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Cone maturation timing and seed germination in *Pinus roxburghii* (Serg.) in the central Himalayan region of Uttarakhand, India

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ABSTRACT

The Himalayan forests are severally affected by several biotic and abiotic disturbance. These disturbances are affecting the stability of the ecosystem and the recurrent fire and grazing are adversely affecting the regeneration of tree species, seed maturation process which contribute to the establishment of the ideal time to harvest when seeds have a better physiological quality for germination. The study site was located at 29°23′15″ N and of 79°30′38″ E between 1720 and 1810 msl elevation in the central Himalayan region of Uttarakhand. Germination was carried out in a dual chamber seed germinator for each collection date in laboratory. The tree density of *Pinus roxburghii* was 295 and 855 ind ha^{-1}. The mean cone size between first and last collection varied from 74.92±2.8 and 202.1±5.7cm^2. The weight of 100 seeds between first and final collection was 8.34±0.05 to 12.84±0.09g. Maximum germination 81.3±1.45% occurred when the seed moisture content was 21.67±0.58%. The natural regeneration of this species is good but the fire is the main cause of death of seedlings.

Key words: *Density, Germination, Himalaya, P. roxburghii, Seed Maturity*

Introduction

P. roxburghii is a principle forest forming tree species of Central Himalaya. Pine forests are found all along the Himalaya with the exception of Kashmir valley between altitudes of 1000 and 1800m and are classified as low montane needle leaf forest with concentrated summer leaf drop (Singh and Singh, 1992). Pine were harvested on a large scale in the 1960s and 1970s for timber and other industrial raw materials and thereafter the continued disturbances, either geological or anthropogenic, are severely threatening the biological diversity (Kumar and Ram, 2005). *P. roxburghii* forests provide a valuable resource for paper and resin-based industries. The pine forests have witnessed severe anthropogenic disturbances. The disturbances were mainly in the form of deforestation, animal grazing, lopping, surface burning and litter removal (Singh *et al.*, 2014). These continued disturbances are affecting the stability of the ecosystem and the recurrent fire and grazing are adversely affecting the regeneration of tree species as a result large forest gaps with stunted trees are the characteristics features of these forests.

In life cycle of forest trees seed ripening is an important part and seed maturity is the critical and the most important factor that determines the size and the quality of the seed (Mittal *et al.,* 2017). Knowledge of the maturation process contributes to the establishment of the ideal time to harvest when seeds have a better physiological quality (Bhatt and Ram, 2015). 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 (Dayal *et al.,* 2017) and are widely indicated through physical indices such as size, colour and weight, which are much useful in perennial crops (Srimathi *et al.,* 2013). Change in seed maturity timing can influence regeneration, development and hence impact stand composition and structure (Tewari *et al.,* 2017). The timing of seed germination plays critical role in the survival and persistence of plants in natural ecosystems, because high seedling mortality is a major limitation in Himalayan forest (Lett and Dorrepaal, 2018). The present study reports the cone maturity time and seed germination status of *P. roxburghii* in the central Himalayan regions.

Materials and Methods

Study site: Three sites of *P. roxburghii* dominated forests were selected in the Nainital forest division of Kumaun Central Himalaya. The study sites were located between $29^{0}23'15''$N and $79^{0}30'38''$E at an elevation ranging between 1720 and 1810 msl. Mean annual temperature was 15.2 ^{o}C and annual precipitation was 2258 mm of which two third occurred during rainy season. In spite of high-annual rainfall, summer and winter are relatively dry, generally with < 10 cm monthly rainfall. Winter snowfall is frequent above 2000 m elevation.

Phytosociological analysis: The phytosociological analysis of tree species was done by placing 30 quadrats of 10 x 10m in the study site. The size and number of the samples were determined following Saxena and Singh (1982) and Singh *et al.* (2014). The importance value index was determined as the sum of the relative frequency, relative density and relative dominance (Ambasht and Ambasht, 2002).

