were grouped into five stages of bud development (Choudhary 2011). In order to record observations on bud development, the buds on each of the three branches per genotype were tagged just after the initiation of bud and the changes that occurred from one stage to another were recorded.

3.2.2.3.1 Stage I

The first stage of catkin development is the visibility of the generative bud swelling that indicates the starting of expansion of inflorescence. The tissues (contents) of the generative buds remain enclosed within the bud scales at this stage.

3.2.2.3.2 Stage II

The next stage was defined by bud bursting and at this stage; the inflorescence gets enlarged while pushing off the bud scale and the inflorescence tissue starts becoming visible through bud scale which got spilt.

3.2.2.3.3 Stage III

At this stage, around 50 per cent of the inflorescence or catkin got elongated and extended considerably beyond the bud scale. Moreover, the bract hairs start becoming visible and there was rise from the inflorescence main axis. At this stage, the development of stamens started in male plants while distinguishable and highly receptive stigmas on fifty per cent of catkins in pistillate genotypes.

3.2.2.3.4 Stage IV

This stage is characterized by anthesis reaching fifty per cent or more of the inflorescence with the male catkins having bright-coloured pollen on their stamen and visibly receptive stigma.

3.2.2.3.5 Stage V

At this stage, around ninety-five per cent or more than that inflorescence reached the maximum elongation and expansion in terms of length. Anthers had started liberating most of the pollens and the stigma started becoming non-receptive. The catkin length was measured with the help of a measuring scale.

3.2.2.4 Flowering period per branch

The date of initiation of flowering and the last flower on selected branches was recorded and days were counted.

3.2.2.5 Anthesis duration per catkin

In order to record the duration of anthesis per catkin the date of the first opening of the floret up to last opening of the floret on a catkin was recorded by selecting five catkins on a branch.

3.2.2.6 Pollen studies

3.2.2.6.1 Pollen collection

a) Direct pollen collection

In order to analyze pollen viability and pollen germination, the male catkins that were ready for pollen dehiscence were bagged in butter paper in the evening and freshly released pollen was collected the next morning. Male catkins that were maturing in a lab setting were taken at the pre-anthesis stage and put on paper sheets that were kept in partial sunshine. The pollens were released onto the filter paper sheets, and these pollens were then dried in some shade and removed from the filter paper using a sterile camel hair brush. To avoid contamination from any other sources, all of the pollen samples were collected separately.

b) Solvent method

The catkins were plucked after the flower bud burst and the majority of the anthers were pollen-shedding. Flowers were taken out and put in a beaker. Solvent was then poured over the flowers, completely covering them, so that they get submerged in a solvent. The pollen from the anthers was then gently stirred out in the beakers for 60 seconds. Pollens were filtered using Whatman filter paper 1 to separate them from the solvent, and then they were removed from the filter paper using a sterile camel hair brush.

3.2.2.6.2 Pollen germination and viability

Pollen germination and viability was studied using *in-vitro* assay and staining method respectively and expressed as germination percentage and viability percentage respectively.

3.2.2.6.2.1 In-vitro assay

Using the hanging drop technique proposed by Mosseler (1989) for *Salix* species with slight modification. Pollen germination of freshly obtained pollen was examined *in-vitro*. A drop of germination media containing 15 per cent (m/v) sucrose, 0.001 per cent (m/v) and H_3BO_4 having pH 5.0 was placed on the cavity slides. Using a sterile, disposable plastic pipette tip, pollen was placed to the media placed on microscope cavity slides by gently rolling it across the medium. To prevent evaporation and ensure enough gas exchange, the slides were placed inverted on wet paper towels inside sealed plastic containers at room temperature. For each replication, the number of pollen grains that germinated after 16–24 hours of incubation was counted. When a pollen tube was visible and had a length that was equal to or larger than the pollen grain's diameter, the pollen grain was considered germinated when observed under a microscope at a 40X magnification.

3.2.2.6.2.2 Staining method

The viability of freshly collected pollens was examined using a solution of 2% acetocarmine and for that, using hair brush; pollen was sprinkled onto clean microscope slides. A drop of acetocarmine solution was then applied, and the slides were left in this state for 10 to 15 minutes to allow the pollen to absorb the stain. With a microscope, the pollen mass was inspected. Viable pollen was defined as having a deep stain and a normal appearance, whereas nonviable pollen as those having a light or no stain as well as being shriveled and was expressed as a percentage.

3.2.2.7 Stigma receptivity

3.2.7.1 Visual observation of stigmatic surface

The change in the appearance of stigma was observed from 24 hours before the opening of the bud till it withered completely. The shiny watery white stigma was considered receptive while dull and dark brown or black coloured was accounted non-receptive.

3.3 STATISTICAL ANALYSIS

The data obtained for crosses (progenies) was subjected to statistical analysis as per the design. The statistical analysis for each parameter was carried out on mean values and the analysis of variance (ANOVA) table was set up as under:

3.3.1 Analysis of variance (ANOVA) Table for RBD

Data will be analysed by using the following model:

$$Y_{ijk}=\mu+ s_i+ r_j+ e_{ij}$$

i = 1,2,……c

j = 1,2,……r

Where,

Y_{ijk} = phenotypic observation of i^{th} entry and j^{th} replication

M = general mean of population

s_i = effect of i^{th} site

r_j = effect of j^{th} replication, and

e_{ij} = error component

Source of Variation	Degree of Freedom	Sum of Squares	Mean Sum of Squares	F Cal	F Tab
Replication	r-1	$S_r=\frac{1}{s}\sum yj^2-C.F.$	$M_r=\frac{Sr}{r-1}$	$\frac{Mr}{Me}$	F(r-1),(t-1)(r-1)
Site	s-1	$S_s=\frac{1}{r}\sum yj^2-C.F.$	$M_s=\frac{Ss}{s-1}$	$\frac{Ms}{Me}$	F(t-1),(t-1)(r-1)
Error	(r-1)(s-1)	By subtraction	$M_e=\frac{Se}{e-1}$		

Similar ANOVA table was used for comparison of genotypes.

Where,

r = No. of replication

s = No. of sites

M_r = Mean sum of squares due to replications

Ms = Mean sum of squares due to sites

M_e = Mean sum of squares due to error

3.3.2 Critical Difference (CD)

The significance of differences among the treatment means was tested by 'F' test. Wherever 'F' test was found significant, (✸) critical difference was calculated to test the significance between any two treatment means

The critical difference (CD) was calculated as under:

CD = SE_d x $t_{0.05}$ error degree of freedom

Where;

SE_d = Standard error of difference calculated as:

SE_d = $\sqrt{2Me/r}$

$t_{0.05}$ error degree of freedom = T value at 5 per cent level of significance.

3.3.3 Variability and genetic parameters

Genotypic, Phenotypic and environmental variances were calculated as follows:

Coefficient of variability were worked out as suggested by Burton and De Vane (1953) and Pillai and Sinha (1968).

PCV (%) = $\sqrt{\frac{Vp}{\overline{X}}}$ x 100 Vp = Phenotypic variance

GCV (%) = $\sqrt{\frac{Vg}{\overline{X}}}$ x 100 Vg = Genotypic variance

ECV (%) = $\sqrt{\frac{Ve}{\overline{X}}}$ x 100 Ve = Environmental variance

Where,

PCV = Phenotypic Coefficient of Variability
GCV = Genotypic Coefficient of Variability
ECV = Environmental Coefficient of Variability
$\overline{X}$ = Population mean of character

$$CV\ (\%) = \left(\frac{SD}{\overline{X}}\right) \times 100$$

Where,

SD = Standard deviation

$\overline{X}$ = Population mean

3.3.4 Heritability (Broad sense)

Heritability in broad sense was calculated as suggested by Burton and De-Vane (1953) and Johnson *et al*. (1955).

$$h^2_{b.s} = \frac{Vg}{Vp} \times 100$$

Where,

$h^2_{b.s}$ = Heritability (broad sense)

Vg = Genotypic variance

Vp = Phenotypic variance

3.3.5 Genetic Advance

The expected genetic advance at 5 per cent selection intensity was calculated by the formula suggested by Lush (1940) and further used by Burton and De-Vane (1953) and Johnson *et al.,* (1955).

$$\left[\frac{Vg}{Vp}\right] \times \left(\sqrt{Vp}\right) \times K$$

Genetic Advance (GA) = Where,

Vg = Genotypic variance

Vp = Phenotypic variance

K = Selection differential at 5 per cent selection intensity.

The value of K = 2.06 (Allard, 1960).

3.3.6 Genetic gain

Genetic gain was worked out following the method suggested by Johnson *et al.* (1955) as under:

$$\text{Genetic Gain (\%)} = \frac{GA}{\overline{X}} \times 100$$

3.3.7 Correlation Coefficient:

The correlation coefficient will be worked out by using following formula (Panse and Sukhatme, 1967)

$$r_{xy} = \frac{COV(XY)}{\sqrt{V(X)\,V(Y)}}$$

Where,

r_{xy} = simple correlation between x and y

V(X) = variance of 'X' variable

V(Y) = variance of 'Y' variable

Chapter-4

RESULTS AND DISCUSSION

The present investigations entitled **"Phenological behaviour and reproductive biology of *Salix tetrasperma* Roxb."** was carried out in the Department of Tree Improvement and Genetic Resources, College of Forestry, Nauni, Solan (H.P.) during 2021-2023 to study the variation in the phenology, pollen viability and germination among the different genotypes of *Salix tetrasperma* Roxb. The results obtained from the study have been presented under the following headings:

4.1 Phenological characters

4.1.1 Vegetative bud characters

4.1.2 Leaf days

4.1.3 Leaf shedding duration

4.2 Reproductive characters

4.2.1 Reproductive bud characters

4.2.2 Flowering characters

4.2.3 Pollen viability and germination

4.2.4 Autumn season flowering

4.3 Estimates of Genetic parameters

4.4 Correlation

4.1 PHENOLOGICAL CHARACTERS

Genotypes collected from different sites located in Uttrakhand, Punjab, Himachal Pradesh and Jammu and Kashmir in 2017 were planted in the year 2018 for evaluation for reproductive and phenological traits under field conditions at Naganji experimental area. Analysis of variance revealed significant variation for characters viz., time taken to bud swell, bud burst, leafing and leaf fall among different genotypes of *Salix tetrasperma* Roxb.

4.1.1 Vegetative characters

4.1.1.1 Vegetative bud swell duration in spring 2022 and 2023

A perusal of Table 4.1 shows that vegetative bud swell in spring 2022 started on 24-12-2021 and lasted up to 12-02-2022 whereas data appended in Table 4.2 showed that vegetative bud swell in spring 2023 started on 22-12-2022 and lasted up to 09-02-2023.

Analysis of Table 4.7 showed that maximum duration (29.00) for vegetative bud swell in spring 2022 was noticed in genotype CH1 that is statistically at par with genotype CH3 (28.67), BN2 (28.00), CH2 (28.0), BN6 (27.67), SP3 (27.00), BN5 (27.00) and CW2 (27.00) whereas minimum duration (22.67) was found in genotype CN2 and CN3. In the spring of the year 2023 spring genotype CH1 showed maximum duration (29.00) for vegetative bud swell spring in the year 2023 which was statistically at par with BN2 (28.67), CH3 (28.67), BN6 (28.00), CH2 (28.00), SP3 (27.67), DM5 (27.33), BN5 (27.33), DE1 (27.33) and DM3 (27.00) whereas genotype BL3 showed minimum bud swell duration (20.33).

On close appraisal of Table 4.3, it was observed that site-wise duration for vegetative bud swell was 24-12-2021 to 12-02-2022 whereas Table 4.4 revealed that, vegetative bud swell started from 22-12-2022 up to 07-02-2023.

It is evident from Table 4.8 that maximum duration (28.56) for vegetative bud swell in spring 2022 was noticed in site Chamba that was statistically at par with site Bhunter (27.55) whereas minimum duration (23.22) was found in Chinani. The maximum duration (28.56) for vegetative bud swell spring in the year 2023 was noticed in site which was statistically at par with site Bhunter (28.00) whereas Balh showed minimum bud swell duration (21.33).

4.1.1.2 Vegetative bud burst in spring 2022 and 2023

Analysis of Table 4.1 revealed that vegetative bud burst in the spring started on 20-01-2022 and last up to 25-02-2022 whereas appraisal of Table 4.2 showed that vegetative bud burst duration was from 09-01-2021 up to 16-02-2023.

A perusal of Table 4.7 revealed that the maximum duration (16.00) for vegetative bud burst during spring 2022 was recorded for genotype DM3 which was followed by NM2 (13.67), RN4 (13.33), HM1 (13.33) and BL1 (13.33) whereas minimum (11.00) was recorded for CH3, CH1, DE1, DE2 and JM1. On the other hand, in the spring of the year 2023, maximum duration (16.00) was recorded for genotype BN6 which was statistically at par

a) Bud Swell

b) Bud Burst initiation

c) Bud Burst stage 2

d) Bud Burst final stage

Plate 1.1: Vegetative Bud Swell and Bud Burst

with CN1 (15.33), CN2 (15.00), NM4 (14.67), CN3 (14.67) and NM2 (14.33). On the other hand, minimum duration (6.33) was recorded for genotype RN3.

Table 4.1: Duration for vegetative bud swell and bud burst in the spring of 2022

Sr. No.	Genotype	Vegetative Bud swell	Vegetative Bud burst
1.	DM3	15-01-2022 to 10-02-2022	9-02-2022 to 25-02-2022
	DM4	13-01-2022 to 8-02-2022	8-02-2022 to 24-02-2022
	DM5	14-01-2022 to 10-02-2022	8-02-2022 to 26-02-2022
2.	JA1	10-01-2022 to 05-02-2022	02-2-2022 to 15-02-2022
	JA3	16-01-2022 to 12-02-2022	09-2-2022 to 24-02-2022
	JA4	06-01-2022 to 01-02-2022	30-01-2022 to 13-02-2022
3.	RP1	12-01-2022 to 05-02-2022	02-02-2022 to 17-02-2022
	RP2	13-01-2022 to 07-02-2022	06-02-2022 to 19-02-2022
	RP3	14-01-2022 to 09-02-2022	07-02-2022 to 13-02-2022
4.	RN1	07-01-2022 to 31-01-2022	29-01-2022 to 13-02-2022
	RN3	06-01-2022 to 31-01-2022	28-01-2022 to 12-02-2022
	RN4	07-01-2022 to 31-01-2022	31-01-2022 to 14-02-2022
5.	TD1	15-01-2022 to 09-02-2022	07-02-2022 to 21-02-2022
	TD2	13-01-2022 to 09-02-2022	08-02-2022 to 22-02-2022
	TD3	13-01-2022 to 09-02-2022	07-02-2022 to 20-02-2022
6.	SP1	07-01-2022 to 02-02-2022	30-01-2022 to 13-02-2022
	SP3	05-01-2022 to 01-02-2022	01-02-2022 to 14-02-2022
	SP4	07-01-2022 to 01-02-2022	31-01-2022 to 13-02-2022
7.	HM1	24-12-2021 to 20-01-2022	20-01-2022 to 02-02-2022
	HM2	26-12-2021 to 21-01-2022	20-02-2022 to 02-02-2022
	HM4	30-12-2021 to 26-01-2022	22-01-2022 to 07-02-2022
8.	NM2	02-01-2022 to 27-01-2022	25-01-2022 to 09-02-2022
	NM4	02-01-2022 to 28-01-2022	24-01-2022 to 08-02-2022
	NM5	02-01-2022 to 27-01-2022	25-01-2022 to 08-02-2022
9.	BN2	07-01-2022 to 04-02-2022	02-02-2022 to 17-02-2022
	BN5	05-01-2022 to 31-01-2022	01-02-2022 to 15-02-2022
	BN6	07-01-2022 to 05-02-2022	02-02-2022 to 17-02-2022
10.	CN1	09-01-2022 to 02-02-2022	01-02-2022 to 16-02-2022
	CN2	10-01-2022 to 02-02-2022	02-02-2022 to 15-02-2022
	CN3	12-01-2022 to 07-02-2022	02-02-2022 to 19-02-2022
11.	JM1	09-01-2022 to 05-02-2022	03-02-2022 to 17-02-2022
	JM2	09-01-2022 to 06-02-2022	31-01-2022 to 18-02-2022
	JM5	09-01-2022 to 05-02-2022	02-02-2022 to 17-02-2022
12.	DE1	06-01-2022 to 02-02-2022	31-01-2022 to 14-02-2022
	DE2	04-01-2022 to 31-01-2022	28-01-2022 to 10-02-2022
	DE5	02-01-2022 to 27-01-2022	24-01-2022 to 09-02-2022
13.	BL1	04-01-2022 to 28-01-2022	27-01-2022 to 11-02-2022
	BL2	03-01-2022 to 29-01-2022	27-01-2022 to 10-02-2022
	BL3	02-01-2022 to 28-01-2022	25-01-2022 to 09-02-2022
14.	CW1	09-01-2022 to 06-02-2022	06-02-2022 to 17-02-2022
	CW2	11-01-2022 to 08-02-2022	07-02-2022 to 20-02-2022
	CW3	12-01-2022 to 08-02-2022	06-02-2022 to 20-02-2022
15.	CH1	10-01-2022 to 10-02-2022	07-02-2022 to 20-02-2022
	CH2	11-01-2022 to 09-02-2022	08-02-2022 to 21-02-2022
	CH3	10-01-2022 to 09-02-2022	06-02-2022 to 20-02-2022

