



## CATKIN MATURATION TIMING AND SEED GERMINATION IN *BETULA UTILIS* (D. DON) IN THE WESTERN HIMALAYAN TREELINE AREA OF UTTARAKHAND

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### ABSTRACT

*B. utilis* is the only broad leaved angiosperm tree species in the Himalayas which dominates an extensive area at subalpine altitudes. Lack of sufficient regeneration is a major problem in *B. utilis* and has been declared endemic in many states of India. Very few studies on seeds and none on maturity indices of *B. utilis* have been conducted particularly in the treeline areas on Indian Himalayan region. The aim of the present study was to assess the seed/catkin maturity time and seed germination status of *B. utilis* in treeline areas. The study site was located at 30°49'22"N and 79°21'47"E between 3340 and 3560m elevation in the western Himalayan region of Uttarakhand. Physical parameters, catkin size, number of catkin, weight of catkin and mass of catkin were taken and germination was carried out in a dual chamber seed germinator for each collection date in laboratory. The tree density of *B. utilis* was 100 ind ha<sup>-1</sup>. The mean catkin size between first and last collection varied from 60.24±0.29 to 114.90±1.62, the change in catkin weight was 13.60 and 11.33 g during the collection period. The mass per 100 catkins during study varied between 16.30±0.74 and 33.27±0.14 g. Maximum germination 25.67±0.67% occurred when the catkin moisture content was 31.60±1.55%. At the treeline areas *B. utilis* is struggling for survival, growth and regeneration due to harsh climatic conditions and excessive anthropogenic pressure in the form of heavy grazing. The species requires proper conservation and management strategy, so that the seedlings and saplings could survive and replace adult trees in future. The present study is useful for assessing the exact catkin maturation time of the species for future multiplication of the species in nurseries.

**Keywords:** Anthropogenic Pressure, *B. utilis*, Germination, Seed Maturity, Treeline

### 1. INTRODUCTION

Himalayan birch or bhojpatra (*Betula utilis* D. Don) belongs to the family *Betulaceae* forms treeline vegetation all along the Himalayas and extensive stands of this species can be found in the cooler parts of the Northern Hemisphere [1]. *B. utilis* is widely distributed in Bhutan, China, Tibet, India and Nepal while the genus *Betula* is found in the northern parts of Europe, Asia, Siberia, Iran, Anatolia, north of Africa and Spain. It is the only broadleaved angiosperm tree species in the Himalayas which dominates an extensive area at subalpine altitudes [2]. Mature birch trees carry large amounts of female and male catkins and the flowers are monoecious which bloom in May-June. Staminate catkins are formed in late summer or autumn, remain naked during winter. Traditionally birch bark has been used for treating rheumatism, gout, malaria [3] by human being. Some of derivatives of betulinic acid also show high anti-HIV and antiviral activities [4].

The species has already been declared as critically endangered in Kashmir by Environmental Information System (ENVIS), Centre on Conservation of Medicinal Plants and Foundation for Revitalization of Local Health Traditions (FRLHT), Bengaluru [5] and as endemic in Arunachal Pradesh, Himachal Pradesh, Sikkim and Uttarakhand. The unscientific management of this species has caused loss of habitat in many of its native groves in the entire Himalayan range [6] as well as the massive overexploitation for fuel wood, fodder and medicines [7]. Lack of sufficient regeneration is a major problem of *B. utilis* in mountain forests [8]. Most studies on subalpine forests have reported poor seedling recruitment in understories of undisturbed old-growth forests [9, 10]. In life cycle of forest trees seed ripening is an important part. Seed maturation refers to the physiological and functional changes that occur from time of anthesis until the seeds are ready for harvest [11]. Maturity is the critical and the most important factor that determines the size and the quality of the seed. These characters could be

achieved with the seed if it is harvested at optimum stage, which is normally designated as the physiological maturity of the seed. Knowledge of the maturation process contributes to the establishment of the ideal time to harvest when seeds have a better physiological quality and a recommended way to study the effect of climatic variations on forest tree species [12]. Physiological maturation is the stage at which the seed attains the maximum dry weight that is accompanied with maximum seed and seedling quality characters in terms of seed germination and seedling vigour [13] and are widely indicated through physical indices such as size, colour and weight, which are much useful in perennial crops [14]. Change in seed maturity timing can influence regeneration, development and hence impact stand composition and structure [15].

