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Reproductive biology of *Populus deltoides* Bartr.

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ABSTRACT

The detailed knowledge on the reproductive biology of plants is required for their successful cultivation and conservation. The reproductive characteristics of trees are required to assess the diversity patterns and community structure of the forests. Understanding the reproductive characteristics, such as, pollination, breeding systems, phenology, and floral biology of *Populus deltoides* is immensely important; because it is one the most important tree species in India. The review documented the physiological basis of the reproductive characteristics of poplar tree. Literature indicated that the dioecious species shows a regular flowering patternin both the male and female trees, and they produces flower (catkin) every year. In all the species, male flower buds bursted earlier than the vegetative bud, whereas, female flower buds bursted after the emergence of leaves. Unisexual flowering occurs between February and April in both male & female, and the dispersal of seeds reported in late May or early June. Most of the pollen grains exhibited reddish colour. The fruits are capsule shaped, splits into 3or 4 parts, and release cotton like seeds, which are viable for only a short period. Poplar mostly regenerates from seed under natural conditions, but are also capable of asexual or vegetative reproduction under artificial conditions. The male and female plant produces a significant number of hybrids seeds; resulting in an excellent growth of the plants. The species displayed cross-pollination characteristics. The information documented in the current literature will be quite useful to understand the reproductive characteristics of the *Populus deltoides.*

Contribution/Originality: Assessment of the reproductive biology of *Populus deltoides* is essential to understand the pollination mechanisms, genetic variation, and propagule dispersal between and within populations. The information documented in current literature will be helpful to analyze the reproductive success, species diversity, and fitness of flowering of the *Populus deltoids.*

1. INTRODUCTION

Genus *Populus* is one of the oldest living angiosperms belongs to family salicaceae, order salicales, group amentiferae and subclass dictoyledenae. The phanerogamae division of angiosperms contains total of 35 species [1-3], and are distributed across the Northern hemisphere's zones. Among these, *Populus deltoides* and *Populus nigra* are the two important species those are extensively planted both inside and outside of their natural ranges.

Populus deltoids Bartramex Marshall, a native of North America, is also referred as Poplar or Eastern Cottonwood. It has a wide geographic range that stretches from the Atlantic coast to the Great Plains region from 70 to 115^0 W longitude from South Texas and the entire Gulf of Mexico coastal plain to the great lakes region of Canada. The latitudinal range extends from 27-30^0 N in East of the United States to 52^0 N in Canada's provinces of Manitoba, Saskatchewan and Alberta in the West [4]. In India, *Populus deltoides* is the most popular tree species under agroforestry system in Punjab and Haryana, Uttarakhand, and Uttar Pradesh states [5].

The tree has a short lifespan, grows quickly, is deciduous in nature, matures at 60 to 120 feet tall and eventually develops into a vase shape. In open spaces, it creates an ovoid crown and grows a single, sturdy trunk up to 4 to 6' across. The abundant branches are ascending above and drooping below; they are somewhat crooked and knobby. Mature trees have thick, grey, and coarsely ridged trunk bark, and is light gray to gray-brown in colour, fairly smooth and divided into thick ridges. Terminal buds 0.6 to 2cm long with 6 to 7 visible scales, and the lateral bud are somewhat smaller and more or less flattened and apprised [6]. Leaves alternate, simple, deltoid or broad-ovate, 4 to 5" long and 3 to 4" across and about as broad, tip acuminate, base sub cordate to truncate, margin coarsely crenate-dentate with curved teeth [7]. The thin petioles are glabrous, light green and somewhat flattened and are almost as long (6 to10 cm) as a leaf blade [8].

The tree grows to its highest size on alluvial soils and prefers moist environments, but it can also endure dry soils, salty environments and a wide range of pH. In 1952, clones of *Populus deltoides* were introduced in India to boost the supply of raw material for the nation's plywood businesses. The wood of species is in demand for plywood, pulp and paper, light constructional timber, matchwood, and packing cases all over the world [9].

*P. deltoides*is the only species of Poplar, which is planted at a large scale in India, and it occupies almost 60,000 hectares equivalent area in the country. *P. deltoids* based agroforestry system is one of the viable alternate land uses systems to prevent the land degradation, enhance productivity, improve ecosystem services, and mitigate climate change [10]. Figure 1 illustrates the morphology leaf and stem of *Populus deltoids*.

Figure 1. Leaf morphology and stem texture of *Populus deltoids*.