Table 4.2: Duration for vegetative bud swell and bud burst during spring of 2023

S. No.	Genotype	Vegetative Bud swell	Vegetative Bud burst
1.	DM3	09-01-2023 to 05-02-2023	27-01-2023 to 10-02-2023
	DM4	08-01-2023 to 03-02-2023	25-01-2023 to 8-02-2023
	DM5	08-02-2023 to 07-02-2023	26-01-2023 to 9-02-2023
2.	JA1	04-02-2023 to 31-01-2023	24-01-2023 to 3-02-2023
	JA3	08-01-2023 to 04-02-2023	24-01-2023 to 5-02-2023
	JA4	05-01-2023 to 30-01-2023	24-01-2023 to 5-02-2023
3.	RP1	07-01-2023 to 01-02-2023	28-01-2023 to 8-02-2023
	RP2	07-01-2023 to 31-01-2023	26-01-2023 to 6-02-2023
	RP3	09-01-2023 to 04-02-2023	24-01-2023 to 4-02-2023
4.	RN1	03-01-2023 to 26-01-2023	17-01-2023 to 24-01-2023
	RN3	01-01-2023 to 26-01-2023	12-01-2023 to 20-01-2023
	RN4	01-01-2023 to 27-01-2023	15-01 2023 to 19-01-2023
5.	TD1	09-01-2023 to 03-02-2023	18-01-2023 to 27-01-2023
	TD2	08-01-2023 to 04-02-2023	19-01-2023 to 26-01-2023
	TD3	08-01-2023 to 04-02-2023	21-01-2023 to 25-1 2023
6.	SP1	06-01-2023 to 02-02-2023	16-01-2023 to 25-01-2023
	SP3	04-01-2023 to 02-02-2023	18-01-2023 to 26-01-2023
	SP4	05-01-2023 to 02-02-2023	17-01-2023 to 24-01-2023
7.	HM1	22-12-2022 to 21-01-2023	15-01-2023 to 23-01-2023
	HM2	25-12-2022 to 20-01-2023	14-01-2023 to 26-01-2023
	HM4	28-12-2022 to 26-01-2023	09-01-2023 to 23-01-2023
8.	NM2	01-01-2023 to 27-01-2023	24-01-2023 to 06-02-2023
	NM4	06-01-2023 to 28-01-2023	18-01-2023 to 02-02-2023
	NM5	31-12-2023 to 27-01-2023	19-01-2023 to 31-01-2023
9.	BN2	04-01-2023 to 01-02-2023	22-01-2023 to 04-02-2023
	BN5	02-01-2023 to 30-01-2023	24-01-2023 to 07-02-2023
	BN6	03-01-2023 to 02-02-2023	26-01-2023 to 09-02-2023
10.	CN1	04-01-2023 to 28-01-2023	03-02-2023 to 19-02-2023
	CN2	05-01-2023 to 31-01-2023	05-02-2023 to 20-02-2023
	CN3	07-01-2023 to 03-02-2023	04-02-2023 to 16-02-2023
11.	JM1	14-01-2023 to 10-02-2023	25-01-2023 to 04-02-2023
	JM2	13-01-2023 to 11-02-2023	22-01-2023 to 05-02-2023
	JM5	13-01-2023 to 09-02-2023	24-01-2023 to 06-02-2023
12.	DE1	04-01-2023 to 02-02-2023	23-01-2023 to 07-02-2023
	DE2	02-01-2023 to 30-01-2023	22-01-2023 to 05-02-2023
	DE5	02-01-2023 to 27-01-2023	20-01-2023 to 04-02-2023
13.	BL1	04-01-2023 to 28-01-2023	21-01-2023 to 31-01-2023
	BL2	03-01-2023 to 29-01-2023	20-01-2023 to 29-01-2023
	BL3	02-01-2023 to 28-01-2023	18-01-2023 to 30-01-2023
14.	CW1	09-01-2023 to 06-02-2023	23-01-2023 to 06-02-2023
	CW2	11-01-2023 to 08-02-2023	21-01-2023 to 05-02-2023
	CW3	12-01-2023 to 08-02-2023	26-01-2023 to 08-02-2023
15.	CH1	05-01-2023 to 07-02-2023	29-01-2023 to 06-02-2023
	CH2	07-01-2023 to 05-02-2023	25-01-2023 to 02-02-2023
	CH3	06-01-2023 to 05-02-2023	23-01-2023 to 07-02-2023

It is evident from Table 4.3 that duration for vegetative bud burst was from 30-01-2022 to 26-02-2022 on site basis whereas Table 4.4 revealed that, vegetative bud burst started from 09-01-2023 up to 19-02-2023.

Close appraisal of Table 4.8 showed that the maximum duration (13.67) for vegetative bud burst during spring 2022 was recorded for site Devamanal which was statistically at par with Rupnagar (13.00) whereas minimum (11.11) was recorded for Chamba. Appraisal of same table showed that for vegetative bud burst in the spring 2023, maximum duration (15.00) was recorded for site Chinani followed by site Namhol, Bhunter (14.00) and Chowari (13.67). On the other hand, minimum duration (7.56) for bud burst was recorded for site Rupnagar.

4.1.1.3 Vegetative bud swell and bud burst during whole year 2022

Analysis of Table 4.5 revealed that vegetative bud swell during the whole year 2022 started from 02-01-2022 up to 31-10-2022 whereas vegetative bud burst duration was from 14-01-2022 to 17-11-2022.

Close appraisal of Table 4.6 showing site-wise analysis revealed that during the whole year 2022 vegetative bud swell started from 02-01-2022 up to 31-10-2022 whereas vegetative bud burst duration was from 14-01-2022 to 18-11-2022.

Data appended in Table 4.7 revealed that maximum duration (300.00) for vegetative bud swell during whole year 2022 was observed in genotype SP3 which was statistically at par with TD2 (299.33), TD3 (298.67), SP1 (298.67) and SP4 (298.67) whereas minimum (231.00) was recorded for CN3. The vegetative bud burst whole year duration was found maximum in genotype SP1 (290.33) followed by SP4 (288.33), SP3 (287.67), TD3 (287.33) and TD1 (285.33) whereas minimum was found in CN3 (218.00).

A perusal of Table 4.8 showed that the maximum duration (299.00) for vegetative bud swell during whole year 2022 was observed in case of site Suhanpur which was statistically at par with site Tandi (298.22) and Rupnagar (296.22) whereas minimum (233.89) was recorded for site Chinani. The maximum duration for vegetative bud burst during whole year duration was found in site Suhanpur (288.78) followed by site Tandi (285.22), Rupnagar (283.00) and Hamirpur (262.45) whereas minimum was found in Chinani (220.89).

Table 4.3: Sitewise vegetative bud swell and bud burst duration for *Salix tetrasperma* genotypes in spring 2022

Sr. No.	Site	Bud swell duration	Bud burst duration
1	Devamanal	13-01-2022 to 10-02-2022	8-02-2022 to 26-02-2022
2	Jakholi	6-01-2022 to 12-02-2022	30-1-2022 to 24-02-2022
3	Rampur	12-01-2022 to 09-02-2022	02-02-2022 to 19-02-2022
4	Rupnagar	06-01-2022 to 31-01-2022	29-01-2022 to 14-02-2022
5	Tandi	13-01-2022 to 09-02-2022	07-02-2022 to 22-02-2022
6	Suhanpur	05-01-2022 to 02-02-2022	30-01-2022 to 14-02-2022
7	Hamirpur	24-12-2021 to 26-01-2022	20-01-2022 to 07-02-2022
8	Namhol	02-01-2022 to 28-01-2022	24-01-2022 to 09-02-2022
9	Bhunter	05-01-2022 to 05-02-2022	01-02-2022 to 17-02-2022
10	Chinani	09-01-2022 to 07-02-2022	01-02-2022 to 19-02-2022
11	Jammu	09-01-2022 to 06-02-2022	31-01-2022 to 18-02-2022
12	Deothi	02-01-2022 to 02-02-2022	24-01-2022 to 14-02-2022
13	Balh	02-01-2022 to 29-01-2022	25-01-2022 to 11-02-2022
14	Chowari	09-01-2022 to 08-02-2022	06-02-2022 to 20-02-2022
15	Chamba	10-01-2022 to 10-02-2022	06-02-2022 to 21-02-2022

Table 4.4: Sitewise vegetative bud swell and bud burst duration for *Salix tetrasperma* genotypes in spring 2023

Sr. No.	Site	Bud swell duration	Bud burst duration
1	Devamanal	08-01-2023 to 7-02-2023	25-01-2023 to 10-02-2023
2	Jakholi	04-02-2023 to 30-01-2023	25-01-2023 to 08-02-2023
3	Rampur	07-01-2023 to 04-02-2023	24-01-2023 to 09-02-2023
4	Rupnagar	03-01-2023 to 27-01-2023	12-01-2023 to 25-01-2023
5	Tandi	09-01-2023 to 04-02-2023	19-01-2023 to 28-01-2023
6	Suhanpur	06-01-2023 to 02-02-2023	16-01-2023 to 26-01-2023
7	Hamirpur	22-12-2022 to 26-01-2023	9-01-2023 to 27-01-2023
8	Namhol	01-01-2023 to 27-01-2023	18-01-2023 to 06-02-2023
9	Bhunter	04-01-2023 to 02-02-2023	24-1-2023 to 10-02-2023
10	Chinani	04-01-2023 to 03-02-2023	03-02-2023 to 19-02-2023
11	Jammu	14-01-2023 to 09-02-2023	22-01-2023 to 08-02-2023
12	Deothi	04-01-2023 to 27-01-2023	20-01-2023 to 07-02-2023
13	Balh	04-01-2023 to 28-01-2023	17-01-2023 to 02-02-2023
14	Chowari	11-01-2023 to 07-02-2023	21-01-2023 to 09-02-2023
15	Chamba	07-01-2023 to 05-02-2023	23-01-2023 to 11-02-2023

Table 4.5: Duration for vegetative bud swell, bud burst and leafy days during whole year 2022

Sr. No.	Genotype	Vegetative Bud swell	Vegetative Bud burst	Leafy days
1.	DM3	15-01-2022 to 15-09-2022	28-01-2022 to 24-09-2022	28-01-2022 to 29-11-2022
	DM4	13-01-2022 to 08-09-2022	25-01-2022 to 18-09-2022	25-01-2022 to 17-11-2022
	DM5	14-01-2022 to 10-09-2022	16-01-2022 to 18-09-2022	16-01-2022 to 12-11-2022
2.	JA1	10-01-2022 to 05-09-2022	26-01-2022 to 15-09-2022	26-01-2022 to 24-11-2022
	JA3	16-01-2022 to 12-09-2022	29-01-2022 to 18-09-2022	29-01-2022 to 28-11-2022
	JA4	05-01-2022 to 21-09-2022	15-01-2022 to 26-09-2022	15-01-2022 to 17-11-2022
3.	RP1	11-01-2022 to 15-09-2022	25-01-2022 to 22-09-2022	25-01-2022 to 07-12-2022
	RP2	13-01-2022 to 19-09-2022	29-01-2022 to 26-09-2022	29-01-2022 to 07-12-2022
	RP3	14-01-2022 to 17-09-2022	30-01-2022 to 25-09-2022	30-01-2022 to 03-12-2022
4.	RN1	07-01-2022 to 31-10-2022	14-01-2022 to 12-11-2022	14-01-2022 to 19-12-2022
	RN3	06-01-2022 to 31-10-2022	15-01-2022 to 14-11-2022	15-01-2022 to 29-12-2022
	RN4	07-01-2022 to 31-10-2022	16-01-2022 to 15-11-2022	16-01-2022 to 17-12-2022
5.	TD1	15-01-2022 to 09-11-2022	22-01-2022 to 18-11-2022	22-01-2022 to 14-11-2022
	TD2	13-01-2022 to 09-11-2022	22-01-2022 to 17-11-2022	22-01-2022 to 15-11-2022
	TD3	13-01-2022 to 09-11-2022	23-01-2022 to 18-11-2022	23-01-2022 to 18-11-2022
6.	SP1	07-01-2022 to 02-11-2022	16-01-2022 to 17-11-2022	16-01-2022 to 01-12-2022
	SP3	05-01-2022 to 01-11-2022	14-01-2022 to 15-11-2022	14-01-2022 to 30-11-2022
	SP4	07-01-2022 to 01-11-2022	16-01-2022 to 16-11-2022	16-01-2022 to 03-12-2022
7.	HM1	04-01-2022 to 11-10-2022	16-01-2022 to 19-10-2022	16-01-2022 to 18-11-2022
	HM2	06-01-2022 to 10-10-2022	18-01-2022 to 22-10-2022	18-01-2022 to 20-11-2022
	HM4	02-01-2022 to 06-10-2022	17-01-2022 to 19-10-2022	17-01-2022 to 20-11-2022
8.	NM2	02-01-2022 to 27-09-2022	17-01-2022 to 03-10-2022	17-01-2022 to 11-11-2022
	NM4	02-01-2022 to 28-09-2022	19-01-2022 to 05-10-2022	19-01-2022 to 13-11-2022
	NM5	02-01-2022 to 27-09-2022	18-01-2022 to 04-10-2022	18-01-2022 to 12-11-2022
9.	BN2	07-01-2022 to 04-09-2022	27-01-2022 to 11-09-2022	27-01-2022 to 25-11-2022
	BN5	05-01-2022 to 02-09-2022	26-01-2022 to 16-09-2022	26-01-2022 to 22-11-2022
	BN6	07-01-2022 to 05-09-2022	26-01-2022 to 15-09-2022	26-01-2022 to 19-11-2022
10.	CN1	09-01-2022 to 05-09-2022	31-01-2022 to 16-09-2022	31-01-2022 to 27-11-2022
	CN2	10-01-2022 to 31-08-2022	02-02-2022 to 11-09-2022	02-02-2022 to 24-11-2022
	CN3	12-01-2022 to 01-09-2022	03-02-2022 to 11-09-2022	03-02-2022 to 26-11-2022
11.	JM1	09-01-2022 to 15-09-2022	21-01-2022 to 25-09-2022	21-01-2022 to 18-11-2022
	JM2	09-01-2022 to 16-09-2022	25-01-2022 to 26-09-2022	25-01-2022 to 18-11-2022
	JM5	09-01-2022 to 15-09-2022	28-01-2022 to 24-09-2022	28-01-2022 to 23-11-2022
12.	DE1	06-01-2022 to 02-09-2022	15-01-2022 to 09-09-2022	15-01-2022 to 13-11-2022
	DE2	04-01-2022 to 09-09-2022	15-01-2022 to 16-09-2022	15-01-2022 to 13-11-2022
	DE5	02-01-2022 to 07-09-2022	22-01-2022 to 16-09-2022	22-01-2022 to 13-11-2022
13.	BL1	04-01-2022 to 08-09-2022	23-01-2022 to 19-09-2022	23-01-2022 to 03-12-2022
	BL2	03-01-2022 to 09-09-2022	18-01-2022 to 24-09-2022	18-01-2022 to 01-12-2022
	BL3	02-01-2022 to 08-09-2022	17-01-2022 to 24-09-2022	17-01-2022 to 01-12-2022
14.	CW1	09-01-2022 to 06-09-2022	24-01-2022 to 18-09-2022	24-01-2022 to 25-11-2022
	CW2	11-01-2022 to 08-09-2022	25-01-2022 to 19-09-2022	25-01-2022 to 26-11-2022
	CW3	12-01-2022 to 08-09-2022	29-01-2022 to 19-09-2022	29-01-2022 to 28-11-2022
15.	CH1	10-01-2022 to 12-09-2022	24-01-2022 to 23-09-2022	24-01-2022 to 17-11-2022
	CH2	11-01-2022 to 14-09-2022	24-01-2022 to 24-09-2022	24-01-2022 to 20-11-2022
	CH3	10-01-2022 to 15-09-2022	27-01-2022 to 21-09-2022	27-01-2022 to 19-11-2022

Table 4.6: Sitewise vegetative bud swell and bud burst duration and number of leafy days for *Salix tetrasperma* genotypes for whole year 2022

Sr. No.	Site	Bud swell duration	Bud burst duration	Leafy Days
1	Devamanal	13-01-2022 to 15-09-2022	16-01-2022 to 24-09-2022	16-01-2022 to 29-11-2022
2	Jakholi	05-01-2022 to 21-09-2022	15-01-2022 to 26-09-2022	15-01-2022 to 28-11-2022
3	Rampur	11-01-2022 to 19-09-2022	25-01-2022 to 26-09-2022	25-01-2022 to 07-12-2022
4	Rupnagar	06-01-2022 to 31-10-2022	14-01-2022 to 15-11-2022	14-01-2022 to 29-12-2022
5	Tandi	13-01-2022 to 09-02-2022	22-01-2022 to 18-11-2022	22-01-2022 to 18-11-2022
6	Suhanpur	05-01-2022 to 02-02-2022	14-01-2022 to 17-11-2022	14-01-2022 to 03-12-2022
7	Hamirpur	02-01-2022 to 11-10-2022	16-01-2022 to 22-10-2022	16-01-2022 to 20-11-2022
8	Namhol	02-01-2022 to 28-09-2022	17-01-2022 to 05-10-2022	17-01-2022 to 13-11-2022
9	Bhunter	05-01-2022 to 05-09-2022	27-01-2022 to 16-09-2022	27-01-2022 to 25-11-2022
10	Chinani	09-01-2022 to 05-09-2022	31-01-2022 to 16-09-2022	31-01-2022 to 27-11-2022
11	Jammu	09-01-2022 to 06-09-2022	22-01-2022 to 26-09-2022	22-01-2022 to 23-11-2022
12	Deothi	02-01-2022 to 09-09-2022	15-01-2022 to 16-09-2022	15-01-2022 to 13-11-2022
13	Balh	02-01-2022 to 09-09-2022	17-01-2022 to 24-09-2022	17-01-2022 to 03-12-2022
14	Chowari	09-01-2022 to 08-09-2022	24-01-2022 to 19-09-2022	24-01-2022 to 23-11-2022
15	Chamba	10-01-2022 to 18-09-2022	24-01-2022 to 24-09-2022	24-01-2022 to 20-11-2022

4.1.2 Leafy days in the year 2022

In case of leafy days (Table 4.7), the results recorded for leafy days were found to be significantly different among the genotypes maximum number of days (348.33) was found in RN3 followed by RN1 (337.67), RN4 (333.33), SP4 (320.67) and SP3 (319.33) whereas minimum was found in genotype DE5 (292.00).

The results recorded for leafy days (Table 4.8) were found to be significantly different among all the sites. Maximum number of leafy days (339.78) was recorded for site Rupnagar which was followed by site Suhanpur (319.67), Balh (315.33), Rampur (311.11) and Hamirpur (305.33) whereas minimum number of days was recorded for site Tandi (295.22).