The timing of seed germination plays critical roles in the survival and persistence of plants in natural ecosystems, because high seedling mortality is a major limitation in the treeline areas [16, 17]. Seed size or weight, reflects potential food reserves for seedling growth and are considered as important traits determining the successful establishment of individual plants [18]. A study by Lett S [19] found that birch seeds are viable for 2-3 years under field conditions but still lack of sufficient regeneration is a major problem of species in entire Himalayan region. Before 5-6 decades it was distributed over a wide range in the Himalayan range but now-a-days it is found only in a certain patches. The species needs special attention for its conservation and scientific management otherwise it will become extinct in near future. The seed maturation study of *B. utilis* in recent warming climatic condition in the treeline areas of Himalayan region is very less studied or untouched. The present study reports the catkin maturity time and seed germination status of *B. utilis* in treeline areas of Indian Himalayan regions.

## 2. MATERIAL AND METHODS

### 2.1. Study Site

The studied treeline site (Tungnath) 30°49'22"N latitude and of 79°21'47"E longitude occurs between 3340 and 3560m asl. The study site is situated in the sub-alpine zone, where snow cover remains till April. Soil is generally brown in colour, sandy loam in texture, with high proportion of sand and silt, and generally acidic with pH value 4 to 5 [20]. The mean annual temperature of the sites varied from -8.91 (January) and +25.6°C (May) and mean annual precipitation was 2410.5±432.2mm [21]. Anthropogenic disturbance in these treeline sites

mainly occurs in the form of migratory grazing and tourist activities [20].

### 2.2. Phytosociological analysis

The phytosociological analysis of treeline tree species was done by placing 40 quadrats of 10 x 10m in the study site. The size and number of the samples were determined following [22, 23]. The quadrats were laid randomly. The importance value index was determined as the sum of the relative frequency, relative density and relative dominance [24].

### 2.3. Maturity Indices

Twenty five trees were selected which had a clear bole, well developed crown, buttresses free with sufficient number of catkin and free from any disease in 1.5 ha area. Catkin collection of *B. utilis* was started from first week of October up to the availability of catkin till third week of November. The catkin collection was made directly from the tree and then seed germinated in the laboratory. For catkin physical parameters three replicate of 100 catkin each were taken and the different parameters includes catkin size (mm<sup>2</sup>) (length × width), number of catkin per 100g and weight of 100 catkin. For weight parameters, digital electronic balance (Model No. PGB 301 accuracy ±0.001 mg Wensar) and for the size, electronic digital vernier caliper (Model No. CD-6" accuracy ±0.02 mm Mitutoyo Co.) were used. For catkin mass the collected catkin were air-dried till the weight of air-dried catkin become constant [25]. For calculating moisture content of catkin three replicates of 25 catkin were taken for each collection date. The moisture content was expressed on fresh weight basis in which catkins were dried at 103±2°C for 16±1 hour and then reweighed [26].

Seeds were extracted from the catkin at each collection date. Germination was carried out in a dual chamber seed germinator for each collection date. The petri dishes and germination paper was sterilized at high temperature (130°C) for 4 hours to make it free from fungal infection. For germination, 4 replicates of 1 gm (4200 to 5500 seed per gram) seeds each were used [27]. The germination of seeds was carried out at 25±1°C on top of the paper in seed germinator under dark condition. Daily observation was taken and germination was counted when visible protrusion of radical (1 mm) occurred. Water was added as required during the experiment. After completion of experiment germination percent was calculated as the

total number of germinated seeds out of tested seeds within the test period, following [28].