2. REPRODUCTIVE CHARACTERS

2.1. Flowering and Fruiting

Early in the spring, sprouting in the Eastern Cottonwood is initiated before the appearance of leaves. Individual trees produce either all female (pistillate) or all male (staminate) flowers. The sex ratio is approximately 1:1. Male buds grow substantially bigger and develop a little earlier than female buds. Before leaves emerge, flowering happens between February and April in both the male and female. The female catkins are green, while the male catkins are long and reddish-purple in colour. Male blooms are only 8 to 13 cm (3 to 5 in) long, while, female flowers grow 15 to 30 cm longer (6 to12 in) [11].

The male flowers have a disk-shaped arrangement of four to 60 stamens, but lack a calyx or corolla. The filaments are short and pale yellow, and the anthers are oblong, purple or red, introrse and two-celled, with the

cells opening longitudinally. The single-celled ovary is enclosed in a cup-shaped disc in the female flower, which is devoid of a calyx and a corolla. The short style has two to four stigmata, and is differently lobed and has a large number of ovules. Among trees in a stand, the difference in flowering may varies by one month. Early flowering trees are therefore prevented from crossing paths with late flowering trees. Young trees are 4 to 5 years old and have blossomed flowers [12].

In the period between pollination and maturity, the length of female catkins significantly increases. The fruit is a 3 to 4 valved dehiscent capsule that matures in mid-summer and is green to reddish-brown in colour. It contains numerous tiny light brown seeds that are surrounded by tufts of long, soft, white hairs, which help the winddispersal of the seed [11].

According to Dhiman and Gandhi [13] the gender ratio of *P. deltoides* trees is equal in both lowland and highland habitats and it does not change over time within the populations. Compared to female trees, male trees grows to greater highest but do not have thicker trunks [14]. As estimated by Bessey [15], Cottonwood trees are very floriferous and produce 32,000 female catkins and 28,000,000 seeds each year. Figure 2 illustrates the male and female catkin of *P. deltoides* tree.

Figure 2. Male and female catkin (Flower) of *P. deltoides* tree.

2.2. Pollen Studies

Strong anemophilous adaptations in flowers results in the large pollen production. For understanding the pollen viability and morphology, male *Populus deltoides* catkins that are ready for pollen dehiscence are placed into butter paper bags, which are then let to dehisce at room temperature (22 to 24°C). To avoid contamination from any other sources, all of the pollen samples should be collected separately. Pollen that had been spread out on glass plates are taken, scooped and transferred into oven dried glass vials. The vials are then placed in a glass desiccation chamber that had been impregnated with silica and kept in a refrigerator at 4°C [16]. Using an acetocarmine dye, pollen viability can be assessed periodically in accordance with normal microscopy sample procedures. Under a light microscope, the pollen sizes are measured using an ocular micrometer [17]. The concentrations of sucrose used for the pollen germination experiments were 1%, 2%, 5%, 10% and 15%. A little amount of pollen grains and 10ml of distilled water are present in each concentration. The following day, 10ml of each concentration's solution are kept on the cavity slide and pollen tube germination was observed under a light microscope. All of these concentrations have to be kept at room temperature for one day. Sucrose concentrations have been observed to have an impact on pollen germination rates [18]. In one of the study, proteomics technique was used to examine the 3929 total proteins in *P. deltoides* pollen and the World Health Organization/International Union of Immunological Societiesdatabase & online database retrieval were used to find the 46 probable allergens. Bioinformatics was used to screen one target protein, which was then produced in *Escherichia coli*. A significant antigenic component of *P. deltoides* pollen is B9N9W6 [19]. Figure 3 illustrates the pollen of *P. deltoides* under electron microscope.

Figure 3. Pollen of *P. deltoides* under microscope.

2.3. Seed Production

A capsule contains 30 to 60 seeds that grow on short stalks on long catkins. There are 3 or 4 valves on each capsule. The female catkins are 15 to 25 cm long, contains several 8 to 12 mm long capsules, and at time of maturity these break into three or more halves and release tufted seeds similar to white "cotton" that is visible during the dispersal [20]. When the trees are 5 to 10 years old, the production of lightweight winged seeds initiated, and the quantity of production increases quickly as the tree become older and bigger. A single open grown tree has been estimated to produce up to 48 million seeds annually. Poplars yield huge quantity of seeds as more than 50 million seeds can be produced by old trees in a single season [21].