Maturity Indices: Twenty trees were select which had a clear bole, well developed crown, with sufficient number of cone. Cone collection of *P. roxburghii* was started from first week of March up to the availability of cone till first week of May. The cone collection was made directly from the tree and seeds extracted germinated in the laboratory. For cone and seed physical parameters three replicate of 10 cone and 100 seed each were taken and the different parameters includes cone and seed size (mm^2) (length ×width) and weight of 10 cone and 100 seed. 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.) was used. For calculating moisture content of cone and seed three replicates of 25 cone and seed were taken for each collection date. The moisture content was expressed on fresh weight basis in which cone and seed were dried at 103±2°C for 16±1 hour and then reweighed (ISTA, 1981).

Seeds were extracted from the cone at each collection date. Germination was carried out in a dual chamber seed germinator for each collection date. The petridishes and germination paper was sterilized at high temperature (130^{o}C) for 4 hours to make it free from fungal infection. For germination 4 replicates of 100 seeds each were used (Tamta and Singh, 2018). The germination of seeds was carried out at 25±1^{o}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 (1mm) 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 Paul (1972).

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.

Results

Tree layer analysis: At studied sites the total tree density varied between 295 and 855 ind ha^{-1} and total basal area ranged from 43.76 to 69.14 m^2 ha^{-1}. The tree density of *P. roxburghii* ranged between 220 and 480 ind ha^{-1}, sapling density ranged from 62 to 120 ind ha^{-1} and seedlings density 320 to 758 ind ha^{-1}. The total basal area of *P. roxburghii* varied between 41.28 and 45.58 m^2 ha^{-1} and IVI was 112.45 to 232.65. The density of associated species was between 25 and 180 ind ha^{-1} while the total basal area ranged from 18.92 to 66.18 m^2 ha^{-1}.

Cone characteristics: ANOVA showed that the cone size, weight of 10 cones and cone moisture content varied significantly across the dates at 0.05 significant level ($p<0.05$). The cone colour of *P. roxburghii* during the first collection was yellowish brown and at final collection it changed to dark brown. The mean cone size between first and last collection varied between 74.92±2.8 and 109.94±1.84cm^2 in Yr1 and 125.39±1.2 and 202.1±5.7cm^2 in Yr2 and the change was 35.02±5.84cm^2 in Yr1 and 76.71±3.76cm^2 in Yr2 across collection dates. Weight of 10 cones varied between 152.3±0.88 to 165.3±0.8g in Yr1 and 151.0±1.7g to 170.0±0.58g in Yr2 and the changes was 13.0±0.89g in Yr1 and 19.0±0.86g in Yr2 during the collection period (Table 1).

Seed Characteristics

ANOVA showed that seed size, weight of 100 seeds, number of seeds in 100g, moisture content and germination varied significantly across dates of collection ($p<0.05$) but did not vary significantly across two years of study. The interaction between Yr × dates was non-significant for seed size, weight of 100 seeds, number of seeds in 100g, moisture content and germination. The seed color was grayish brown during the first collection and dark brown at last collection in both the Yrs. The mean seed size between first and last collection varied between 0.61±0.01 and 0.69±0.01mm^2 in Yr1 and 0.59±0.01 and 0.71±0.01mm^2 in Yr2 and the changes was 0.8±0.01mm^2 in Yr1 and 0.12±0.03mm^2 in Yr2. The mean weight of 100 seeds ranged from 8.34±0.05 to 12.84±0.09g in Yr1 and 8.72±0.21 to 12.74±0.04g in Yr2 the changes was 4.5±0.82g in Yr1 and 4.02±0.46g in Yr2 during the collection period (Table 2).

A significantly positive correlation was found between seed size, weight of 100 seeds and germination percent of seeds; weight of 100 seeds and germination percent of seeds ($p<0.01$). A significant negative correlation was found between moisture content and germination; seed size and weight of 100 seeds.

Germination

During the first collection the mean germination was 29.0±0.58% in Yr1 and 31.0±0.5% in Yr2 which gradually increased with each collection date. The maximum germination was observed 81.3±1.45% in Yr1 and 79.0.0±1.7% in Yr2 during the fifth collection. The moisture content of seeds across all collection dates varied from 21.67±0.58 and 31.67±0.7% in Yr1 and 22.33±0.3 and 33.67±0.6% in Yr2. The maximum germination occurred when the seed moisture content was 21 and 22% across the year. *P. roxburghii* attained maturity in the first week of May (Fig. 1).