#### 2.4. Statistical Test

The data were subjected to analysis of variance with a 95% confidence level using SPSS version 2016. Correlation coefficient was used for expressing relationship between different variables.

### 3. RESULTS

#### 3.1. Tree layer analysis

At studied treeline site, the total tree density was 760 ind ha<sup>-1</sup> and total basal area 54.31 m<sup>2</sup> ha<sup>-1</sup>. The tree density of *B. utilis* was 100 ind ha<sup>-1</sup> and sapling density was 90 ind ha<sup>-1</sup> while seedlings were completely absent. The total basal area of *B. utilis* was 3.35 m<sup>2</sup> ha<sup>-1</sup> and the IVI was 38.33. The density of associated species was between 60 and 260 ind ha<sup>-1</sup> while the total basal area ranged from 0.79 to 29.98 m<sup>2</sup> ha<sup>-1</sup>.

#### 3.2. Catkin colour

The catkin colour of *B. utilis* was dark green during the initial collection and changed to brown at final collection in both the years (Table 1).

#### 3.3. Catkin characteristics

The mean catkin size between first and last collection varied from 61.20±0.80 to 114.90±1.62 mm<sup>2</sup> in Yr1 and 60.24±0.29 to 111.48±4.32 mm<sup>2</sup> in Yr2 and the change was 53.70 mm<sup>2</sup> in Yr1 and 51.24 mm<sup>2</sup> in Yr2 across dates (Table 1). The weight of 100 catkins across collections ranged from 21.50±0.97 to 35.10±0.15 g in Yr1 and 22.40±0.21 to 33.73±1.32 g in Yr2 and the change was 13.60 g in Yr1 and 11.33 g in Yr2 during the collection period (Table 1). During the first collection the number of catkins per 100 g was 499.90±1.89 in Yr1 and 502.00±2.67 in Yr2 which gradually declined and at final collection it was 325.00±2.89 in Yr1 and 318.33±9.28 in Yr2 (Table 1). The catkin mass per 100 catkins from initial to final collection varied between 16.30±0.74 and 33.27±0.14 g in Yr1 and 16.52±0.67 and 33.12±0.77 g in Yr2 and the change was 16.97 g in Yr1 and 16.60 g in Yr2 across dates (Table 1).

**Table 1: Variations in physical attributes of catkins of *B. utilis* over the collection period in Yr1 and Yr**

Collection number	Collection date	Catkin colour	Catkin size (mm <sup>2</sup> )	Weight of 100 catkins (g)	Number of catkins/100 (g)	Mass of 100 catkins (g)
<b>Yr 1</b>						
I <sup>st</sup>	II <sup>nd</sup> week of October	DG	61.20±0.80	21.50±0.97	499.90±1.89	16.30±0.74
II <sup>nd</sup>	III <sup>rd</sup> week of October	DG	77.80±6.24	21.50±0.97	498.89±2.65	17.16±0.78
III <sup>rd</sup>	IV <sup>th</sup> week of October	DG	81.06±2.00	23.14±0.34	466.67±3.33	19.13±0.29
IV <sup>th</sup>	I <sup>st</sup> week of November	BG	108.56±2.25	30.85±0.23	393.33±3.33	27.38±0.24
V <sup>th</sup>	II <sup>nd</sup> week of November	B	110.19±2.81	31.97±0.73	350.00±5.77	29.67±0.68
VI <sup>th</sup>	III <sup>rd</sup> week of November	B	114.90±1.62	35.10±0.15	325.00±2.89	33.27±0.14
<b>Yr 2</b>						
I <sup>st</sup>	I <sup>st</sup> week of October	DG	60.24±0.29	22.40±0.21	502.00±2.67	16.52±0.67
II <sup>nd</sup>	II <sup>nd</sup> week of October	DG	80.17±5.74	22.90±0.49	483.33±8.82	17.58±1.42
III <sup>rd</sup>	III <sup>rd</sup> week of October	DG	83.33±2.67	23.38±0.58	456.67±8.82	19.93±0.32
IV <sup>th</sup>	IV <sup>th</sup> week of October	BG	105.89±0.43	28.95±0.89	406.67±12.0	25.93±0.22
V <sup>th</sup>	I <sup>st</sup> week of November	B	107.85±4.89	30.63±0.90	360.00±5.77	29.60±0.65
VI <sup>th</sup>	II <sup>nd</sup> week of November	B	111.48±4.32	33.73±1.32	318.33±9.28	33.12±0.77