2.3.1. Seed Dispersal

In Southern populations, the seed dispersal starts about two months after flowering and in Northern populations it starts a little sooner. It is distinguished by wide variance within trees as well as a protracted dispersal period for some specific trees. In the South, seed dispersal takes place from May through mid-July, whereas in the North, it happens from in late May or early June. As the spring flood waters recede, the distribution pattern causes substantial deposits of seeds along waterways. With the help of the "cotton" affixed to the seed, wind can carry seeds hundreds of feet. When seeds fall into water, they may travel far from the parent tree before landing on silt deposits [20]. The seeds are dispersed via wind over long distances, resulting in the high rates of migration, high gene flow, and greater genetic variety. The first several weeks are sensitive for seedlings as many of them are killed by rain, intense sunlight and damping-off fungi. It needs wet and open mineral soils, like new silt deposits. Epigeal germination occurs and seedlings grow slowly during the first three weeks, then possibly very quickly. After the first few weeks, full sunshine for a significant portion of each day is needed [22].

Within the riparian corridor, seedlings settle on moist, recently exposed soil that was previously flooded in the winter along gravel bars, sandbars and riverbanks. Poplar seeds quickly lose viability due to their small size. Seed germination requires constant wetness for maintaining the viability. When the level of river drops too quickly, seedlings become vulnerable to drought stress. At lower river levels, seedlings that germinate on moist soil are vulnerable to eventual removal or damage at the hands of floodwaters. With regard to climatic conditions and channel morphology, these environmental restrictions as a whole lead to the infrequent establishment of seedlings, on the scale of every 10 to 20 years. In some natural populations, reproductive maturity may not occur until the trees are 15 to 20 years old, which limits the occurrence of introgression events. Reproductive maturity in some species ranges from five to ten years. On the other hand, Hybrid poplars cultivated under plantations typically

reach reproductive maturity in four years [23].

2.3.2. Seed Storage

Fully developed seeds can remain viable for several months if they are quickly dried to a moisture content of 5 to 8% and stored at temperatures just below freezing. Viability may be extended for five or more years if stored at -20° C (-4° F). There is gradual need to increase the moisture content to germinate a very dry seed. Cottonwood seeds require a good seedbed and germinate quickly if they aren't floating on or submerged in water. The germination percentage of seeds are as high as 90% [24]. The only viable alternative for the long-term conservation of intermediate and short-lived seeds is cryopreservation. Although *Salix* and *Populus* seeds can be kept in cryo-storage for longer than under temperature conditions, the ageing was not totally prevented and the seed lifetime was less than that of many other species. For instance, after 20 years of cryo-storage, the seeds exhibited germination rates of 19% and 33% only (original germination rates were 90%)[25].

3. CONTROLLED POLLINATION/ HYBRIDIZATION

Natural hybridization has significant evolutionary repercussions and has long been acknowledged as a way for genes to move between species. Despite the fact that both species that hybridize are equally likely to be the male or female parent, hybridization is commonly thought to be symmetrical [11]. A flower is naturally dichogamous (producing male and female reproductive elements at various times by a hermaphroditic organism to ensure cross fertilization) and dioecious (containing the male and female reproductive organs in distinct individuals). Clones that are both male and female have pendulous catkins (hanging flowering spike of trees such willow) and hazel, pollinated by the wind [26]. In a study on control crossing (hybridization), blooming branches of both the male and female clones of *Populus deltoides* were used [27]. To achieve artificial pollination, male catkins were removed along with pollen at the time of anthesis. At the stigma receptivity stage, all of the female clones were manually pollinated with each of the male clones. In another study, the vitality of the pollen utilized in controlled crossing was examined in-vitro. There was never a pollen mixture employed; each controlled cross only contained single pure pollen. The flowers were bagged and marked after pollination. The seeds ripe after controlled crosses were gathered and sown right away Singh, et al. [28]. Dhir and Mohn [29] observed that no seed set in the bagged inflorescence, indicate cross pollination in plants. Under cross pollination, the percentage of capsules and seeds set was quite high, however, led to a lower seed set than that open pollination. Figure 4 shows the mature fruit (Capsule) of *P. deltoids*.

Figure 4. Mature fruit (Capsule) of *P. deltoides*.

3.1. Vegetative Propagation

Low cut stumps from trees as old as 25 years have successfully sprouted. It is uncommon for root suckers to reproduce. Cuttings from one year old nursery trees are collected and stem growth are typically used in artificially propagating the species. Before out planting, these may or may not produce roots. In the North, the planting season of cuttings starts in the month of December to February. Commonly, rooted cuttings are employed in these circumstances [30]. Un-rooted cuttings sized between 30 and 50cm (12 and 20 inches) in length offer a reliable, affordable method of planting. Depending on the genetics of the clones and quality of the cuttings, the field circumstances and survival rates of 70% to 90% are typically attained [31].