In the present study, significant variation was found between sites as well as among the genotypes with respect to vegetative bud swell and bud burst during spring of 2022 and 2023 which is comparable with the study conducted by Weih (2009) in he found significant variation among the genotypes of *Salix* for bud burst date and leafy period and that variation were related to the source habitats of the parental genotypes. According to McKown *et al.* (2018), bud break showed a quadratic relationship with latitude, and provenances from the southern and northernmost regions of species range typically breaking bud earlier than those from middle regions. This pattern contrasted with study carried on phenology of *Populus trichocarpa*, by Evans *et al*. (2014) and McKown *et al*. (2014). In aspen (*Populus tremula*),

Luquez *et al*. (2008) noticed a very high clinal variation pattern with respect to the date of bud set and leaf area duration, but no change in the date of bud flush along a latitudinal cline.

Table 4.7: Number of days for vegetative bud swell, bud burst during spring of 2022 and 2023 and number of leafy days for *Salix tetrasperma* genotypes in 2022

Sr. No.	Genotype	Vegetative Bud swell in spring2022 (Days)	Vegetative Bud burst spring 2022 (Days)	Vegetative Bud swell in spring2023 (Days)	Vegetative Bud burst spring 2023 (Days)	Vegetative Bud swell whole year duration	Vegetative Bud burst whole year duration	Leafy days
1.	DM3	26.00	16.00	27.00	13.67	239.67	234.33	304.00
	DM4	25.00	12.67	25.33	11.67	237.00	234.67	293.67
	DM5	26.00	12.33	27.33	12.33	238.00	239.33	293.33
2.	JA1	25.33	11.33	26.33	11.67	237.00	231.33	300.33
	JA3	24.67	12.67	25.00	12.67	236.67	231.67	302.00
	JA4	24.33	12.67	24.33	12.00	257.67	250.00	301.00
3.	RP1	23.67	12.33	24.00	11.33	245.67	238.00	314.67
	RP2	25.00	11.67	25.33	12.00	247.00	238.67	312.00
	RP3	25.00	12.00	25.67	11.33	247.00	237.67	306.67
4.	RN1	23.33	12.67	22.67	8.33	296.33	281.00	337.67
	RN3	23.00	13.00	23.33	6.33	296.00	283.67	348.33
	RN4	23.33	13.33	24.00	8.00	296.33	284.33	333.33
5.	TD1	24.00	12.33	24.33	8.67	296.67	285.33	293.67
	TD2	26.33	12.33	26.33	8.00	299.33	283.00	293.67
	TD3	25.67	12.33	26.33	7.67	298.67	287.33	298.33
6.	SP1	26.33	12.67	26.33	8.67	298.67	290.33	319.00
	SP3	27.00	12.33	27.67	8.33	300.00	287.67	319.33
	SP4	25.00	12.33	26.00	9.33	298.33	288.33	320.67
7.	HM1	26.67	13.33	24.67	11.67	279.00	262.33	306.00
	HM2	25.00	12.00	23.33	12.67	276.00	263.00	304.00
	HM4	25.00	13.00	26.33	12.00	277.67	262.00	306.00
8.	NM2	23.67	13.67	25.00	14.33	266.67	230.67	297.67
	NM4	23.33	12.00	24.67	14.67	266.33	231.67	296.00
	NM5	24.67	12.33	26.33	13.00	267.33	229.00	294.00
9.	BN2	28.00	13.00	28.67	12.33	238.00	226.00	300.33
	BN5	27.00	12.33	27.33	13.67	238.33	230.33	299.67
	BN6	27.67	11.67	28.00	16.00	239.67	231.00	297.67
10.	CN1	24.33	12.00	26.67	15.33	237.00	224.67	299.00
	CN2	22.67	12.67	26.67	15.00	233.67	220.00	294.67
	CN3	22.67	12.67	25.67	14.67	231.00	218.00	293.67
11.	JM1	26.00	11.00	25.33	13.33	249.00	241.33	296.33
	JM2	25.67	12.00	24.33	12.00	249.00	241.00	294.67
	JM5	25.00	12.33	24.00	13.67	247.67	237.00	297.33
12.	DE1	26.33	11.00	27.33	13.00	237.67	233.00	297.33
	DE2	26.00	11.00	26.33	13.00	242.33	237.00	301.67
	DE5	23.00	12.67	23.00	12.33	246.00	233.00	292.00
13.	BL1	24.00	13.33	21.67	12.00	247.33	238.00	313.33
	BL2	24.00	12.33	22.00	11.33	247.00	245.33	315.33
	BL3	24.00	12.67	20.33	13.33	247.00	246.33	317.33
14.	CW1	26.67	11.33	24.33	14.00	238.67	237.00	304.67
	CW2	27.00	11.67	23.67	13.00	239.00	236.00	303.67
	CW3	25.33	12.67	25.67	14.00	237.33	232.00	302.67
15.	CH1	29.00	11.00	29.00	11.67	242.33	239.00	296.67
	CH2	28.00	11.33	28.00	11.00	245.33	242.67	299.33
	CH3	28.67	11.00	28.67	11.33	247.67	237.33	296.67
	Mean	**25.30**	**12.33**	**25.43**	**11.92**	**257.33**	**246.92**	**304.65**
	$CD_{(0.05)}$	1.86	1.46	2.18	1.71	2.88	5.01	3.79

Ducousso *et al.* (1996) found significant variation among provenances for bud burst of sessile oak (*Quercus petraea*) that was mainly clinal and related to altitude and latitude of origin. They also noticed the late flush in northern provenances than southern which was similar to results our study. While Rossi and Bousquet (2014) obtained opposite trend in black spruce due to phenological adaptations of the species to its local environmental conditions. Ro¨nnberg - Wa¨stljung (2001) and Pellis *et al.* (2004) observed that timing of critical stages for growth initiation i.e. bud and leaf phonologies as well as that of growth termination (e.g. growth cessation and leaf abscission) were highly influenced by environmental conditions and therefore were given importance in breeding for biomass production in willows and Poplars. Species and clonal difference in bud flushing in *Salix* was documented by Ro¨nnberg - Wa¨stljung and Gull-berg (1999). Whittet *et al*. (2021) demonstrated that timing of bud burst was negatively associated with latitude of in provenance trial of sycamore (*Acer pseudoplatanus* L.) in England showing adaptive differentiation among provenances.

Table 4.8: Sitewise vegetative bud swell, bud burst duration and number of days of during spring of 2022 and 2023 and number of leafy days for *Salix tetrasperma* genotypes in 2022

Sr No.	Site	Vegetative Bud swell in spring2022 (Days)		Vegetative Bud burst spring 2022 (Days)		Vegetative Bud swell whole year duration	Vegetative Bud burst whole year duration	Leafy days
		2022	**2023**	**2022**	**2023**	**2022**	**2022**	**2022**
1	Devamanal	25.67	26.56	13.67	12.56	238.22	236.11	297.00
2	Jakholi	24.78	25.22	12.22	12.11	243.78	237.67	301.11
3	Rampur	24.56	25.00	12.00	11.56	246.56	238.11	311.11
4	Rupnagar	23.22	23.33	13.00	7.56	296.22	283.00	339.78
5	Tandi	25.30	25.67	12.33	8.11	298.22	285.22	295.22
6	Suhanpur	26.111	26.67	12.44	8.78	299.00	288.78	319.67
7	Hamirpur	25.56	24.78	12.78	12.11	277.56	262.45	305.33
8	Namhol	23.89	25.33	12.67	14.00	266.78	230.44	295.89
9	Bhunter	27.56	28.00	12.33	14.00	238.67	229.11	299.22
10	Chinani	23.22	26.33	12.44	15.00	233.89	220.89	295.78
11	Jammu	25.56	24.56	11.78	13.00	248.56	239.78	296.11
12	Deothi	25.11	25.56	11.56	12.78	242.00	234.33	297.00
13	Balh	24.00	21.33	12.78	12.22	247.11	243.22	315.33
14	Chowari	26.33	24.56	11.89	13.67	238.33	235.00	303.67
15	Chamba	28.56	28.56	11.11	11.33	245.11	239.67	297.56
	Mean	**25.30**	**25.43**	**12.33**	**11.92**	**257.33**	246.92	304.65
	$CD_{(0.05)}$	1.06	1.26	0.83	0.99	1.64	2.89	2.19

4.1.3 Leaf shedding durations

4.1.3.1 Duration for 50 per cent leaf shedding (Days)

Genotypes of different sites were found to be statistically different from each other for the time taken for 50 per cent leaf shedding. Maximum no. of days for 50 per cent leaf shedding was observed in Genotype RN3 (42 days) which was followed by genotype RN4 (38.33), RN1 (36.67) and RP2 (34.67). Minimum number of days for 50 per cent leaf shedding (13.33) was recorded in genotype TD1 as showed in table 4.9.

The results in Table 4.10 showed that Rupnagar site recorded maximum duration (39 days) for 50 per cent leaf shedding which was followed by site Rampur (32.78), Chinani (24.89) and Jakholi (24.78). Minimum duration for 50 per cent leaf shedding was recorded in case of site Tandi (14.33 days) which was statistically at par with site Deothi (15.78 days).

4.1.3.2 Duration for 80 per cent leaf shedding (Days)

A perusal of table 4.9 revealed that genotypes of different sites found statistically different from each other for the time taken for 80 per cent leaf shedding. Maximum number of days for 80 per cent leaf shedding was observed in Genotype RN3(62.00 days) which was followed by genotype RN1 (54.00), RN4 (54.00) and RP2 (46.33). Minimum number of days for 80 per cent leaf shedding (20.67 days) was recorded in Genotype TD1 which was statistically at par with DE1 (21.00), DE5 (21.33), NM5 (21.33), DE2 (22.33), NM2 (22.67), TD2 (23.67) and CH1 (23.67).

The maximum duration for 80 per cent leaf shedding (Table 4.10) was found in site Rupnagar (56.67 days) followed by site Rampur (45.00), Suhanpur (37.33) and Balh (35.00) whereas minimum duration was observed in case of site Deothi (21.56 days) which was statistically at par with site Namhol (22.78 days) and Tandi (23.11 days).

4.1.3.3 Duration for complete leaf shedding (Days)

Data appended in Table 4.9 showed that genotypes of different sites were statistically different from each other in the case of the time taken for complete leaf fall. The duration of leaf fall in all genotypes was found statistically different from each other. Maximum number of days for complete leaf fall was observed in genotype RN3 (78.67 days) followed by RN1 (67.33), RN4 (65.67) and RP2 (56.33). The minimum number of days was observed in Genotype DE1 (26.00) which was statistically at par with DE2 (26.67), NM5 (26.67), DE5 (27.0), NM2 (27.67), NM4 (29.0), TD1 (29.33), CH1 (29.67), CH2 (31.67), CH3 (31.67), DM5 (32.0), TD2 (32.33) and HM1 (33.33).

Table 4.9: Variation in *Salix tetrasperma* genotypes in respect of days taken from full maturation to complete leaf fall, 50 per cent to complete leaf fall, 80 per cent to complete leaf fall

Sr. No.	Genotype	50 per cent leaf fall	80 per cent leaf fall	Complete leaf fall
1.	DM3	28.33	41.00	48.67
	DM4	20.67	29.00	36.33
	DM5	19.00	27.67	32.00
2.	JA1	26.00	35.00	42.33
	JA3	27.67	38.33	47.67
	JA4	20.67	30.67	36.67
3.	RP1	33.33	46.00	56.00
	RP2	34.67	46.33	56.33
	RP3	30.33	42.67	52.33
4.	RN1	36.67	54.00	67.33
	RN3	42.00	62.00	78.67
	RN4	38.33	54.00	65.67
5.	TD1	13.33	20.67	29.33
	TD2	15.00	23.67	32.33
	TD3	14.67	25.00	33.67
6.	SP1	21.33	37.33	47.00
	SP3	24.67	35.67	45.67
	SP4	25.67	39.00	49.33
7.	HM1	17.00	24.00	33.33
	HM2	18.33	26.33	35.00
	HM4	17.00	27.00	35.00
8.	NM2	17.00	22.67	27.67
	NM4	16.33	24.33	29.00
	NM5	15.67	21.33	26.67
9.	BN2	23.33	33.67	40.33
	BN5	19.67	29.33	37.00
	BN6	19.67	29.00	51.33
10.	CN1	24.00	33.33	41.33
	CN2	24.33	33.33	39.33
	CN3	26.33	34.00	40.67
11.	JM1	21.00	28.33	34.33
	JM2	18.67	25.33	32.00
	JM5	21.00	29.33	37.00
12.	DE1	15.33	21.00	26.00
	DE2	16.00	22.33	26.67
	DE5	16.00	21.33	27.00
13.	BL1	25.33	36.33	47.67
	BL2	23.67	34.00	44.00
	BL3	24.33	34.67	45.00
14.	CW1	21.67	31.00	39.67
	CW2	21.67	30.67	39.00
	CW3	21.00	30.67	40.67
15.	CH1	17.00	23.67	29.67
	CH2	18.00	24.67	31.67
	CH3	18.67	25.33	31.67
	Mean	22.45	32.11	40.58
	$CD_{(0.05)}$	2.77	3.07	7.42

Among sites (Table 4.10), Rupnagar recorded maximum duration (70.56 days) for complete leaf fall which was followed by site Rampur (54.89), Suhanpur (47.33) and Balh (45.56) and minimum duration was recorded in site Deothi (26.56) which was statistically at par with site Namhol (27.78).

Table 4.10 Sitewise variation of *Salix tetrasperma* genotypes in respect of days taken from full maturation to complete leaf shedding, 50 per cent to complete leaf shedding, 80 per cent to complete leaf shedding

Sr No.	Site	Complete leaf shedding	50 per cent Complete leaf shedding	80 per cent Complete leaf shedding
1	Devamanal	39.00	22.67	32.56
2	Jakholi	42.22	24.78	34.67
3	Rampur	54.89	32.78	45.00
4	Rupnagar	70.56	39.00	56.67
5	Tandi	31.78	14.33	23.11
6	Suhanpur	47.33	23.89	37.33
7	Hamirpur	34.44	17.44	25.78
8	Namhol	27.78	16.33	22.78
9	Bhunter	42.89	20.89	30.67
10	Chinani	40.44	24.89	33.56
11	Jammu	34.44	20.22	27.67
12	Deothi	26.56	15.78	21.56
13	Balh	45.56	24.44	35.00
14	Chowari	39.78	21.44	30.78
15	Chamba	31.00	17.89	24.56
	Mean	40.58	22.45	32.11
	$CD_{(0.05)}$	4.28	1.60	1.78

4.1.3.4 Duration from 50 to 80 per cent leaf shedding (Days)

A perusal of Table 4.11 revealed that for 50-80 per cent leaf shedding, the maximum number of days (20.00) was noticed in genotype RN3 followed by RN1 (17.33), SP1 (16.00) and RN4 (15.67) whereas minimum (5.33) was noticed in genotype DE5. Minimum number of days was observed in Genotype DE5 (5.33) followed by DE1 (5.67), NM5 (5.67), DE2 (5.67), CH3 (6.67), CH2 (6.67) CH1 (6.67), JM2 (6.67) and HM1 (7.0)

Maximum duration for 50-80 per cent leaf shedding (Table 4.12) was found in site Rupnagar (17.67 days) which is statistically at par with site), Suhanpur (13.44), Rampur (12.22), Balh (10.56), Devamanal (9.89), Jakholi (9.89), Bhunter (9.78), Chowari (9.33), Tandi (8.78), Chinani (8.67) and Hamirpur (8.33). Minimum duration for 50% - 80% leaf fall was observed in case of site Deothi (5.78 days) which was statistically at par with site Namhol (6.44 days) and Chamba (6.67).

4.1.3.5 Duration from 80 to 100 per cent leaf shedding (Days)

For 80-100 per cent leaf shedding (Table 4.11), the maximum number of days (22.33) was noticed in genotype BN6 which was statistically at par with RN3 (16.67) whereas minimum number of days was observed in genotype DE2 (4.33) which is statistically at par with all other genotypes having values 4.33 and 10.68 except BL1 (11.33) and RN1 (13.33)**.**

Data appended in Table 4.12 revealed that maximum duration for 80 – 100 per cent leaf shedding was found in site Rupnagar (13.89 days) which is statistically at par with site Bhunter (12.22) and Balh (10.56). Minimum duration for 80 – 100 per cent leaf shedding was observed in case of site Deothi and Namhol (5.00) which was statistically at par with site Chamba and Devamanal (6.44).

4.1.3.5 Duration from 50 to complete leaf shedding (Days)

In case of 50 per cent to complete leaf shedding (Table 4.11), genotype RN3 showed maximum number of days (36.67) which was statistically at par with BN6 (31.67) and RN1 (30.67) while genotype DE2 showed a minimum number of days (10.67) which is statistically at par DE1 (10.67), NM2 (10.67), DE5 (11.00), NM5 (11.00), CH1 (12.67), NM4 (12.67), CH3 (13.00), DM5 (13.00), JM2 (13.33), JM1 (13.33) and CH2 (13.67).

Maximum duration for 50 per cent to complete leaf shedding (Table 4.12) was found in site Rupnagar (31.56 days) which is followed by with site Suhanpur (23.44 days) and Rampur (22.11 days). Minimum duration for 50% - 100 per cent leaf shedding was observed in case of site Deothi (10.78 days) which was statistically at par with site Namhol (11.44 days), Chamba (13.11 days) and Jammu (14.22 days).