DG = Dark Green, BG = Brownish Green and B = Brown

#### 3.4. Germination

In both the years the seeds of *B. utilis* failed to germinate for the first four collections. The germination started from fifth collection. During fifth and sixth collection the mean germination was 13.67±0.88 and 23.33±0.88% in Yr1 and 17.00±1.15 and 25.67±0.67% in Yr2. The moisture content was 31.60±1.55 and 37.19±0.90% in Yr1 and 32.06±2.42 and 39.21±3.33% in Yr2 during fifth and sixth collection. *B. utilis* attained maturity in the

second week of November (Fig. 1). ANOVA showed that the catkin size, weight of 100 catkins, number of catkins per 100 g, mass of 100 catkins, moisture content of catkins and seed germination varied significantly across dates ( $p < 0.05$ ), whereas seed germination varied significantly across year ( $p < 0.05$ ), but not with other catkin parameters. The interactions between year × dates varied significantly with germination at 95% confidence level ( $p < 0.05$ ) (Table 2).

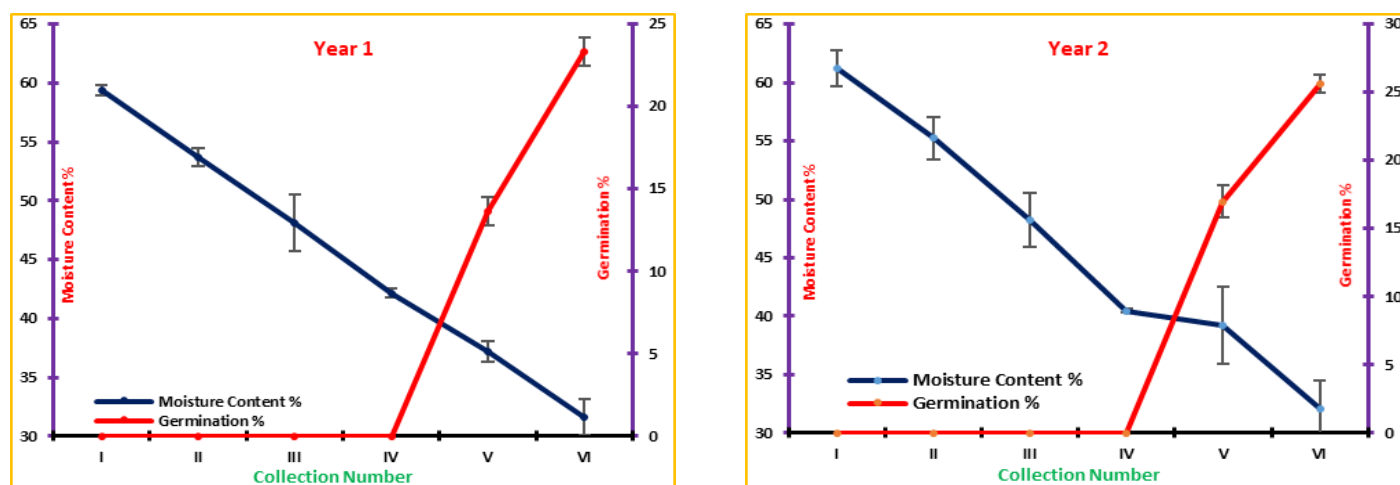


Fig. 1: Relationship between moisture content of catkin and germination of seeds of *B. utilis* over the collection dates in Yr1 and Yr2