Figure 5. Steps and methodology for conducing cross pollination in *P. deltoides.*

Normally, root-inducing hormones are not employed. Long cuttings, either rooted or un-rooted, areoccasionally utilized to access moisture, lessen deer damage, enable less intensive site preparation and provides flood tolerance. Rooting ability is very important because operational usage of Cottonwood asexual proliferation allows for the quick and complete utilization of superior genotypes [32].

Even though propagation from one year old trees is frequently challenging, some success is usually seen in this aspect. Re-propagation from the resultant material frequently yields positive results. Clones that may be traced back to older trees typically have the smooth, slightly thin bark that marks the crowns of those trees [33].

In addition to regeneration from seed, *Populus deltoids* are also capable of asexual or vegetative reproduction. Asexual reproduction is only encouraged by flood disturbances when dormant primordial in roots and shoots are triggered to create new shoots and roots by prolonged durations of submersion and/or mechanical damage to

parent plants. As a result, genotypes can stay on a location for far longer than individual trees. Furthermore, there is proof of cladoptosis, a process by which small shoots abscise and can be transported over great distances by water courses before eventually taking root [33]. Figure 5 shows the steps and methodology for conducing cross pollination in *P. deltoides*.

3.2. Cultivation

Full sunlight, humid air, and soil made up of silty or sandy loam are preferred. Young trees grow and develop fairly quickly. However, mature trees typically have a short life span. In the spring, brief flooding is accepted. The fragile and brittle wood of the limbs makes this tree susceptible to storm and ice damage. The cottony hairs of the seeds may be discharged by female trees in such large quantities that they can block air conditioner filters and gutters. Individual trees shouldn't be placed next to sewers or water lines since their spreading roots will always in search of water [34].

Fast growth, good form, and ease of vegetative propagation are only a few of the notable silvicultural traits of cultivated hybrids that have encouraged widespread production of cultivated Poplar plantations, mostly in Europe, North America and China. The European Black Poplar (*Populus nigra* L.) and the North American Cottonwood *P. deltoides*and *P. trichocarpa* are the two Poplar species that are widely utilized in breeding programmes in India to create hybrids [35].

A clone is the Poplar unit of cultivation & breeding and one clone typically represents one cultivar. To reduce the diversity in growth and wood quality within the plantation, Poplar plantations typically consist of just one cultivar or clone. They constitute a very small genetic basis dispersed over a very big area and they may greatly contribute to pollen and seed pools. The huge spread of a few narrow base cultivars is therefore the greatest concern in this situation [36].

3.3. Genetics and Tree Improvement

In India, 90% of the Poplar plantations are based on the clones G-48, D-121, S_7C_{15} and G-3. Due to the leaf blight disease's attack, the yield of clone G-3 is decreasing, which was once the most common clone. Other clones, including S_7C_8, Uday, L-34/82, etc., are taking its place. However, individual plantations are monoclonal [37]. In 1997, Forest Research Institute, Dehradun launched the National Poplar Improvement Programme with the goal of boosting poplar output [21]. The Forest Research Institute, Dehradun and fifteen research institutions, including universities and state forest departments, worked together on this programme [37].

There has been intra-specific hybridization from among top 40 clones. For field tests, 289 clones from control pollination and 111 from open pollination have been chosen. The Forest Research Institute, Dehradun has received seed from 104 candidate plus trees that are currently thriving in 44 natural groups in the USA. 100 clones have been chosen from the new germplasm for additional testing [38]. At Forest Research Institute, Dehradun, a novel method of simultaneous replication and multi-step selecting has been devised, allowing for the multiplication of improved clone material at 2 years of age. As a result, the procedure of clonal testing and multiplication takes 4 years less time [37].

To combine the fast growth of *P. deltoids* and the stress tolerance of *P. euphratica*, hybrid seedlings of *P. deltoides*' G-48' x *P. euphratica* have been created [39]. Wimco Seedling Ltd. registered six novel clones with the International Poplar Commission in Italy in 2000: WSL-22, WSL-27, WSL-32, WSL-39, WSL-A26 and WSL-A49. The Uttarakhand Forest Department has discovered more than twenty novel replicas that produce noticeably more fruit than clones that were previously planted. Poplar plantations will become more productive as more of these new clones are used [40].