Table 4.11: Variation in *Salix tetrasperma* genotypes in respect of days taken from 50-80 per cent leaf shedding, 80 per cent to complete leaf shedding, 50 per cent to complete leaf shedding

S. No	Genotype	50 to 80 per cent Leaf shedding (days)	80 to 100 per cent Leaf shedding (days)	50 to100 per cent Leaf shedding (days)
1.	DM3	12.67	7.67	20.33
	DM4	8.33	7.33	15.67
	DM5	8.67	4.33	13.00
2.	JA1	9.00	7.33	16.33
	JA3	10.67	9.33	20.00
	JA4	10.00	6.00	16.00
3.	RP1	12.67	10.00	22.67
	RP2	11.67	10.00	21.67
	RP3	12.33	9.67	22.00
4.	RN1	17.33	13.33	30.67
	RN3	20.00	16.67	36.67
	RN4	15.67	11.67	27.33
5.	TD1	7.33	8.67	16.00
	TD2	8.67	8.67	17.33
	TD3	10.33	8.67	19.00
6.	SP1	16.00	9.67	25.67
	SP3	11.00	10.00	21.00
	SP4	13.33	10.33	23.67
7.	HM1	7.00	9.33	16.33
	HM2	8.00	8.67	16.67
	HM4	10.00	8.00	18.00
8.	NM2	5.67	5.00	10.67
	NM4	8.00	4.67	12.67
	NM5	5.67	5.33	11.00
9.	BN2	10.33	6.67	17.00
	BN5	9.67	7.67	17.33
	BN6	9.33	22.33	31.67
10.	CN1	9.33	8.00	17.33
	CN2	9.00	6.00	15.00
	CN3	7.67	6.67	14.33
11.	JM1	7.33	6.00	13.33
	JM2	6.67	6.67	13.33
	JM5	8.33	7.67	16.00
12.	DE1	5.67	5.00	10.67
	DE2	6.33	4.33	10.67
	DE5	5.33	5.67	11.00
13.	BL1	11.00	11.33	22.33
	BL2	10.33	10.00	20.33
	BL3	10.33	10.33	20.67
14.	CW1	9.33	8.67	18.00
	CW2	9.00	8.33	17.33
	CW3	9.67	10.00	19.67
15.	CH1	6.67	6.00	12.67
	CH2	6.67	7.00	13.67
	CH3	6.67	6.33	13.00
	Mean	9.66	8.47	18.13
	$CD_{(0.05)}$	1.69	6.35	6.79

Table 4.12: Site-wise variation in *Salix tetrasperma* genotypes in respect of days taken from 50-80 per cent leaf shedding, 80 per cent to complete leaf shedding, 50 per cent to complete leaf shedding

Sr No.	Site	50 to 80 per cent Leaf shedding (days)	80 to 100per cent Leaf shedding (days)	50to100 per cent Leaf shedding (days)
1	Devamanal	9.89	6.44	16.33
2	Jakholi	9.89	7.56	17.44
3	Rampur	12.22	9.89	22.11
4	Rupnagar	17.67	13.89	31.56
5	Tandi	8.78	8.67	17.44
6	Suhanpur	13.44	10.00	23.44
7	Hamirpur	8.33	8.67	17.00
8	Namhol	6.44	5.00	11.44
9	Bhunter	9.78	12.22	22.00
10	Chinani	8.67	6.89	15.56
11	Jammu	7.44	6.78	14.22
12	Deothi	5.78	5.00	10.78
13	Balh	10.56	10.56	21.11
14	Chowari	9.33	9.00	18.33
15	Chamba	6.67	6.44	13.11
	Mean	9.66	8.47	18.13
	$CD_{(0.05)}$	0.98	3.67	3.92

The result of the present study showed significant variations for leaf shedding traits which was similar to the observations noticed in *Salix acutifolia* Wild., *Salix smithiana* Wild., and *Salix viminalisi* L. trees evaluated by Orlandi *et al*. (2021) in a phenological garden in central Italy. They discovered that during spring time leaf development advanced and leaf senescence was delayed after the summer.

Similarly, for the leafy period, Weih (2009) observed significant variance among the *Salix* genotypes and found that genotypically specific variation was correlated with the parental genotypes' source environments or habitat. In the case of *Populus tremula*, Fracheboud *et al*. (2009) observed considerable variation in the timing of leaf senescence both within local populations and among trees that originated from various populations, and they noticed that this variation was correlated with the latitude of the respective origins.

According to Ceulemans *et al*. (1992) and Yu *et al.* (2001), higher shoot production may be responsible or connected to the delay in growth cessation. However, it can be seen that in high-latitude areas, growing conditions, such as light and temperature, quickly deteriorate as late autumn approaches, and there must be a critical time period in autumn beyond which a delay in growth cessation and leaf fall is no longer related to any type of gain in biomass. In contrast, in lower latitude areas, light and temperature conditions are more favourable during late autumn, which caused a delay in growth cessation. But within the site, microclimate was a reason for significant variation in case of beech provenance as recorded by Gömöry *et al.* (2011).

4.2 REPRODUCTIVE CHARACTERS

4.2.1 Duration of bud swell (Days)

A close appraisal of the table 4.13 showed that maximum duration for bud swell (37.00) was recorded in genotype CN3 which was followed by genotypes CN1 (35.67), CN2 (35.33) and BN6 (31.33) while minimum bud swell duration was recorded in genotype JA3 (14.33) which was statistically at par with genotypes JA1 (14.67) and JA4 (15.00).

Table 4.13: Reproductive Bud swell duration and number of days of *Salix tetrasperma* genotypes.

S.No.	Site	Genotypes	Bud swell duration	Mean Days
1	Devamanal	DM3	01-01-2023 to 26-01-2023	26.67
		DM4	03-01-2023 to 25-01-2023	23.33
		DM5	04-01-2023 to 26-01-2023	21.67
2	Jakholi	JA1	06-01-2023 to 20-01-2023	14.67
		JA3	06-01-2023 to 20-01-2023	14.33
		JA4	05-01-2023 to 20-01-2023	15.00
3	Rampur	RP1	02-01-2023 to 26-01-2023	26.00
		RP2	04-01-2023 to 27-01-2023	23.33
		RP3	04-01-2023 to 27-01-2023	22.33
4	Rupnagar	RN1	01-01-2023 to 28-01-2023	26.00
		RN3	02-01-2023 to 26-01-2023	24.67
		RN4	01-01-2023 to 28-01-2023	25.00
5	Tandi	TD1	01-01-2023 to 28-01-2023	26.00
		TD2	01-01-2023 to 26-01-2023	25.33
		TD3	01-01-2023 to 26-01-2023	24.33
6	Suhanpur	SP1	01-01-2023 to 29-01-2023	27.67
		SP3	01-01-2023 to 27-01-2023	26.67
		SP4	01-01-2023 to 28-01-2023	26.00
7	Hamirpur	HM1	09-01-2023 to 06-02-2023	27.33
		HM2	12-01-2023 to 07-02-2023	28.33
		HM4	10-01-2023 to 06-02-2023	26.67
8	Namhol	NM2	13-01-2023 to 07-02-2023	26.33
		NM4	13-01-2023 to 08-02-2023	27.00
		NM5	13-01-2023 to 09-02-2023	28.00
9	Bhunter	BN2	13-01-2023 to 09-02-2023	27.00
		BN5	14-01-2023 to 11-02-2023	27.67
		BN6	15-01-2023 to 13-02-2023	31.33
10	Chinani	CN1	16-01-2023 to 24-02-2023	35.67
		CN2	19-01-2023 to 26-02-2023	35.33
		CN3	18-01-2023 to 26-02-2023	37.00
11	Jammu	JM1	02-01-2023 to 09-02-2023	30.67
		JM2	06-01-2023 to 11-02-2023	30.67
		JM5	01-01-2023 to 30-01-2023	30.00
12	Deothi	DE1	02-01-2023 to 27-01-2023	26.33
		DE2	03-01-2023 to 28-01-2023	24.00
		DE5	04-01-2023 to 28-01-2023	23.33
13	Balh	BL1	12-01-2023 to 09-01-2023	27.33
		BL2	13-01-2023 to 10-01-2023	27.67
		BL3	13-01-2023 to 12-01-2023	28.67
14	Chowari	CW1	15-01-2023 to 12-02-2023	27.67
		CW2	13-01-2023 to 12-02-2023	29.00
		CW3	13-01-2023 to 13-02-2023	30.67
15	Chamba	CH1	15-01-2023 to 12-02-2023	27.67
		CH2	14-01-2023 to 12-02-2023	28.00
		CH3	13-01-2023 to 14-02-2023	30.33
			Mean	26.64
			$CD_{(0.05)}$	2.29

Comparisons of sites in Table 4.14 depicted that genotypes of site Chinani had maximum bud swell duration i.e 36 days which is followed by Jammu (30.44), Chowari (29.11) and Bhunter (28.67) Minimum bud swell duration was recorded in site Jakholi (14.67).

Table 4.14: Sitewise reproductive bud swell duration and number of days for *Salix tetrasperma* genotypes.

Sr. No.	Site	Bud swell duration	Mean Days
1	Devamanal	01-01-2023 to 26-01-2023	23.89
2	Jakholi	05-01-2023 to 20-01-2023	14.67
3	Rampur	02-01-2023 to 27-01-2023	23.89
4	Rupnagar	01-01-2023 to 28-01-2023	25.22
5	Tandi	01-01-2023 to 28-01-2023	25.22
6	Suhanpur	01-01-2023 to 29-01-2023	26.78
7	Hamirpur	09-01-2023 to 07-02-2023	27.44
8	Namhol	13-01-2023 to 09-02-2023	27.11
9	Bhunter	13-01-2023 to 13-02-2023	28.67
10	Chinani	16-01-2023 to 26-02-2023	36.00
11	Jammu	01-01-2023 to 09-02-2023	30.44
12	Deothi	02-01-2023 to 28-01-2023	24.56
13	Balh	12-01-2023 to 12-02-2023	27.89
14	Chowari	13-01-2023 to 12-02-2023	29.11
15	Chamba	13-01-2023 to 14-02-2023	28.67
	Mean		26.64
	$CD_{(0.05)}$		1.31

4.2.2 Duration of reproductive bud burst (Days)

Table 4.15 showed that the genotype JA4 recorded maximum (25.67) duration of bud burst which was statistically at par with genotype JA3 (25.00), JA1 (24.33), DE2 (23.33) and RP2 (23.00) as showed in table 4.9. Minimum bud swell duration was recorded in genotype CN3 (10.33) which was statistically at par with genotype CN2 (10.67), JM1 (12.00) and CN1 (13.00). Verma *et al.* (2011) studied floral biology and pollen handling of *Grewia optiva* Drummond and found that flowering buds started to appear along with the appearance of new leaves during end of March.

Among the sites (Table 4.16), Jakholi recorded maximum (25.00) bud burst duration followed by site Deothi (22.11), Rampur (21.11) and Suhanpur (20.33). Minimum bud burst duration was recorded in site Chinani (11.33) which was followed by Jammu (13.78), Chowari (15.22) and Chamba (15.89). Reproductive bud swell and bud burst are shown in Plate 1.2.

a) Bud Swell

b) Initial Bud Burst

c) Bud Burst stage 2

d) Bud Burst final stage

Plate 1.2: Reproductive Bud Swell and Bud Burst

Table 4.15: Reproductive Bud burst duration and number of days of *Salix tetrasperma* genotypes.

Sr. No.	Site	Genotypes	Bud burst duration	Mean Days
1	Devamanal	DM3	21-01-2023 to 11-02-2023	20.33
		DM4	21-01-2023 to 14-02-2023	21.00
		DM5	22-01-2023 to 11-02-2023	19.33
2	Jakholi	JA1	16-01-2023 to 09-02-2023	24.33
		JA3	15-01-2023 to 08-02-2023	25.00
		JA4	10-01-2023 to 07-02-2023	25.67
3	Rampur	RP1	21-01-2023 to 11-02-2023	21.00
		RP2	21-01-2023 to 14-02-2023	23.00
		RP3	22-01-2023 to 11-02-2023	19.33
4	Rupnagar	RN1	21-01-2023 to 08-02-2023	16.33
		RN3	20-01-2023 to 11-02-2023	19.33
		RN4	21-01-2023 to 11-02-2023	20.33
5	Tandi	TD1	21-01-2023 to 08-02-2023	16.33
		TD2	20-01-2023 to 11-02-2023	20.33
		TD3	20-01-2023 to 10-02-2023	20.67
6	Suhanpur	SP1	21-01-2023 to 10-02-2023	18.33
		SP3	21-01-2023 to 11-02-2023	21.33
		SP4	20-01-2023 to 11-02-2023	21.33
7	Hamirpur	HM1	02-02-2023 to 22-02-2023	19.00
		HM2	03-02-2023 to 21-02-2023	16.67
		HM4	02-02-2023 to 22-02-2023	21.00
8	Namhol	NM2	03-02-2023 to 21-02-2023	18.33
		NM4	05-02-2023 to 23-02-2023	17.00
		NM5	07-02-2023 to 27-02-2023	19.33
9	Bhunter	BN2	03-02-2023 to 21-02-2023	18.33
		BN5	06-02-2023 to 23-02-2023	17.00
		BN6	07-02-2023 to 27-02-2023	18.33
10	Chinani	CN1	15-02-2023 to 02-03-2023	13.00
		CN2	17-02-2023 to 03-03-2023	10.67
		CN3	20-02-2023 to 05-03-2023	10.33
11	Jammu	JM1	29-01-2023 to 18-02-2023	12.00
		JM2	06-02-2023 to 17-02-2023	14.67
		JM5	26-01-2023 to 10-02-2023	14.67
12	Deothi	DE1	21-01-2023 to 13-02-2023	22.33
		DE2	20-01-2023 to 13-02-2023	23.33
		DE5	22-01-2023 to 13-02-2023	20.67
13	Balh	BL1	03-02-2023 to 21-02-2023	18.33
		BL2	05-02-2023 to 25-02-2023	19.00
		BL3	07-02-2023 to 27-02-2023	19.00
14	Chowari	CW1	06-02-2023 to 22-02-2023	15.67
		CW2	09-02-2023 to 23-02-2023	16.00
		CW3	09-02-2023 to 25-02-2023	14.00
15	Chamba	CH1	06-02-2023 to 23-02-2023	16.67
		CH2	07-02-2023 to 23-02-2023	16.00
		CH3	07-02-2023 to 25-02-2023	15.00
			Mean	18.44
			$CD_{(0.05)}$	2.85

Table 4.16: Sitewise reproductive bud burst duration and mean number of days for *Salix tetrasperma* genotypes

Sr. No.	Site	Bud burst duration	Mean Days
1	Devamanal	21-01-2023 to 14-02-2023	20.22
2	Jakholi	10-1-2023 to 09-02-2023	25.00
3	Rampur	21-01-2023 to 14-02-2023	21.11
4	Rupnagar	20-01-2023 to 11-02-2023	18.67
5	Tandi	20-01-2023 to 11-02-2023	19.11
6	Suhanpur	20-01-2023 to 11-02-2023	20.33
7	Hamirpur	02-02-2023 to 22-02-2023	18.89
8	Namhol	03-02-2023 to 27-02-2023	18.22
9	Bhunter	03-02-2023 to 27-02-2023	17.89
10	Chinani	15-02-2023 to 05-03-2023	11.33
11	Jammu	26-01-2023 to 18-02-2023	13.78
12	Deothi	20-01-2023 to 13-02-2023	22.11
13	Balh	03-02-2023 to 27-02-2023	18.78
14	Chowari	06-02-2023 to 25-02-2023	15.22
15	Chamba	06-02-2023 to 25-02-2023	15.89
		Mean	**18.44**
		$CD_{(0.05)}$	1.63

Gu *et al*. (2003) found that the rise in photosynthetic activity during the spring could be related to the pace of bud flushing or bursting, which measures how quickly trees react to temperature changes. On the other hand, Azad (2012) studied the bud burst and spring phenology of poplar (*Populus tremula* and *P. tremuloides*) from eight distinct provenances and noticed that there was a substantial difference in the timing of bud flushing between the saplings from the various provenances like bud flushing started and finished earlier in case of one provenance than those from other different provenance.

In northwest Quebec, Elferjani *et al.* (2016) studied the plasticity of four hybrid poplar clones along a latitudinal gradient and discovered that growth duration between the southernmost and northernmost sites ranged from 21 to 32 days. They also observed that bud phenology plasticity was clearly evident and correlated to stem volume growth along the latitudinal growth, and that bud burst and bud set duration were negatively correlated with growth season duration. They discovered that in the boreal zone, bud burst began early at lower latitudes where the early spring climate was warmer, indicating the start of biomass production. They also observed that the productive clones' growing season was extended due to later bud set and faster (but not earlier) bud burst development. The floral and vegetative buds may have different temperature sensitivity, with inflorescence buds having a lower

cumulative temperature requirement (heat sum, for instance in degree days) required for development (Cronk *et al*., 2015).

4.2.3 Flowering Characters

4.2.3.1 Catkin development Stages

Categorization of flower bud into different stages not only helped in the effective evaluation of phenological development but also in making comparative study among the different genotypes of *Salix tetrasperma* Roxb. and earlier also Bangarwa (1996) undertook study on *Dalbergia sissoo* and divided the stages from bud appearance to flower opening into five stages, Pant *et al.* (2003) categorized the developmental stages of *Grewia optiva* flowers into eight stages and in the same way Wani (2005) divided the different flower development stages into twelve stages. Various catkin development stages observed in the present study are shown in Plate no. 1.3. The sex of the most of the genotypes was staminate while only genotypes from Balh and Chowari were pistillate (Table 4.17).

Stage I

Data presented in Table 4.17 revealed that during stage 1 fully developed bud/catkin attained a maximum length of 1.03 cm in genotype TD3 which was statistically at par with genotypes TD2 (1.01), RN4 (1.00), SP4 (0.99) and TD1 (0.98). However, genotype CN1 recorded bud length of 0.48 cm which was minimum among the genotypes for the stage that was statistically at par with genotypes CN2 (0.51), CN3 (0.53) and BN6 (0.53).

Among the sites (Table 4.19), site Tandi showed maximum (1.01 cm) bud length which was statistically at par with site Suhanpur (0.98 cm). Minimum bud / catkin length was recorded in site Chinani (0.51 cm) followed by Bhunter (0.55 cm) and Namhol (0.60 cm).

Stage II

During this stage (Table 4.17) the inflorescence among the genotypes attained maximum catkin length of 1.57 cm in genotype SP4 which was statistically at par with genotype RN4 (1.56 cm), RN3 (1.54 cm) and SP3 (1.54 cm). However, minimum catkin length of 1.06 cm at this stage was recorded in genotypes CN2 and CN1 which was statistically at par with CN3 (1.1).

Among the sites (Table 4.19), site Suhanpur showed a maximum catkin length of 1.53 cm at this stage which was statistically at par with Rupnagar (1.52 cm). Minimum catkin

length was recorded in site Chinani (1.07 cm) which was followed by Chamba (1.15 cm) and Bhunter (1.19cm).