Table 2: Analysis of variance (ANOVA) for different catkin attributes of *B. utilis* across collection dates and years

ANOVA				
Parameter	Source of variation	DF	Mean square	F-value
Catkin size	Years	1	5.62	0.158 <sup>NS</sup>
	Dates	5	2657.29	74.808 <sup>**</sup>
	Years × Dates	5	9.65	0.272 <sup>NS</sup>
Weight of 100 catkins	Years	1	1.06	0.646 <sup>NS</sup>
	Dates	5	173.10	105.35 <sup>**</sup>
	Years × Dates	5	2.81	1.715 <sup>NS</sup>
Number of catkins/100 (g)	Years	1	25.00	0.207 <sup>NS</sup>
	Dates	5	33060.00	273.600 <sup>**</sup>
	Years × Dates	5	205.00	1.697 <sup>NS</sup>
Mass of 100 catkins	Years	1	0.01	0.009 <sup>NS</sup>
	Dates	5	293.65	218.09 <sup>**</sup>
	Years × Dates	5	0.89	0.66 <sup>NS</sup>
Moisture content of catkins	Years	1	2.850	0.301 <sup>NS</sup>
	Dates	5	572.44	60.533 <sup>**</sup>
	Years × Dates	5	3.39	0.359 <sup>NS</sup>
Seed germination	Years	1	8.02	9.633 <sup>**</sup>
	Dates	5	685.09	822.113 <sup>**</sup>
	Years × Dates	5	3.36	4.033 <sup>**</sup>

NS = Non-Significant, \*\* = Significant at 5% ( $P < 0.05$ )

A significant positive co-relation existed between catkin size and weight of 100 catkins ( $r = 0.904$ ), catkin size and mass of 100 catkins ( $r = 0.890$ ), catkin size and seed germination ( $r = 0.658$ ), weight of 100 catkins and mass of 100 catkins ( $r = 0.960$ ), weight of 100 catkins and seed germination ( $r = 0.801$ ), number of catkins per 100 g and moisture content of catkins ( $r = 0.915$ ) and mass of 100 catkins and seed germination ( $r = 0.821$ ) at 95% confidence level ( $p < 0.05$ ). A significant negative co-relation was found between catkin size and number of

catkins per 100 g ( $r = 0.892$ ), catkin size and moisture content ( $r = 0.933$ ), weight of 100 catkins and number of catkins per 100 g ( $r = 0.957$ ), weight of 100 catkins and moisture content of catkins ( $r = 0.905$ ), number of catkins per 100 g and mass of 100 catkins ( $r = 0.959$ ), number of catkins per 100 g and seed germination ( $r = 0.861$ ), mass of 100 catkins and moisture content of catkins ( $r = 0.877$ ) and moisture content of catkins and seed germination ( $r = 0.713$ ) at 95% confidence level ( $p < 0.05$ ) (Table 3).

**Table 3: Co-relations between different catkin parameters and seed germination of *B. utilis***

Co-Relations						
Parameters	Catkin size	Weight of 100 catkins	Number of catkins/100 (g)	Mass of 100 catkins	Moisture content	Seed Germination
Catkin size	1	<b>0.904**</b>	-0.892**	<b>0.890**</b>	-0.933**	<b>0.658**</b>
Weight of 100 catkins		1	-0.957**	<b>0.960**</b>	-0.905**	<b>0.801**</b>
Number of catkins/100 (g)			1	-0.959**	<b>0.915**</b>	-0.861**
Mass of 100 catkins				1	-0.877**	<b>0.821**</b>
Moisture content					1	-0.713**
Seed Germination						1

\*\*Co-relation is significant at the 0.05 level ( $p < 0.05$ ).