The *P. deltoides* chromosomal number is 2n = BB. For study on trees using molecular genetics and genomics, the *Populus* genus has emerged as the standard [41]. Since each plant is either male or female, a significant number of hybrids are produced by its seeds [42]. Excellent options exist for development and selection with this character. For growing large-scale commercial plantations above 2B"N latitude, a substantial number of replicas have been examined, evaluated, and suggested in India. There are locations that are suitable for planting American selections (D-l2l, D-66, D-61, D-7b, D-181, D-100, $37C_2O$, S_7C_4, S_7C_8, SR-67 and Lux 69/55) as well as Australian selections (G-3 and G-48). The L series clones created at Haldwani, India from open-pollinated G-48 and D-l2l seeds have also shown excellent outcomes. At the State Forest Research Institute field zone in Tamil Nadu, 83 various clones were assembled [43]. Figure 6 shows the ariation in bud size among the different male and female clones of *P. deltoides*

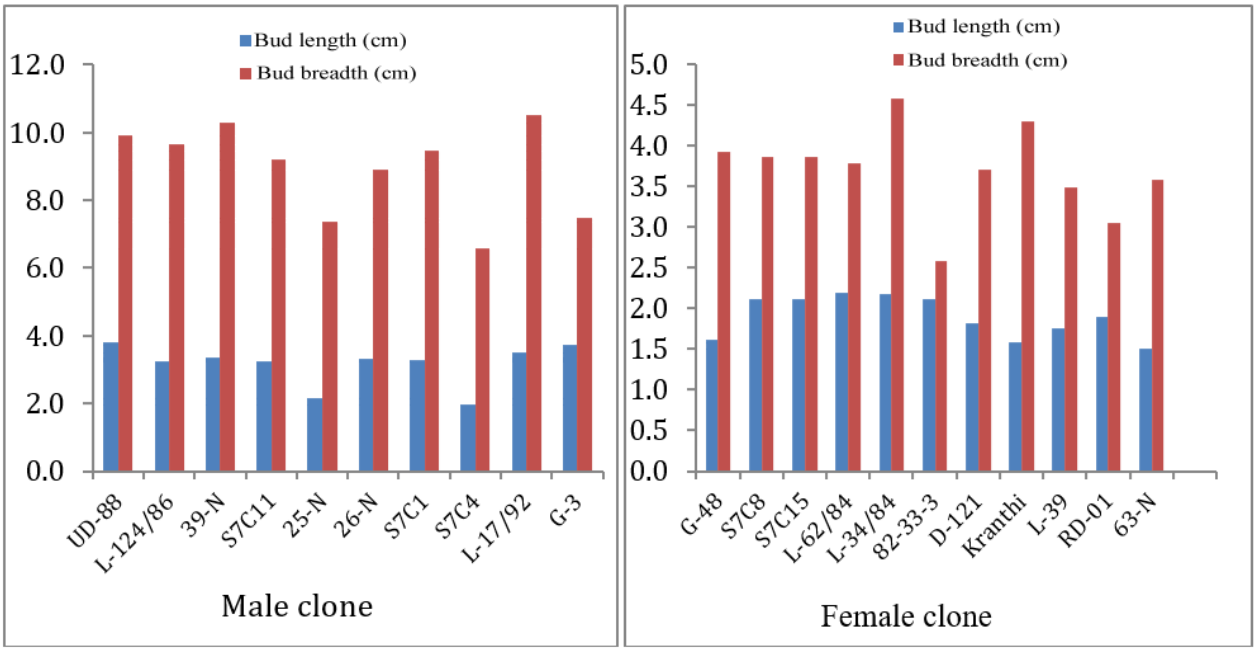

Figure 6. Variation in bud size among the different male and female clones of *P. deltoides*.

4. CONCLUSION

Our study presented the detailed information on the reproductive biology aspect of *Populus deltoids*, and this will be highly helpful in the cultivation, conservation, and genetic improvement of the species. *Populus deltoides* are mostly assessed for phenotypic selections, and the reproductive biology also offers a vast potentiality for improvement of the species. The controlled crossing in poplar can result in the production of quality and viable seedlings. Therefore, the elite genotypes/accessions of this species can be multiplied either by seed or vegetative means to get the quality planting material for the different afforestation programmes.

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Transparency: The authors state that the manuscript is honest, truthful, and transparent, that no key aspects of the investigation have been omitted, and that any differences from the study as planned have been clarified. This study followed all writing ethics.
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