Table 4.17: Sex of genotype and bud length (cm) during catkin development stages 1&II in the genotypes of *Salix tetrasperma*

Sr. No.	Site	Genotypes	Sex	Stage I	Stage II
1	Devamanal	DM3	Male	0.65	1.22
		DM4	Male	0.65	1.22
		DM5	Male	0.64	1.20
2	Jakholi	JA1	Male	0.58	1.30
		JA3	Male	0.64	1.28
		JA4	Male	0.60	1.30
3	Rampur	RP1	Male	0.68	1.23
		RP2	Male	0.68	1.19
		RP3	Male	0.64	1.19
4	Rupnagar	RN1	Male	0.94	1.47
		RN3	Male	0.94	1.54
		RN4	Male	1.00	1.56
5	Tandi	TD1	Male	0.98	1.45
		TD2	Male	1.01	1.51
		TD3	Male	1.03	1.48
6	Suhanpur	SP1	Male	0.97	1.48
		SP3	Male	0.97	1.54
		SP4	Male	0.99	1.57
7	Hamirpur	HM1	Male	0.72	1.36
		HM2	Male	0.69	1.34
		HM4	Male	0.72	1.33
8	Namhol	NM2	Male	0.61	1.23
		NM4	Male	0.60	1.24
		NM5	Male	0.60	1.24
9	Bhunter	BN2	Male	0.57	1.26
		BN5	Male	0.56	1.18
		BN6	Male	0.53	1.14
10	Chinani	CN1	Male	0.48	1.06
		CN2	Male	0.51	1.06
		CN3	Male	0.53	1.10
11	Jammu	JM1	Male	0.72	1.27
		JM2	Male	0.73	1.32
		JM5	Male	0.69	1.35
12	Deothi	DE1	Male	0.63	1.28
		DE2	Male	0.66	1.25
		DE5	Male	0.66	1.24
13	Balh	BL1	Female	0.65	1.23
		BL2	Female	0.67	1.25
		BL3	Female	0.62	1.22
14	Chowari	CW1	Female	0.65	1.26
		CW2	Female	0.72	1.24
		CW3	Female	0.70	1.15
15	Chamba	CH1	Male	0.59	1.15
		CH2	Male	0.66	1.15
		CH3	Male	0.70	1.15
			Mean	**0.71**	**1.28**
			$CD_{(0.05)}$	0.05	0.05

Stage -I

Stage -2

Stage -3

Stage -4

Stage -5

Plate 1.3: Different stages of catkin development

Stage III

Data presented in Table 4.18 revealed that during stage III catkin attained maximum length of 1.95 cm in genotype TD1 which was statistically at par with genotypes SP4 (1.95), SP3 (1.94) and RN4 (1.92) However, genotype CN3 recorded a bud length of 1.45 cm which was the minimum among the genotypes for the stage that was statistically at par with genotype CN1 (1.47).

Among the sites (Table 4.19), site Suhanpur showed maximum (1.93 cm) catkin length which was followed by Rupnagar (1.88 cm) and Tandi (1.88 cm). Minimum catkin length at this stage was recorded in Chinani (1.48 cm) followed by Jammu (1.56 cm) and Bhunter (1.59 cm).

Stage IV

Data presented in Table 4.18 revealed that during stage IV catkin attained maximum length of 3.28 cm in genotype SP4 which was statistically at par with genotypes RN4 (3.26 cm), TD2 (3.26 cm), TD1 (3.25 cm) and RN3 (3.21 cm) . However, genotype JM5 recorded bud length of 1.79 cm which was minimum among the genotypes for this stage which was followed by genotype BL3 (1.91 cm), CW3 (1.97 cm) and BL2 (1.98 cm).

Among the sites (Table 4.19), site Suhanpur showed maximum (3.26 cm) catkin length which was followed by Tandi (3.25 cm) and Rupnagar (3.23 cm). Minimum catkin length at this stage was recorded in Chinani (2.62 cm) followed by Jammu (2.64 cm) and Bhunter (1.59 cm).

Stage V

Data presented in Table 4.18 revealed that during stage V catkin attained maximum length of 6.8 cm in genotype RN4 which was followed by genotypes SP1 (5.81 cm), SP3 (5.68 cm) and RN1 (5.45 cm). However, genotype JM5 recorded bud length of 2.03 cm which was minimum among the genotypes for the stage that was statistically at par with genotypes BL1 (2.11 cm), BL2 (2.18 cm) and BL3 (2.33 cm).

Table 4.19 showed the site variation for catkin length at stage V and revealed that maximum length (5.85) was recorded for site Rupnagar which was statistically at par with site Suhanpur (5.62) whereas minimum length at this stage was recorded for site Balh (2.21). Genotypes from Punjab RN1, RN3, RN4, TD1, TD2, TD3, SP1, SP3 and SP4 were observed to have flowering in two times i.e once in October 2022 and other in February 2023 (Table

4.22). Catkin development were also observed in the month of October 2022 and January to February 2023

Table 4.18: Reproductive bud length (cm) catkin development stages at III, IV & V stages in the genotypes of *Salix tetrasperma*

Sr No.	Site	Genotypes	Stage III	Stage IV	Stage V
1	Devamanal	DM3	1.63	2.84	3.53
		DM4	1.59	2.85	3.52
		DM5	1.61	2.90	3.61
2	Jakholi	JA1	1.68	2.97	4.52
		JA3	1.69	2.96	4.26
		JA4	1.66	2.96	4.69
3	Rampur	RP1	1.66	2.95	3.64
		RP2	1.65	3.00	3.64
		RP3	1.61	2.99	4.19
4	Rupnagar	RN1	1.86	3.22	5.45
		RN3	1.87	3.21	5.31
		RN4	1.92	3.26	6.80
5	Tandi	TD1	1.95	3.25	4.66
		TD2	1.86	3.26	4.58
		TD3	1.84	3.25	4.81
6	Suhanpur	SP1	1.89	3.25	5.81
		SP3	1.94	3.25	5.68
		SP4	1.95	3.28	5.38
7	Hamirpur	HM1	1.69	3.21	4.27
		HM2	1.67	3.02	4.27
		HM4	1.71	3.05	4.10
8	Namhol	NM2	1.62	2.99	4.09
		NM4	1.60	3.00	4.07
		NM5	1.63	2.97	4.04
9	Bhunter	BN2	1.63	2.93	4.05
		BN5	1.61	2.85	3.71
		BN6	1.52	2.81	3.76
10	Chinani	CN1	1.47	2.69	3.73
		CN2	1.52	2.62	3.69
		CN3	1.45	2.56	3.77
11	Jammu	JM1	1.55	3.04	3.88
		JM2	1.58	3.08	3.93
		JM5	1.56	1.79	2.03
12	Deothi	DE1	1.56	2.70	4.05
		DE2	1.62	2.75	4.10
		DE5	1.64	2.81	4.13
13	Balh	BL1	1.61	1.87	2.11
		BL2	1.62	1.98	2.18
		BL3	1.64	1.91	2.33
14	Chowari	CW1	1.63	2.96	3.78
		CW2	1.60	2.93	3.58
		CW3	1.57	1.97	2.41
15	Chamba	CH1	1.64	2.95	4.01
		CH2	1.64	2.97	3.80
		CH3	1.63	3.03	3.79
		Mean	**1.67**	**2.87**	**4.04**
		$CD_{(0.05)}$	0.04	0.07	0.37

Table 4.19: Sitewise Catkin length (cm) recorded at I, II, III, IV & V stages in the genotypes of *Salix tetrasperma*

Sr No.	Site	Stage I	Stage II	Stage III	Stage IV	Stage V
1	Devamanal	0.65	1.22	1.61	2.86	3.55
2	Jakholi	0.61	1.30	1.68	2.96	4.49
3	Rampur	0.67	1.20	1.64	2.98	3.83
4	Rupnagar	0.96	1.52	1.88	3.23	5.85
5	Tandi	1.01	1.48	1.88	3.25	4.68
6	Suhanpur	0.98	1.53	1.93	3.26	5.62
7	Hamirpur	0.71	1.34	1.69	3.10	4.21
8	Namhol	0.60	1.24	1.62	2.99	4.07
9	Bhunter	0.55	1.19	1.59	2.87	3.84
10	Chinani	0.51	1.07	1.48	2.62	3.73
11	Jammu	0.71	1.31	1.56	2.64	3.28
12	Deothi	0.65	1.26	1.60	2.75	4.09
13	Balh	0.65	1.23	1.62	1.92	2.21
14	Chowari	0.69	1.22	1.60	2.62	3.26
15	Chamba	0.65	1.15	1.64	2.98	3.87
	Mean	**0.71**	**1.28**	**1.67**	**2.87**	**4.04**
	$CD_{(0.05)}$	0.03	0.03	0.026	0.043	0.36

4.2.3.2 Duration of flowering per branch (Days)

Flowering in trees is a complex process that includes many different developmental stages. Trees interact with environmental conditions throughout the year and especially flowering is highly influenced by seasonal climatic changes. In general, the time duration between initiation and completion of flowering can be correlated with the growth habit of the tree that in turn influenced by the climatic range of species (Sedgley and Griffin, 1989). A knowledge of flower bud development and maturation is essential in any breeding programme, especially in willows in which inflorescence is there instead of complete flower.

Data appended in Table 4.20 revealed that maximum days (14.33) of flowering per branch was noticed in genotype RN3 which was statistically at par with genotypes RN1 (13.67), RN4 (13.00) and TD1 (12.67). Minimum (5.33) duration of flowering per branch was recorded in CW2 which was statistically at par with BL3 (5.33), CW1 (5.67), BL2 (6.00), JM5 (6.67), JM2 (6.67), CN3 (6.67) and NM5 (7.00).

Among the sites (Table 4.21), maximum (13.67) duration of flowering was recorded in site Rupnagar which was followed by site Tandi (12.11), Suhanpur (11.22) and Devamanal (10.22). Minimum (5.78) duration of flowering was recorded in site Chowari which is statistically at par with site Balh (5.89).

4.2.3.3 Anthesis duration per catkin (Days)

Data presented in Table 4.20 revealed that genotype BL1, CH3 and CW1 showed maximum days i.e 5.67 days for anthesis duration per catkin which was followed by genotypes DE2 (5.33), CH1 (5.33) and CH2 (5.00).

Among the sites (Table 4.21), the site Chamba and Balh showed maximum (5.33) anthesis duration per catkin which was statistically at par with genotype Deothi (5.00). Minimum anthesis duration per catkin (4.11) was recorded in Jakholi and Devamanal.

4.2.4 Pollen viability and germination

4.2.4.1 Pollen collection

Pollen grains of different genotypes of *Salix tetrasperma* were collected in the month of March during 2023 both by direct extraction and by a solvent method using toluene and are shown in Plate No. 1.4. Choudhary and Singh (2013) conducted study on pollen of *Salix* species and collected pollen grains of different species / clone in bulk from January to March by both direct extraction from catkins and solvent method.

4.2.4.2 Pollen viability (%)

Data presented in Table 4.20 revealed that pollen viability was found to be maximum (96.43%) for genotype RN1 which was statistically at par with genotype DM3 (92.22%) DM4 (92.68%) DM5 (92.38%) JA1(93.95%), JA3 (94.09%), JA4 (94.66%), RN3 (95.09%), RN4 (94.37%), SP1 (93.72%), SP3 (91.69%), SP4 (90.54%), HM1 (91.40%), HM2(94.67%), HM4 (92.00%), BN2 (93.11%), BN5 (92.66%), BN6 (94.05%), CN1 (92.70%), CN2 (92.07%), CN3 (92.61%), JM1 (92.46%), JM2 (92.76%), JM5 (91.76%), DE1 (93.96 %), DE2 (93.76%), DE5 (94.91%), CH1(87.44%), CH2 (86.83%) and CH3 (89.13%). The minimum value for pollen viability was recorded for genotype TD3 (83.30%). The results of the present study are comparable with results obtained by Verma *et al.* (2011) who studied floral biology and pollen handling of *Grewia optiva* Drummond and found that pollen grains were dark yellow in colour and the size of pollen varied between 43-53.1 microns in size among different genotypes. They further observed pollen viability varying from 77.19-80.30 per cent.

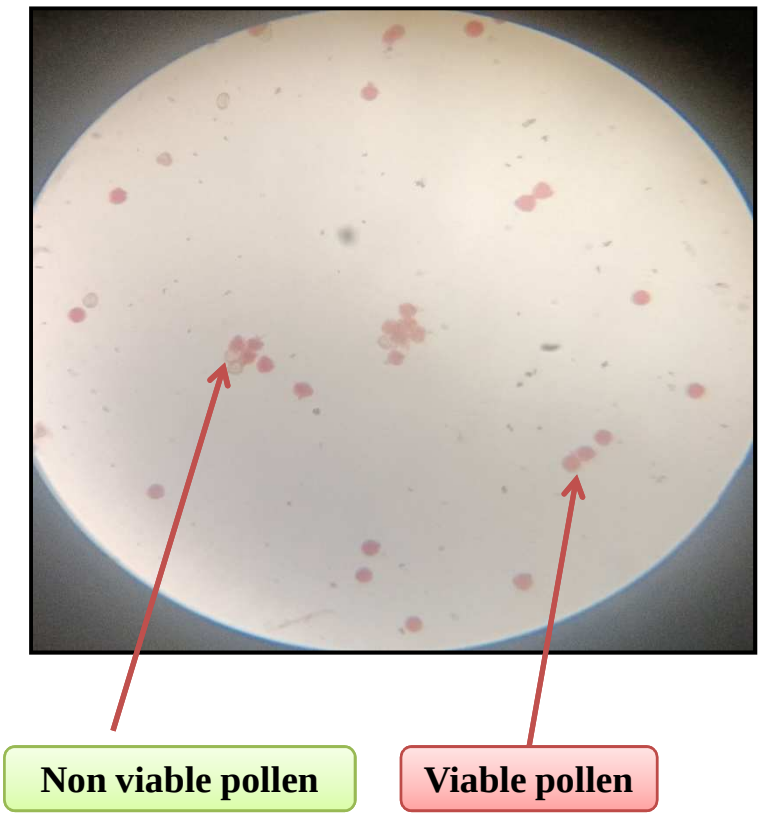
a) Catkin collection in field

b) Collected catkin in polybags

c) Catkin dehiscence in lab

d) Pollen collection by toulene method

Plate 1.4: Collection of Pollens



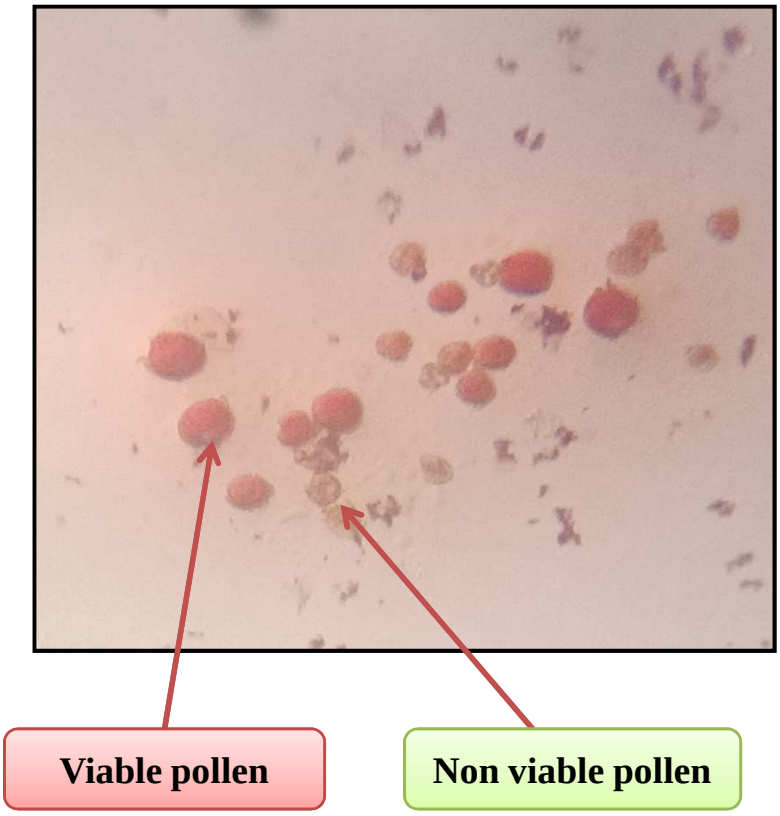


Plate 1.5: Pollen Viability

Table 4.20: Flowering period per branch, anthesis duration per catkin (days), pollen viability and pollen germination in the genotypes of *Salix tetrasperma*

Sr. No.	Site	Genotypes	Flowering period per branch (days)	Anthesis duration per catkin (days)	Pollen viability (%)	Pollen germination (%)
1	Devamanal	DM3	11.00	4.00	92.22 (73.97)	75.27(60.38)
		DM4	9.67	4.00	92.68 (74.40)	73.07(58.75)
		DM5	10.00	4.33	92.38 (74.61)	71.53(57.86)
2	Jakholi	JA1	8.00	4.00	93.95 (76.12)	72.30(58.31)
		JA3	9.00	4.33	94.09 (76.04)	73.97(59.57)
		JA4	9.67	4.00	94.66 (76.66)	75.47(60.63)
3	Rampur	RP1	9.00	4.33	88.77 (70.77)	75.77(60.91)
		RP2	8.33	4.33	88.80 (70.63)	73.90(59.42)
		RP3	8.67	4.67	88.94 (70.77)	74.30(59.57)
4	Rupnagar	RN1	13.67	4.33	96.43 (79.17)	78.90(62.68)
		RN3	14.33	4.33	95.09 (77.36)	75.73(60.47)
		RN4	13.00	4.67	94.37 (76.51)	72.13(58.28)
5	Tandi	TD1	12.67	4.00	86.54 (68.93)	67.17(55.04)
		TD2	12.33	4.33	85.91 (67.99)	67.40(55.17)
		TD3	11.33	4.33	83.30 (65.93)	64.27(53.27)
6	Suhanpur	SP1	12.00	4.33	93.72 (75.80)	72.90(58.74)
		SP3	11.00	4.33	91.69 (73.35)	71.67(57.83)
		SP4	10.67	4.33	90.54 (72.52)	71.63(57.84)
7	Hamirpur	HM1	8.33	4.00	91.40 (73.07)	68.63(55.95)
		HM2	8.00	5.00	94.67 (76.78)	73.17(58.93)
		HM4	7.67	4.67	92.00 (73.59)	74.00(59.48)
8	Namhol	NM2	8.00	4.33	87.86 (69.90)	69.39(56.39)
		NM4	7.67	4.33	88.05 (69.83)	67.88(55.47)
		NM5	7.00	4.33	90.41 (72.06)	72.33(58.28)
9	Bhunter	BN2	10.00	4.33	93.11 (74.96)	77.20(61.46)
		BN5	8.00	4.67	92.66 (74.52)	70.93(57.49)
		BN6	9.00	4.00	94.05 (75.98)	73.33(59.04)
10	Chinani	CN1	7.33	4.00	92.70 (74.68)	73.10(58.86)
		CN2	8.00	4.33	92.07 (73.70)	70.57(57.31)
		CN3	6.67	4.33	92.61 (74.53)	71.97(58.15)
11	Jammu	JM1	8.00	4.33	92.46 (74.30)	71.93(58.14)
		JM2	6.67	4.67	92.76 (74.57)	69.63(56.60)
		JM5	6.67	4.33	91.76 (73.99)	72.57(58.51)
12	Deothi	DE1	7.33	5.00	93.96 (75.63)	71.53(57.85)
		DE2	8.00	5.33	93.76 (75.63)	72.50(58.50)
		DE5	8.67	4.67	94.91 (76.98)	69.67(56.68)
13	Balh	BL1	6.33	5.67*	-	-
		BL2	6.00	5.00*	-	-
		BL3	5.33	5.33*	-	-
14	Chowari	CW1	5.67	5.67*	-	-
		CW2	5.33	4.00*	-	-
		CW3	6.33	4.33*	-	-
15	Chamba	CH1	7.67	5.33	87.44 (69.28)	67.43(55.25)
		CH2	7.67	5.00	86.83 (68.84)	71.00(57.43)
		CH3	7.67	5.67	89.13 (70.92)	71.73(57.91)
		Mean	8.74	4.53	91.50 (73.46)	91.49
		$CD_{(0.05)}$	1.71	NS	5.07	NS

Values in parenthesis are arcsine transformed values

*Pistillate flowers were observed for stigma receptivity duration

Data appended in Table 4.21 showed that the maximum pollen viability percentage (95.30 %) was recorded for site Rupnagar whereas the minimum (85.25%) recorded for Tandi site.