#### 4. DISCUSSION

The regeneration of a forest is a vital process in which old trees die and are replaced by young ones in perpetuity. The entire Himalayan treeline forest species face a major problem of lack of regeneration which may further aggravate in a warming climate. In the treeline areas *B. utilis* is facing survival problem due to severe anthropogenic pressure and harsh climatic conditions [29]. Disturbance in the form of grazing, especially by cattle (buffalo and cow), horse and mule, has negative effect on tree regeneration and growth of this species [20, 30]. The seeds of *B. utilis* are viable for 2-3 years under field conditions but still it is facing a major regeneration problem and facing the threat of extinction. In Himalayan treeline region there are many forest tree species in which seed maturation is synchronized with the commencement of monsoon, and their seed viability is very low. Low germination of small seed such as of *Betula* and *Rhododendron* species under low light and high humidity conditions prevail in such closed stands [31], low light reduces seedling emergence of birch (*Betula* species) by 43%, seedling growth by 99%, and survival by 94% [32]. According to [33] lack of birch regeneration can be attributable to poor seedling growth and survival rather than inadequate seed dispersal.

Several workers throughout the world have investigated colour as a workable indicator for several species [15, 34]. Distinct colour changes have been associated with seed maturity in hard wood fruits. In the present study colour change of *B. utilis* catkins from dark green to brown was found to be a good maturity indicator. Distinct colour change have always been associated with attainment of maturity, the main reason is that other indices offer no advantages over this easily observed indicator [35]. A number of researchers observed that the colour change has always been recommended as ripeness indicator in many species viz, *Prunus serotina* by [36], *Acer*

*oblongum* by [37], *S. robusta* by [38], *M. esculenta* by [35, 39], *Prunus cerasoides* by [40, 41], *Q. leucotrichophora* by [12, 15, 34]. After fruit/seed colour, weight and mass of fruit/seed have been associated with maturation time. The species attain maximum weight or mass at the time of maturation. In the present study during the catkin maturity and maximum seed germination time the weight of 100 catkin was 35.10 g in Yr1 and 33.73 g in Yr2 and mass of 100 catkin was 33.27 g in Yr1 and 33.12 g in Yr2. [42] in *Q. semecarpifolia* have reported weight of 100 acorn was 332.7 g at the time maximum seed germination. Similarly, in studies by [43] for *C. macrophylla* maximum seed germination was observed when the weight of 100 fruits was 21.03 g and weight of 100 seeds was 16.33 g.

Moisture plays a vital role throughout the life of seeds, change in fruit and seed moisture content were strong manifestations that ripeness is progressing [42]. As for fleshy fruits, increase in moisture is accompanied with ripening, while maturing dry fruits lose moisture [43]. Decline in moisture content in maturing seeds is closely related to seed maturity [35]. In the present study, at the time of maximum germination (23.33% in Yr1 and 25.67% in Yr2) of seed was in the second week of November in Yr1 and third week of November in Yr2, the moisture content of catkin ranged from 31 to 32%. [44] in *P. cerasoides* have reported seed moisture content between 29.8% and 34.13% as reliable indicator of maturity. Similarly, in studies by [15, 34] for *Q. leucotrichophora* acorns maximum germination was observed when the acorn moisture content was between 36 to 37%. The onset of moisture loss triggers a decline in the physiological activity of maturity cotyledons as well as embryo before the seed falls [38, 45]. Negative correlation existed between seed germination and seed moisture content ( $P < 0.05$ ).

## 5. CONCLUSION

The seed germination in this treeline species is low. In such a case knowledge of the exact time of seed/catkin maturation is essential for future multiplication of the species and undertaking massive afforestation/reforestation programmes of the species in the Himalayan region. The study can be useful for undertaking such programmes that will avoid large scale failure of such plantation drives due to collection of immature catkins.

## 6. ACKNOWLEDGEMENT

The authors are thankful to National Mission on Himalayan Studies (NMHS), Ministry of Environment, Forest and Climate Change, Government of India, for providing financial support.

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