4.2.4.3 Pollen germination

Appraisal of Table 4.20 showed that maximum germination percentage (78.90 %) was recorded for genotype RN1 while minimum (64.27 %) was recorded for genotype TD3.

Data appended in Table 4.21 showed that maximum pollen germination percentage (75.59 %) was recorded for site Rupnagar whereas minimum (66.28 %) recorded in site Tandi. Pollen germination and pollen tube growth are shown in Plate 1.6.

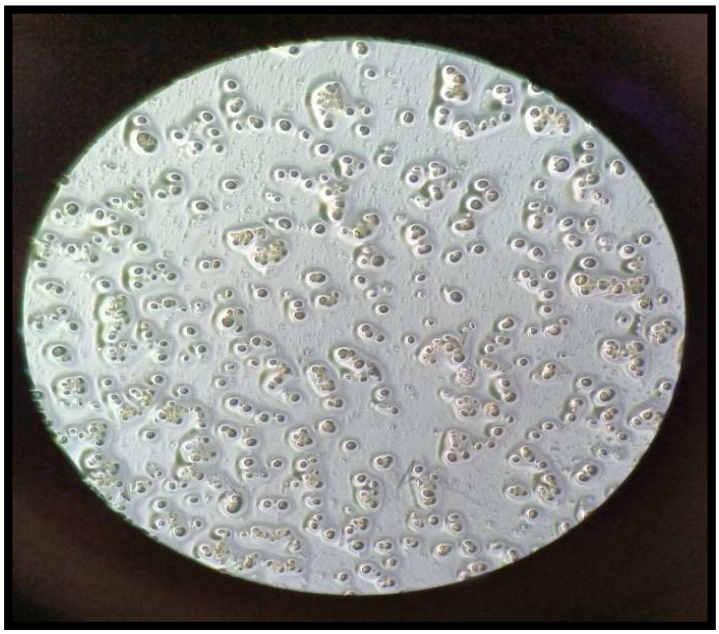
Table 4.21: Sitewise flowering period per branch and anthesis duration per catkin and pollen viability in the genotypes of *Salix tetrasperma*

Sr. No.	Site	Flowering period per branch (days)	Anthesis duration per catkin (days)	Pollen viability (%)	Pollen germination (%)
1	Devamanal	10.22	4.11	92.42 (74.33)	73.29(59.00)
2	Jakholi	8.89	4.11	94.23 (76.27)	73.91(59.50)
3	Rampur	8.67	4.44	88.84 (70.61)	74.66(59.97)
4	Rupnagar	13.67	4.44	95.30 (77.68)	75.59(60.48)
5	Tandi	12.11	4.22	85.25 (67.50)	66.28(54.49)
6	Suhanpur	11.22	4.33	91.98 (73.89)	72.07(58.14)
7	Hamirpur	8.00	4.56	92.69 (74.48)	71.93(58.12)
8	Namhol	7.56	4.33	88.78 (70.60)	69.87(56.71)
9	Bhunter	9.00	4.33	93.27 (75.15)	73.82(59.33)
10	Chinani	7.33	4.22	92.46 (74.31)	71.88(58.11)
11	Jammu	7.11	4.44	92.33 (74.29)	71.38(57.75)
12	Deothi	8.00	5.00	94.21 (76.15)	71.23(57.68)
13	Balh	5.89	5.33*	-	70.06(56.86)
14	Chowari	5.78	4.67*	-	73.29(59.00)
15	Chamba	7.67	5.33	87.80 (69.68)	73.91(59.50)
	Mean	8.74	4.53	91.51(73.46)	72.00(58.16)
	$CD_{(0.05)}$	0.99	0.72	2.93	NS

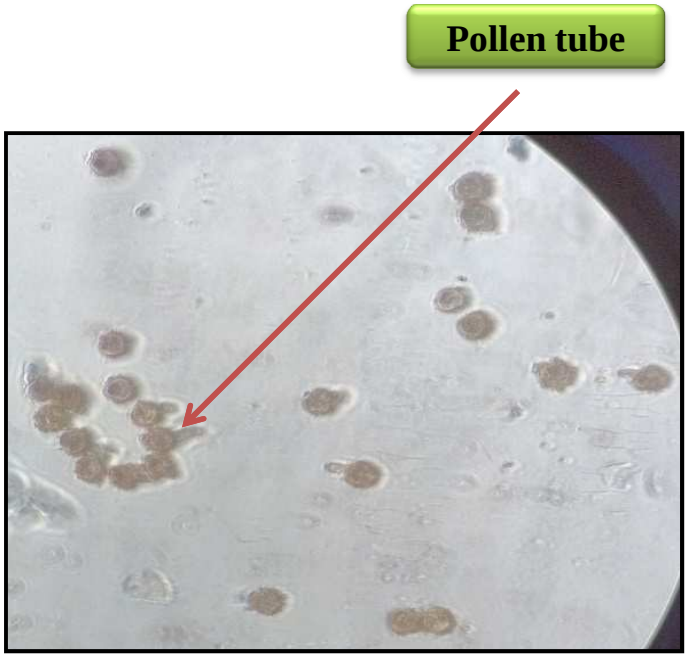
Values in parenthesis are arcsine transformed values
*Pistillate flowers were observed for stigma receptivity duration

Table 4.2.5 Autumn season flowering

Genotypes from Punjab RN1, RN3, RN4, TD1, TD2, TD3, SP1, SP3 and SP4 were observed to have flowering two times i.e once in month of October 2022 and other in the month of January- February 2023.



a) Pollen germination initiation



b) Pollen germination 15% sucrose

Plate 1.6: Pollen germination

A perusal of Table 4.22 revealed that genotype TD2 showed maximum duration (23.66) for reproductive bud swell whereas minimum bud swell duration (22.33) was recorded for genotype RN1 and TD3. Site Suhanpur showed maximum reproductive bud swell duration (23.88) whereas Rupnagar showed minimum duration (22.77) for reproductive bud swell.

Table 4.22 Autumn season flowering in some genotypes of *Salix tetrasperma*

S. No.	Site	Genotype	Bud swell duration	No. of Days	Bud burst duration	No. of Days	Flowering duration	No. of days
1	Rupnagar	RN1	11-09-2022 to 6-10-2022	22.33	1-10-2022 to 12-10-2022	12.66	8-10-2022 to 19-10-2022	12.00
		RN3	12-09-2022 to 6-10-2022	23.33	1-10-2022 to 13-10-2022	12.66	5-10-2022 to 19-0-2022	12.33
		RN4	11-09-2022 to 6-10-2022	22.66	3-10-2022 to 13-10-2022	13.33	05-10-2022 to 17-10-2022	12.00
			Mean	**22.77**		**12.88**		**12.11**
2	Tandi	TD1	11-09-2022 to 5-10-2022	23	30-09-2022 to 11-10-2022	12.66	7-10-2022 to 18-10-2022	11.66
		TD2	10-09-2022 to 6-10-2022	23.66	1-10-2022 to 12-10-2022	11.33	5-10-2022 to 16-10-2022	11.00
		TD3	10-9-2022 To 4-10-2022	22.33	1-10-2022 to 13-10-2022	13.00	5-10-2022 to 17-10-2022	12.33
			Mean	**22.99**		**12.33**		**11.66**
3	Suhanpur	SP1	13-09-2022 to 7-10-2022	23.66	3-10-2022 to 12-10-2022	13.66	5-10-2022 to 17-10-2022	13.66
		SP3	12-09-2022 to 7-10-2022	24.66	2-10-2022 to 13-10-2022	13.00	4-10-2022 to 16-10-2022	12.66
		SP4	12-09-2022 to 6-10-2022	23.33	2-10-2022 to 12-10-2022	12.66	6-10-2022 to 17-10-2022	12.00
			Mean	**23.88**		**13.11**		**12.77**

Table 4.22 showed that maximum bud burst duration (13.66) was recorded for SP1 whereas minimum (11.33) was showed by genotype TD2. Maximum bud burst duration (13.11) was recorded for site Suhanpur while minimum was for site Tandi (12.33).

Flowering duration was maximum (13.66) for genotype SP1 and minimum (11.00) for the genotype TD2. Site Suhanpur showed maximum flowering duration (12.77) whereas Tandi showed minimum (11.66).

In their study of the phenological stages of willow (*Salix*), Margaret and Kuzovkina (2010) found that the stage of the bud covered by closed scales was obtained after leaf fall and that *Salix gracilistyla* bud scales initially appeared glossy red before turning purple or tan. The change in bark coloration may be caused by anthocyanin content when exposed to sunlight. It was concluded that depending on the species, the colour of generative bud scales varies from green to reddish, brownish, or yellowish. Further observation revealed that at the time of generative bud swell stage, the colour may change as well as surface also alter (from glabrous to pubescent), indicating a transition from dormancy to flower development. Species and clonal difference in *Salix* with regard to bud flushing was documented by Ro¨nnberg-Wa¨stljung and Gull-berg (1999).

The pattern of flowering observed in present study is similar to that obtained by Choudhary *et al.* (2011) who studied phenology and reproductive biology of important nine willow species and observed that *Salix tetrasperma*, *S. alba* and *S. nigra* flowered in staggered pattern from end of January to the last week of March. Pant *et al.* (2003) undertook study on anthesis in *Grewia optiva* and they noticed that temperature had a significant impact on the flowering. According to Chauhan *et al*. (2004), the increase in temperature had an effect on flowering which occurred from March to April in *Dalbergia sissoo.* The anthesis, according to their observations, lasted from 10:00 am until 2:00 pm. The anthers dehisced in the morning hours when the flower was in the bud stage, just before it bloomed. In our study, the anthesis duration per catkin was found to be non-significant. Similarly, Maloof (2000), who studied longevity of flowers in *Corydalis caseana* and found that the flowers stayed open and healthy for about 4 days, followed the mixed mating system, were self-fertile, produced fewer seeds per fruit and supposed to have a chance of inbreeding depression.

Parallel to our study, Kopp (2002) investigated the viability and extraction of pollen of willow clones. He discovered that toluene can easily extract pollen and high pollen viability was obtained that persisted even after 18 months of storage at -20°C. The pollen study of *Salix* species conducted by Singh *et al.* (2012) obtained results that are comparable to those of our current study in terms of pollen viability and germination. In their study, pollen grains of various species/clones were collected in bulk from January to March in

2009–2010, both by solvent and by direct extraction from catkins method. They reported slightly lower pollen viability than what we obtained.

The results of pollen germination in the current study are statistically non-significant and somewhat higher than those obtained in the study carried out by Choudhary and Singh (2013), in which germination percentages varied from 46.45 to 54.28 per cent in 2009 and 46.88 to 62.88 per cent in 2012. Pogorzelec *et al*. (2016) determined the pollen viability for both fresh and pollen stored for one year in *Salix lapponum.* The results obtained in present study are parallel to the findings of Nagarajan *et al*. (2006) who undertook study on floral biology, phenology and seed production in *Casuarina equisetifolia* and found that pollen was binucleate and viable up to 99 per cent and can be stored (at 4°C) without loss in fertility until three months.

The findings of the present investigation are consistent with those obtained by Kadri *et al*. (2021) who reported that fresh pollen of *Phoenix dactlifera* L. had the maximum viability (96.3%) and germination (85%), which declined with increasing storage temperature (28, 4, and -300°C) and length (3, 6, 9 and 12 months). Flowering in *Shorea robusta* was observed twice a year by Singh and Kushwah (2006). Similarly, Kigomo *et al.* (1994) reported that flowering and fruiting in dioecious tree, *Brachylaena huillensis* O. Hoffm occurred twice a year and that was affected by rainfall.

4.3 ESTIMATES OF GENETIC VARIABILITY PARAMETERS

Characters to be selected for improvement are outcome of heritable and non-heritable components. So there is a need for portioning the overall variability into its heritable and non-heritable components. Genetic improvement of any character cannot be done without having sufficient knowledge of heritability, genetic advance and genetic variability.. Hence, heritability and genetic advance play important role in selecting a genotype that exhibits more effectiveness of selection by excluding environmental influence from total variability.

4.3.1 Vegetative and reproductive bud characters and leafy days parameters

It is evident from Table 4.23 that Vegetative bud burst during whole year duration showed the widest range of values (218-290) and reproductive bud burst duration had maximum value of coefficient of variation (9.41). The maximum phenotypic coefficient of variation (20.61) was exhibited by reproductive bud burst duration while minimum (4.12)

was estimated for total number of leafy days. Similarly, as regards to genotypic coefficient of variation, reproductive bud burst duration exhibited the maximum (18.34), while the minimum was noticed for total number of leafy days (4.05).

Highest heritability was recorded for vegetative bud swell whole year duration (99.44 %) followed by vegetative bud burst whole year duration (97.99 %), total leafy days (96.55%), vegetative bud burst in spring 2023 (80.85%) reproductive bud burst duration (79.17 %), vegetative bud swell in spring 2023 (63.49 %), vegetative bud swell in spring 2022 (62.80%), while moderate heritability was recorded in vegetative bud burst in spring 2022 (39.79%). The timing or duration of Bud flush has repeatedly been shown to be under strong genetic control in *Populus* (Bradshaw and Stettler 1995).

The low genetic advance as percentage of mean was observed for vegetative bud burst spring 2023 (2.79 %), reproductive bud burst (2.42 %), reproductive bud swell (0.98 %), vegetative bud burst spring 2022 (0.65), vegetative bud burst spring (0.31%), vegetative bud swell whole year (0.08 %), vegetative bud burst whole year (0.08 %), total number of leafy days (0.03 %).

4.3.2 Leaf shedding parameters

Critical appraisal of Table 4.24 revealed that the widest range of values (26.00- 78.67) was showed by complete leaf fall. Leaf shedding characters had coefficient of variation range from 04.39 percent in complete leaf fall to13.26 percent in 80 per cent to complete leaf fall. The maximum phenotypic variance (133.06) was found in character complete leaf fall whereas minimum phenotypic variance was recorded in 80 per cent complete leaf fall (06.81). The maximum magnitude of genotypic variance (129.94) was found in complete leaf fall character, while minimum (05.65) for 80 per cent to complete leaf fall.

Genetic parameters for leaf shedding characters depicted the highest magnitude of PCV for character 50 percent to 80 per cent leaf shedding (34.18%) followed by 80 per cent to complete leaf shedding (32.18%). Similarly highest magnitude of GCV was showed for character 50 percent to 80 per cent leaf shedding (32.51 %) followed by 50 percent to complete leaf shedding (30.16 %).

All the characters depicted high heritability except vegetative bud burst in spring 2022. The highest heritability was estimated for complete leaf fall (97.66 %) followed by 80 per cent leaf shedding (96.08 %).

The moderate genetic advance as percentage of mean was observed for 80% to 100% leaf fall (17.85%) and 50 per cent to 80 per cent leaf shedding (12.53%). The low genetic advance as percentage of mean was observed for 50 per cent to complete leaf fall (7.47 %), 50 per cent leaf shedding (03.62 %), complete leaf shedding (02.41 %) and 80 per cent leaf shedding (0.08 %).

4.3.3 Catkin length (cm) at different stages, flowering period per branch and pollen viability of *Salix tetrasperma* genotypes.

Table 4.25 revealed that the widest range of values (83.30-96.43) was recorded in pollen viability and maximum coefficient of variation (12.04) was found in flowering period per branch while minimum (1.60) in stage IV. Phenotypic variance (15.57) was recorded highest in pollen viability followed by flowering per branch and lowest in catkin length at stage III, II and I. Similarly maximum genotypic variance (5.89) was recorded in pollen viability and minimum in catkin length at stage I, II, III.

Maximum phenotypic coefficient of variation (27.40 %) was exhibited by flowering per branch while minimum (5.37 %) by pollen viability. As regards to the genotypic coefficient of variation, flowering period per branch exhibited the maximum (24.62 %), while the minimum was noticed in pollen viability (3.30 %).

Highest heritability was recorded for stage IV (98.62 %) followed by stage 1 (95.61 %), stage III (95.50 %), stage II (95.04 %), stage V (94.64 %) and moderate by pollen viability (37.86 %). Similarly high genetic advance as percentage of mean was observed for stage I (100.19 %) , moderate genetic advance as percentage of mean by stage II (20.23 %), stage V (11.78 %) and low genetic advance as percentage of mean by stage III (9.52 %), stage IV (9.10 %), flowering period per branch (6.22 %) and pollen viability (0.07 %).

Alder (2021) evaluated *Populus trichocarpa* hybrids collected from six distinct locations in Sweden and the Baltics to determine broad-sense heritabilities and genotype-by-environment interactions for phenology and growth parameters. Using GxE interaction analysis, they found that the heritabilities for phenology characteristics ranged from 0.31 to

Table 4.23: Genetic parameters for vegetative and reproductive bud characters and leafy days among *Salix tetrasperma* genotypes.

GENETC PARAMETERS	Vegetative Bud swell in spring duration		Vegetative Bud burst spring duration		Vegetative Bud swell whole year duration	Vegetative Bud burst whole year duration	Reproductive Bud swell duration	Reproductive Bud burst duration	Total number of leafy days
Year	**2022**	**2023**	**2022**	**2023**	**2022**	**2022**	**2022**	**2022**	**2022**
Range	23.67-29.00	20.33-29	11.00-16.00	6.33-16	231-300	218-290	14.33-37.00	10.33-25.67	292-348
Coefficient of Variation	4.48	5.28	7.19	2.79	0.68	1.23	3.35	9.41	0.76
Phenotypic Variance	3.46	4.94	1.31	5.81	549.83	473.99	22.01	14.44	157.44
Genotypic variance	2.17	3.13	0.52	4.70	546.77	464.48	20.07	11.44	152.01
Phenotypic coefficient of Variation	4.49	5.28	7.19	8.85	9.11	8.82	17.61	20.61	4.12
Genotypic coefficient of Variation	5.83	6.96	5.85	18.18	9.09	8.73	16.82	18.34	4.05
Heritability (%)	62.80	63.49	39.79	80.85	99.44	97.99	91.17	79.17	96.55
Genetic advance	2.41	2.91	0.94	4.01	48.03	43.95	8.81	6.20	24.96
Genetic Gain (%)	0.31	0.37	0.65	2.79	0.08	0.08	0.98	2.42	0.03

Table 4.24: Genetic parameters for leaf shedding characters among *Salix tetrasperma* genotypes.

GENETIC PARAMETERS	50%Complete leaf fall	80%Complete leaf fall	Complete leaf fall	50% to 80% leaf fall	50% to 100% leaf fall	80% to 100% leaf fall
Range	13.33-42.00	20.67-62.00	26.00-78.67	05.33-20.00	10.33-36.67	04.33-16.67
Coefficient of Variation	7.57	5.88	04.39	10.55	06.71	13.26
Phenotypic Variance	45.80	90.93	133.06	10.85	30.07	06.811
Genotypic variance	42.91	87.36	129.94	9.82	28.65	05.65
Phenotypic coefficient of Variation	30.13	29.71	28.69	34.18	30.89	32.18
Genotypic coefficient of Variation	29.16	29.12	28.35	32.51	30.16	29.32
Heritability (%)	93.68	96.08	97.66	90.48	95.28	83.01
Genetic advance	06.77	18.87	23.20	06.14	10.77	4.46
Genetic Gain (%)	03.62	0.08	02.41	12.53	7.47	17.85

Table 4.25: Genetic parameters for catkin length (cm) in different development stages, flowering period per branch and pollen viability among *Salix tetrasperma* genotypes.

GENETIC PARAMETERS	Stage I	Stage II	Stage III	Stage IV	Stage V	Flowering period per branch (days)	Pollen viability (%)
Range	0.48-1.03	1.06-1.57	1.45-1.95	01.79-03.28	02.03-06.78	5.33 – 14.33	83.30 (65.93) -96.43 (79.17)
Coefficient of Variation	4.56	2.37	1.65	1.60	5.54	12.04	4.23
Phenotypic Variance	0.02	0.02	0.02	0.15	0.94	5.74	15.57
Genotypic Variance	0.02	0.02	0.02	0.15	0.88	4.63	5.89
Phenotypic coefficient of Variation	21.79	10.64	7.81	13.59	23.94	27.40	5.37
Genotypic coefficient of Variation	21.30	10.37	7.63	13.49	23.29	24.62	3.30
Heritability (%)	95.61	95.04	95.50	98.62	94.64	80.71	37.86
Genetic advance	0.30	0.27	0.26	0.79	1.89	3.98	3.08
Genetic Gain (%)	100.19	20.23	9.52	9.10	11.78	6.22	0.07

Table 4.26. Correlation of different character

	Lat	Alt	VBS22	VBB22	VBS23	VBB23	VBSW	VBBW	LD	50LS	80LS	100LS	RBS	RBB	CatL	FB
Lat	1	-0.216*	0.218*	-0.251**	0.156	0.417**	-0.351**	-0.190*	-0.256**	-0.146	-0.204*	-0.206*	0.797**	-0.787**	-0.366**	-0.509**
Alt		1	-0.029	0.080	0.088	-0.575**	-0.753**	-0.628**	-0.428**	-0.133	-0.211*	-0.282**	-0.250**	0.234**	-0.451**	-0.374**
VBS22			1	-.449**	**.595****	0.009	-0.117	-0.031	-0.247**	-0.328**	-0.312**	-0.314**	0.027	-0.001	-0.011	-0.088
VBB 22				1	-.265**	-0.011	0.125	0.088	0.187*	0.208*	.0241**	0.241**	0.001	0.101	0.015	0.254**
VBS23					1	0.062	-0.128	-0.148	-0.365**	-0.261**	-0.252**	-0.305**	0.083	-0.021	0.178*	0.076
VBB23						1	-.734**	-0.807**	-0.535**	-.280**	-0.366**	-0.427**	0.319**	-0.290**	-0.578**	-0.635**
VBSW							1	0.716**	0.501**	0.078	0.201*	0.269**	-0.120	0.181*	0.682**	0.652**
VBBW								1	0.462**	0.131	0.222**	0.277**	-0.085	0.133	0.479**	0.475**
LD									1	0.760**	0.830**	0.871**	-0.122	0.189*	0.390**	0.446**
50LS										1	0.975**	0.953**	-0.118	0.117	0.224**	0.330**
80LS											1	0.987**	-0.135	0.142	0.292**	0.426**
100LS												1	-0.133	0.132	0.291**	0.434**
RBS													1	-0.805**	-0.275**	-0.299**
RBB														1	0.259**	0.269**
CatL															1	0.680**
FB																1

Lat= Latitude, Alt= Altitude, VBS22= Vegetative bud swell in year 2022, VBS23= Vegetative bud swell in year 2023, VBB22= Vegetative bud burst in year 2022, VBB23= Vegetative bud burst in year 2023, VBSW=Vegetative Bud swell whole year duration, VBBW=Vegetative Bud burst whole year duration, LD=Leafy days, 50LS= 50% leaf shedding days, 80LS= 80% leaf shedding days, 100LS= Complete leaf shedding days, RBS=Reproductive Bud swell, RBB= Reproductive Bud burst, CatL= Catkin length at satage V, FB= Flowering per branch

0.91. He came to the conclusion that, following growth features, phenology was a key selection criterion. In aspen, Luquez *et al*. (2008) found that phenological traits had moderate within-populations heritabilities and which is greater than heritability showed by growth traits in *Populus tremula.* The numerous documents showed that the bud-timing within families in conifers is moderately to strongly under genetic control (Aitken and Adams 1997). Bud flush in poplars showed a broad sense heritability ranging from 0.80 to 0.94 (Howe *et al*. 2000). Different selection pressure on these two types of traits may be one explanation for the genetic structural differences between the growth and bud flush characters that were seen in this study. Since the climate can change not only between years but also between various habitats, such as valleys and slopes, where the risk of frost might fluctuate significantly (Rönnberg-Wästljung 2001), the environmental influence on bud flush is presumably of substantial relevance.

4.4. CORRELATION:

Correlation is very important as it helps in formulating suitable selection criteria for the traits by providing idea regarding associations between the dependent and independent variables.

4.4.1 Latitude

It is evident from Table 4.26 that with latitude of collection sites showed significantly positive correlation with reproductive bud swell duration (0.797) followed by vegetative bud burst in 2023 (0.417) and vegetative bud swell in 2022 (0.218). It had significant negative correlation with reproductive bud burst duration (-0.787) followed by flowering per branch (-0.509), catkin length (-0.366), vegetative bud swell duration (-0.351), leafy days (-0.256), vegetative bud burst in 2022 (-0.251), complete leaf fall (-0.206), 80 per cent leaf fall (-0.204) and vegetative bud burst duration (-0.190).

4.4.2 Altitude

Table 4.26 showed that there was significantly positive correlation between altitude of collection sites and reproductive bud burst duration (0.234) whereas there was significant negative correlation with vegetative bud swell duration (-0.753), vegetative bud burst duration (-0.628), leafy days (-0.428), 80 per cent leaf shedding (-0.211), complete leaf shedding (-0.282), reproductive bud swell duration (-0.250) and flowering per branch (-0.374).

4.4.3 Vegetative and reproductive characters

Vegetative bud swell 2022 had significant positive correlation (0.595) with vegetative bud swell 2023 whereas it had significant negative correlation with vegetative bud burst 2022 (-0.449), 50 per cent leaf shedding (-0.328), complete leaf shedding (-0.314), 80 per cent leaf shedding (-0.312) and leafy days (-0.247).

There was negative and significant correlation of vegetative bud swell 2023 with leaf parameters i.e with leafy days (-0.365), complete leaf shedding (-0.305), 50 per cent leaf shedding (-0.261) and 80 per cent leaf shedding (-0.252) and had positive significant correlation with catkin length at stage V (0.178).

On studying table 4.26, it was revealed that vegetative bud swell during whole year had only positive significant correlation with other characters i.e with vegetative bud burst duration (0.716) followed by catkin length at stage V (0.682), flowering per branch (0.652) and leafy days (0.501).

Table 4.26 showed that vegetative bud burst 2022 had maximum significant negative correlation with vegetative bud swell 2023 (-0.265) only. Positive correlation was observed between vegetative bud burst 2022 with (0.254), complete leaf shedding (0.241), 50 per cent leaf shedding (0.208), leafy days (0.187) and 80 per cent leaf shedding (0.0241).

The vegetative bud burst 2023 had significant correlation with all other characters i.e positive and significant correlation only with reproductive bud swell duration (0.319) and significant negative correlation with vegetative bud burst duration whole year (-0.807) followed by vegetative bud swell duration whole year (-0.734), flowering per branch (-0.635), catkin length at stage V (-0.578), leafy days (-0.535), complete leaf shedding (-0.427), 80 per cent leaf shedding (-0.366), reproductive bud burst duration (-0.290) and 50 per cent leaf shedding (-0.280).

Vegetative bud burst whole year showed maximum positive significant correlation with catkin length at stage V (0.479) followed by flowering per branch (0.475), leafy days (0.462), complete leaf shedding (0.277) and 80 per cent leaf shedding (0.222).

Leafy days showed only significant positive correlation with complete leaf fall (0.871), 80 per cent leaf shedding (0.830), 50 per cent leaf shedding (0.760), flowering per branch (0.446) catkin length at stage V (0.390) and reproductive bud burst duration (0.189).

Fifty per cent leaf shedding had significant positive correlation with complete leaf shedding (0.953), 80 per cent leaf fall (0.975), flowering per branch (0.330) and catkin length at stage V (0.224). Complete leaf shedding was positively correlated with flowering per branch (0.434) and catkin length at stage V (0.291). There was significant positive correlation of 50 per cent leaf shedding with 80 per cent leaf shedding and complete leaf shedding. The highest positive correlation (0.957) was found between 80 per cent leaf shedding and complete leaf shedding which was followed by correlation with flowering per branch (0.426), catkin length at stage V (0.292).

The results in Table 4.26 showed that there was negative and significant correlation of reproductive bud swell duration with reproductive bud burst duration (-0.805) followed by catkin length at stage V (-0.275). Reproductive bud burst had positive and significant correlation with flowering per branch (0.269) followed by catkin length at stage V (0.259). On the other hand, catkin length (stage 5) had significant and positive correlation with flowering per branch (0.680).

According to McKown *et al*. (2018), bud break showed a quadratic relationship with latitude, and provenances from the southern and northernmost regions of species range typically breaking bud earlier than those from middle regions. This pattern contrasted with study carried on phenology of *Populus trichocarpa*, by Evans *et al*. (2014) and Mckown *et al*. (2014). According to Luquez *et al*. (2008) observed a substantial clinal variation with latitude in case of the date of bud set and the duration of the leaf area in aspen (*Populus tremula*) but there was no change in the date of bud flush along a latitudinal cline. Whittet *et al*. (2021) showed that in a provenance experiment of sycamore (*Acer pseudoplatanus* L.) in England exhibiting adaptive difference among provenances, the timing of bud burst was negatively linked with latitude. Sessile oak (*Quercus petraea*) bud burst showed significant provenance-based variation according to Ducousso *et al*. (1996), which was primarily clinal and connected to the origin's altitude and latitude. They also observed that in contrast to northern provenances, southern provenance had early flush, which was consistent with the findings of our study. Rossi and Bousquet (2014) found an opposite tendency in black spruce, which they attribute to the species' phenological adaptations to the local environment. Similar results

were obtained by Luquez *et al.* (2008) who observed that the traits like the date of bud set and leaf area duration showed strong clinal variation patterns with latitude in both field trials, but bud burst did not change along a latitudinal cline between populations. However, there were significant differences in bud flushing between individuals. Diatta *et al.* (2021) undertook study on phenology of *Acacia senegal* trees procured from different range wide populations and grown in a common garden to study correlation between phenology and climate of site of origin. They noticed that the trees with early flushing had longer growing period. They concluded that differences in phenology among trees appeared to be linked to climate differences at their site of origin as there was significant correlation between duration of leaf development in common garden and timing as well as duration of rainy season at site of origin.

Chapter-5

SUMMARY AND CONCLUSION

The present investigation entitled "**Phenological Behaviour and Reproductive Biology of *Salix tetrasperma* Roxb.**" was carried out in the Department of Tree Improvement and Genetic Resources, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) during 2021-2023. The experimental material consisted of genotypes of *Salix tetrasperma* collected from 15 different sites and planted in Naganji nursery of the department. The vegetative bud swell, bud burst, leafy days, leaf fall, reproductive bud swell, reproductive bud burst, flowering, anthesis duration per catkin, pollen viability and pollen germination were observed.

5.1 VEGETATIVE CHARACTERS

5.1.1 Vegetative bud swell and bud burst

Vegetative bud swell during spring started on 24-12-2021 and last up to 12-02-2022 whereas vegetative bud swell during spring of the year 2023 started on 22-12-2022 and last up to 09-02-2023. The maximum duration for vegetative bud swell (29.00 days) during spring of the year 2022 was noticed in genotype CH1 that was statistically at par with genotype CH3, BN2, CH2, BN6, SP3, BN5 and CW2 whereas minimum duration (22.67 days) was found in genotype CN2 and CN3. In the spring of the year 2023, genotype CH1 showed maximum duration (29.00 days) for vegetative bud swell spring during the year 2023 which was statistically at par with BN2, CH3, BN6, CH2 (28.00), SP3 (27.67), DM5 (27.33), BN5 (27.33), DE1 (27.33) and DM3 (27.00) whereas genotype BL3 showed minimum bud swell duration (20.33).

Site Chamba showed a maximum duration (28.56 days) for vegetative bud swell during spring 2022 that was statistically at par with site Bhunter whereas minimum duration (23.22 days) was found in Chinani. The maximum duration (28.56 days) for vegetative bud swell spring during the year 2023 was noticed in site Chamba which was statistically at par with site Bhunter (21.33) whereas Balh showed minimum bud swell duration (21.33 days).

The vegetative bud burst during spring started on 20-01-2022 and last up to 26-02-2022 whereas the vegetative bud burst duration was from 09-01-2023 up to 19-02-2023.

The longest vegetative bud burst duration (16.00 days) in spring 2022 was recorded for genotype DM3 followed by NM2, RN4, HM1 and BL1 whereas shortest (11.00 days) was recorded for CH3, CH1, DE1, DE2 and JM1. On the other hand, in the spring of 2023, maximum duration (16.00 days) was recorded for genotype BN6 which was statistically at par with CN1, CN2, NM4, CN3 and NM2 whereas minimum duration (6.33 days) was recorded for genotype RN3.

The maximum duration for vegetative bud burst (13.67 days) during spring 2022 was recorded for site Devamanal which was statistically at par with Rupnagar whereas the minimum (11.11 days) was recorded for Chamba. For vegetative bud burst during spring 2023, maximum duration (15.00 days) was recorded for site Chinani followed by site Namhol, Bhunter and Chowari. On the other hand, the minimum duration for bud burst (7.56 days) was recorded for site Rupnagar.

5.1.2 LEAFY DAYS

In case of leafy days, the results recorded were significantly different among the genotypes as well as among sites and the maximum number of leafy days (348.33) was found in RN3 followed by RN1, RN4, SP4 and SP3 whereas the minimum (292.00) was found in genotype DE5. The maximum number of leafy days (339.78) was recorded for site Rupnagar which was followed by site Suhanpur, Balh, Rampur and Hamirpur whereas a minimum number of days (295.22) was recorded for site Tandi.

5.1.3 LEAF SHEDDING

Genotypes of different sites were found statistically different from each other for the time taken for 50 per cent, 80 per cent and complete leaf shedding. Maximum no. of days for 50 per cent leaf shedding (42.00) was observed in Genotype RN3 whereas a minimum number of days (13.33) was recorded in genotype TD1. Rupnagar site recorded a maximum duration for 50 per cent leaf shedding (39.00) and the minimum number of days (14.33) was recorded in the case of site Tandi which was statistically at par with site Deothi.

A maximum number of days (62.00) for 80 per cent leaf shedding was observed in Genotype RN3 whereas genotype TD1 recorded a minimum number of days (20.67) for 80 per cent leaf shedding which was statistically at par with DE1, DE5, NM5, DE2, NM2, TD2

and CH1. Site Rupnagar showed a maximum duration (56.67days) for 80 per cent leaf shedding followed by site Rampur, Suhanpur and Balh whereas minimum duration (21.56 days) was observed in the case of site Deothi which was statistically at par with site Namhol and Tandi.

Genotype RN3 recorded a maximum number of days (78.67) for complete leaf fall followed by RN1, RN4 and RP2 and the minimum number of days was observed in Genotype DE1(26.00) which was statistically at par with DE2, NM5, DE5, NM2, NM4, TD1, CH1, CH2, CH3, DM5, TD2 and HM1. Among sites, Rupnagar recorded maximum duration for complete leaf fall (70.56 days) which was followed by site Rampur, Suhanpur and Balh and minimum duration (26.56 days) was recorded in site Deothi which was statistically at par with site Namhol.

Genotype RN3 retained leaves up to maximum number of days for leaf shedding from 50 to 80 per cent (20.00 days) as well as from 50 per cent to complete leaf shedding (36.67 days). While BN6 genotype required maximum days (22.33) for shedding leaves from 80 per cent to complete leaf shedding. Among sites, Rupnagar site required maximum number of days (17.67) for leaf shedding from 50 to 80 per cent, (13.89 days) for 80 to complete and (31.56 days) for 50 per cent to complete leaf shedding.

This indicates that the timing of critical stages for growth initiation i.e. bud and leaf phonologies as well as that of growth (e.g. growth cessation and leaf abscission) are highly influenced by environmental conditions of origin and therefore were given importance in breeding.

5.2 REPRODUCTIVE CHARACTERS

5.2.1 Reproductive bud swell and bud burst

Reproductive bud swells in different genotypes of *Salix tetrasperma* started from 01-01-2023 to 26-02-2023. The maximum duration for bud swell (37.00 days) was recorded in genotype CN3 while minimum bud swell duration (14.33 days) was recorded in genotype JA3 which was statistically at par with genotypes JA1(14.67) and JA4(15.00). Comparisons of sites depicted that genotypes of site Chinani had maximum bud swell duration (36.00 days) while site Jakholi recorded minimum bud swell duration (14.67 days).

Reproductive bud burst in different genotypes of *Salix tetrasperma* started from 10-01-2023 to 05-03-2023. The genotype JA4 recorded maximum duration of bud burst (25.67 days) which was statistically at par with genotype JA3, JA1, DE2 and RP2. Minimum bud swell duration (10.33 days) was recorded in genotype CN3 which was statistically at par with genotype CN2, JM1 and CN1. Among the sites, Jakholi recorded maximum bud burst duration (25.00 days) followed by site Deothi, Rampur and Suhanpur. Minimum bud burst duration (11.33 days) was recorded in site Chinani which was followed by Jammu, Chowari and Chamba.

5.2.2 Flowering and anthesis duration per catkin

Out of forty five genotypes, only six genotypes namely BL1, BL2, BL3 (from site Balh) and CW1, CW2, CW3 (from site Chowari) were pistillate, while others were staminate. Different catkin development stages were studied and it was observed that catkin of genotype TD3 attained maximum length (1.03 cm) at stage I of catkin development while catkins of genotype SP4, TD1, SP4 and RN4 attained maximum length (1.57 cm), (1.95 cm), (3.28 cm) and (6.8 cm) at stage II, III, IV and V respectively whereas genotype CN1 had minimum catkin size (0.48 cm), (1.06 cm), (1.45 cm), (1.79 cm) and (2.03 cm) at stage I, CN2 and CN1 at stage II, CN3 at stage III while JM5 at stage IV and V respectively.

Site wise analysis showed that site Tandi recorded maximum catkin length (1.01 cm) at stage I, Suhanpur at stage II as well as stage III and IV (1.53 cm, 1.93cm and 3.26 cm respectively) while Rupnagar at stage V (5.85 cm). Chinani recorded minimum catkin length for stage I, II, III and IV (0.51 cm, 1.07 cm, 1.48 cm and 2.62 cm respectively) whereas Balh for stage V (2.21 cm).

Flowering in the genotypes collected from Punjab, RN1, RN3, RN4, TD1, TD2, TD3, SP1, SP3 and SP4 were observed to occurred two times i.e once in month of October 2022 and other in the month of January- February 2023 whereas in other genotypes it occurred only once during January-February 2023.

In case of flowering, maximum duration for flowering per branch (14.33 days) was recorded for genotype RN3 maximum duration of flowering was recorded in site Rupnagar whereas minimum duration of flowering per branch (5.33 days) was recorded in CW2 which was statistically at par with BL3, CW1, BL2, JM5, JM2, CN3 and NM5.

Among the sites, maximum (13.67) duration of flowering was recorded in site Rupnagar whereas minimum (5.78) duration of flowering was recorded in site Chowari which was statistically at par with site Balh (5.89).

Genotype BL1 showed maximum anthesis duration per catkin i.e 5.67 days. On the other hand, site wise analysis revealed that site Balh showed maximum (5.33) anthesis duration per catkin which was statistically at par with genotype Chamba (5.33), Deothi (5.00) and Chowari (4.67). Minimum number of anthesis days (4.11) was recorded in Jakholi which was followed by Devamanal, Chinani, Tandi, Bhunter, Namhol, Suhanpur, Jammu, Rupnagar and Rampur.

5.2.3 Pollen viability and germination

Pollen viability was counted maximum (96.43 %) in RN1 genotype which was statistically at par with DM3, DM4, DM5, JA1, JA3, JA4, RN3, RN4, SP1, SP3, SP4, HM1, HM2, HM4 , BN2, BN5, BN6 , CN1, CN2 , CN3, JM1, JM2, JM5, DE1, DE2, DE5, CH1, CH2 and CH3 whereas minimum (83.30 %) in TD3. Similarly, maximum pollen germination percentage (75.59 %) was recorded for site Rupnagar whereas minimum (66.28 %) recorded in site Tandi.

5.3 GENETIC PARAMETERS AND CORRELATION

The genetic parameters study revealed that for all the studied characters, the phenotypic coefficients of variation (PCV) were higher than analogous genotypic coefficients of variation (GCV), although there was minimal difference between corresponding PCV and GCV values in most of the characters which in turn indicated that the characters were highly influenced by the environment. The highest PCV and GCV were noticed in 50 per cent to 80 per cent leaf fall (34.18 % and 32.51 % respectively). The variability estimation for each trait revealed that there was variability among all the genotypes with maximum coefficient of variation (13.26) in 80 per cent-100 per cent leaf shedding followed by flowering per branch. The high heritability coupled with low genetic advance as percentage mean were recorded in most of the characters with maximum value for catkin length (stage IV) which suggests that these characters are influenced by non additive gene action or were non additive in nature.

Highest positive and significant correlation (0.987) was observed between 80% leaf fall and complete leaf fall and lowest positive and significant correlation was observed between vegetative bud swell 2022 and vegetative bud burst 2023. On the other hand, there was maximum negative and significant correlation of reproductive bud swell duration with reproductive bud burst duration (-0.805).

Correlation studies with latitude of collection sites showed significantly positive correlation with vegetative bud swell in 2022 (0.218), vegetative bud burst in 2023 (0.417) and reproductive bud swell duration (0.797) whereas significantly negative correlation with flowering per branch (-0.509), catkin length (-0.366), vegetative bud swell duration in whole year (-0.351), leafy days (-0.256), vegetative bud burst 2022 (-0.251), complete leaf shedding (-0.206), 80 per cent leaf shedding (-0.204) and vegetative bud burst (-0.190).

There was significant and negative correlation between altitude and most of the characters except reproductive bud burst duration (0.234). The catkin length (stage 5) had maximum significant correlation with vegetative bud swell duration (0.682) followed by flowering per branch (0.680).

CONCLUSIONS

- There were significant variations among genotypes as well as sites of *Salix tetrasperma* for the phenological and reproductive characters.
- All the characters showed the negative correlation with the latitude and altitude of the collection site except reproductive bud swell duration, vegetative bud burst in 2023 and vegetative bud swell in spring 2022 with the latitude and reproductive bud burst duration with the altitude of the collection sites which indicates the adaptiveness of these characters towards collection sites.
- Genotypes from Punjab flowered twice in a year i.e in the month of October (2022) and February (2023) which ensured that these genotypes can be exploited for pollen collection even in the autumn season for the improvement programme.
- All the characters showed high heritability but low genetic gain that means characters are non additive in nature and thus can be improved through hybridization.
- This study will be useful for best utilization of temporal measurements and Germplasm of *Salix tetrasperma* for further improvement programmes of *Salix* species.

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ANOVA of Genotypes for different parameters under study
VEGETATIVE CHARACTERS

APPENDIX – I

ANOVA for vegetative bud swell of the genotypes of *Salix tetrasperma* Roxb. in spring 2022

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	134	472.15	3.523494		
GSS	44	343.4815	7.806397	6.064609	1.514726
RSS	2	15.39259	7.696296	5.979074	3.100069
ESS	88	113.27	1.287205		

APPENDIX – II

ANOVA for vegetative bud swell of the genotypes of *Salix tetrasperma* Roxb. in spring 2023

Source of error	df	Sum of Square	Mean Sum of Square	F- calculated	F-Tabulated
TSS	134	659.08	4.918519		
GSS	44	493.0815	11.2064	6.215893	1.514726
RSS	2	7.348148	3.674074	2.037912	3.100069
ESS	88	158.65	1.802862		

APPENDIX - III

ANOVA for vegetative bud burst of the genotypes of *Salix tetrasperma* Roxb. in spring 2022

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	134	178.000	1.328		
GSS	44	103.333	2.348	2.983	1.515
RSS	2	5.378	2.689	3.415	3.100
ESS	88	69.289	0.787		

APPENDIX – IV

ANOVA for vegetative bud burst of the genotypes of *Salix tetrasperma* Roxb. in spring 2023

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	134	768.104	5.732		
GSS	44	668.770	15.199	13.663	1.515
RSS	2	1.437	0.719	0.646	3.100
ESS	88	97.896	1.112		

APPENDIX - V

ANOVA for vegetative bud swell during whole year 2022 in the genotypes of *Salix tetrasperma* Roxb.

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	134	62592.104	467.105		
GSS	44	61730.104	1402.957	147.612	1.515
RSS	2	25.615	12.807	1.348	3.100
ESS	88	836.385	9.504		

APPENDIX – VI

ANOVA for vegetative bud burst during whole year 2022 in the genotypes of *Salix tetrasperma* Roxb.

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	134	768.104	5.732		
GSS	44	668.770	15.199	13.663	1.515
RSS	2	1.437	0.719	0.646	3.100
ESS	88	97.896	1.112		

APPENDIX – VII

ANOVA for number of leafy days in the year 2022 in the genotypes of *Salix tetrasperma* Roxb.

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	134	20834.637	155.482		
GSS	44	20304.637	461.469	84.951	1.515
RSS	2	51.970	25.985	4.784	3.100
ESS	88	478.030	5.432		

Leaf Shedding Parameters

APPENDIX – VIII

ANOVA for 50 per cent leaf shedding in the genotypes of *Salix tetrasperma* Roxb. in the year 2022

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	134	6051.526	45.161		
GSS	44	5790.859	131.610	45.496	1.515
RSS	2	6.104	3.052	1.055	3.100
ESS	88	254.563	2.893		

APPENDIX – IX

ANOVA for 80 per cent leaf shedding in the genotypes of *Salix tetrasperma* Roxb. in the year 2022

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	134	12011.748	89.640		
GSS	44	11688.415	265.646	74.473	1.515
RSS	2	9.437	4.719	1.323	3.100
ESS	88	313.896	3.567		

APPENDIX – X

ANOVA for complete leaf shedding in the genotypes of *Salix tetrasperma* Roxb. in the year 2022

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	134	17574.193	131.151		
GSS	44	17289.526	392.944	126.003	1.515
RSS	2	10.237	5.119	1.641	3.100
ESS	88	274.430	3.119		

APPENDIX – XI

ANOVA for 50- 80 per cent leaf shedding in the genotypes of *Salix tetrasperma* Roxb. in the year 2022

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	134	1433.215	10.696		
GSS	44	1341.215	30.482	29.504	1.515
RSS	2	1.081	0.541	0.523	3.100
ESS	88	90.919	1.033		

APPENDIX - XII

ANOVA for 50 per cent to complete leaf shedding in the genotypes of *Salix tetrasperma* Roxb. in the year 2022

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	134	3971.437	29.638		
GSS	44	3844.104	87.366	61.590	1.515
RSS	2	2.504	1.252	0.883	3.100
ESS	88	124.830	1.419		

APPENDIX – XIII

ANOVA for 80 per cent to complete leaf shedding 2022 in the genotypes of *Salix tetrasperma* Roxb. in the year

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	134	905.333	6.756		
GSS	44	797.333	18.121	15.661	1.515
RSS	2	6.178	3.089	2.670	3.100
ESS	88	101.822	1.157		

REPRODUCTIVE CHARACTERS

APPENDIX - XIV

ANOVA for Reproductive bud swell in the genotypes of *Salix tetrasperma* Roxb. in spring 2023

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	134	2907.215	21.696		
GSS	44	2734.548	62.149	32.040	1.515
RSS	2	1.970	0.985	0.508	3.100
ESS	88	170.696	1.940		

APPENDIX – XV

ANOVA for Reproductive bud burst in the genotypes of *Salix tetrasperma* Roxb. in spring 2023

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	134	1907.215	14.233		
GSS	44	1641.881	37.315	12.406	1.515
RSS	2	0.637	0.319	0.106	3.100
ESS	88	264.696	3.008		

APPENDIX - XVI

ANOVA for catkin development at stage 1 in the genotypes of *Salix tetrasperma* Roxb.

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	134	3.127	0.023		
GSS	44	3.034	0.069	66.370	1.515
RSS	2	0.001	0.001	0.565	3.100
ESS	88	0.091	0.001		

APPENDIX - XVII

ANOVA for catkin development at stage 2 in the genotypes of *Salix tetrasperma* Roxb.

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	134	2.4665	0.0184		
GSS	44	2.3843	0.0542	58.4284	1.5147
RSS	2	0.0007	0.0003	0.3522	3.1001
ESS	88	0.0816	0.0009		

APPENDIX - XVIII

ANOVA for catkin development at stage 3 in the genotypes of *Salix tetrasperma* Roxb.

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	134	2.2383	0.0167		
GSS	44	2.1698	0.0493	64.6861	1.5147
RSS	2	0.0014	0.0007	0.9046	3.1001
ESS	88	0.0671	0.0008		

APPENDIX – XIX

ANOVA for catkin development at stage 4 in the genotypes of *Salix tetrasperma* Roxb.

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	134	20.0592	0.1497		
GSS	44	19.8652	0.4515	215.0139	1.5147
RSS	2	0.0092	0.0046	2.1953	3.1001
ESS	88	0.1848	0.0021		

APPENDIX – XX

ANOVA for catkin development at stage 5 in the genotypes of *Salix tetrasperma* Roxb.

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	134	113.3554	0.8459		
GSS	44	109.2047	2.4819	54.7462	1.5147
RSS	2	0.1612	0.0806	1.7780	3.1001
ESS	88	3.9895	0.0453		

APPENDIX – XXI

ANOVA for flowering days per branch in the genotypes of *Salix tetrasperma* Roxb.

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	134	759.93	5.671089		
GSS	44	659.9259	14.99832	13.54981	1.514726
RSS	2	2.592593	1.296296	1.171103	3.100069
ESS	88	97.41	1.106902		

APPENDIX – XXII

ANOVA for Pollen viability in the genotypes of *Salix tetrasperma* Roxb.

Source of error	df	Sum of Square	Mean Sum of Square	F-calculated	F-Tabulated
TSS	116	1807.491	15.582		
GSS	38	1039.348	27.351	2.828	1.562
RSS	2	33.048	16.524	1.708	3.117
ESS	76	735.095	9.672		

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Number of words in the abstract	**:**	297

ABSTRACT

The present investigation entitled **"Phenological behaviour and reproductive biology of *Salix tetrasperma* Roxb."** was carried out in the experimental field as well as in the laboratory of the department of Tree Improvement and Genetic Resources of Dr. Y.S. Parmar University of Horticulture and Forestry Nauni, Solan (H.P.) during 2021-23. Phenological and reproductive study conducted in the experimental field and laboratory revealed that significant variation was observed among the genotypes as well as the site of collection of *Salix tetrasperma* for both vegetative and reproductive characters. Maximum duration for vegetative bud swell in spring 2022, vegetative bud burst in spring 2023, reproductive bud swell, reproductive bud burst was recorded in CH1 (29.00 days), BN6 (16.00 days), CN3 (37.00 days) and JA4 (25.67 days), respectively. Maximum duration for 50 per cent and complete leaf shedding was recorded in genotype RN3 (42 days and 78.67 days, respectively). The maximum number of leafy days (348.33) was found in RN3 whereas minimum was found in genotype DE5 (292.00). Maximum duration for flowering per branch was recorded in the genotype RN3 (14.33 days) while minimum was recorded in CW2 (5.33 days). Maximum pollen viability was recorded for genotype RN1 (96.43 %) whereas minimum was recorded in TD3 (83.30 %). Maximum pollen germination percentage (75.59 %) was recorded for site Rupnagar whereas minimum (66.28 %) recorded in site Tandi. Genotypes from Punjab flowered twice a year i.e in the month of October (2022) and February (2023). All the characters showed the negative correlation with the latitude and altitude of the collection site except reproductive bud swell duration, vegetative bud burst in 2023 and vegetative bud swell in spring 2022 with the latitude and reproductive bud burst duration with the altitude of the collection sites which indicates the adaptiveness of these characters towards collection sites.

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Whether sponsored by some state/Central Govt./Univ./SAARC : NO

Scholarship/ Stipend/fellowship any other financial Assistant received during the study period : University Stipend

Any other Information : NA

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