Discussion

The regeneration of different plant species through

Table 1. Variations in physical attributes of *P. roxburghii* cones over the collection period

Collection Numbers	Collection Dates	Yr1		Yr2	
		Weight of 10 cone (g)	Cone Size (cm^2)	Weight of 10 cone (g)	Cone Size (cm^2)
I	Ist week of March	152.3±0.88	74.9±2.89	151.0±1.73	125.4±1.29
II	IIIrd week of March	153.0±1.73	91.1±2.68	153.7±1.20	151.7±2.49
III	Ist week of April	156.3±1.20	95.0±2.83	159.0±0.58	167.3±2.99
IV	IIIrd week of April	160.6±0.88	102.1±2.32	164.3±2.03	180.9±5.0
V	Ist week of May	165.3±0.88	109.9±1.84	170.0±0.58	202.1±5.7

Table 2. Variations in physical attributes of *P. roxburghii* seeds over the collection period

Collection Numbers	Collection Dates	Yr1		Yr2	
		Seed Size (mm^2)	Wt. of 100 Seeds (g)	Seed Size (mm^2)	Wt. of 100 Seeds (g)
I	Ist week of March	0.61±0.01	8.34±0.05	0.59±0.01	8.72±0.21
II	IIIrd week of March	0.61±0.01	9.42±0.03	0.64±0.01	9.84±0.19
III	Ist week of April	0.62±0.02	11.20±0.35	0.64±0.02	10.92±0.30
IV	IIIrd week of April	0.64±0.01	12.35±0.34	0.70±0.01	12.20±0.21
V	Ist week of May	0.69±0.01	12.84±0.09	0.71±0.01	12.74±0.04

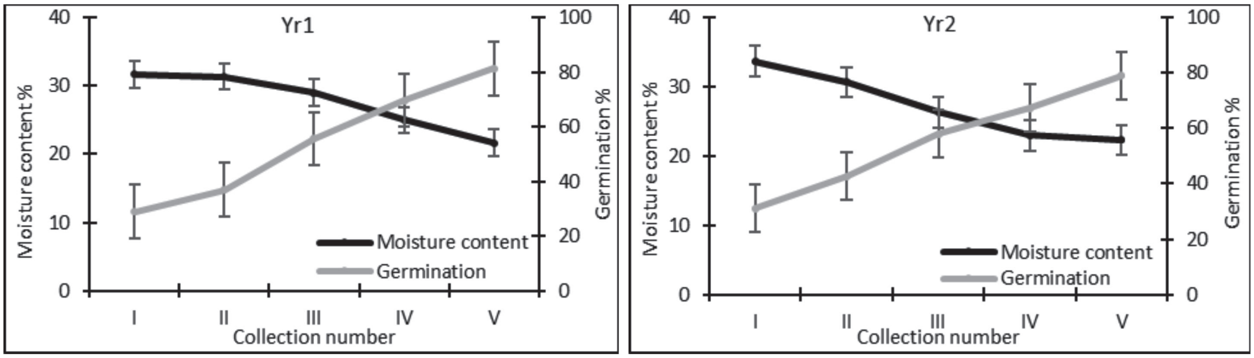

Fig. 1. Effect of moisture content on germination of seeds *P. roxburghii* over the collection period

seeds depends upon the production and germination of the seeds and the successful establishment of the seedlings (Ghildiya *et al.*, 2009). Seed collectors have long been aware that most mature and immature fruits and seeds can be distinguished in a numbers of ways like by colour difference, increased firmness or brittleness, decreased moisture content and specific gravity or by change in physical dimensions (Edwards, 1980; Shah, 2005; Tewari *et al.*, 2017 and Tamta and Singh, 2018). Physical indices like colour change have been widely used as a workable indicator of maturity for several species. In the present study colour change from yellowish brown to dark brown of cone and grayish brown to dark brown of seed in *P. roxburghii* 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 (Tewari, 2005; Mittal *et al.*, 2017). Several authors have observed that the colour change has always been recommended as ripeness indicator in many species such as *Fraxinus pennsylyanica* (Bonner, 1973), *Acer oblungum*, *Kydia calyciana, Terminalia belerica*, *T. chebula* (Negi and Todaria, 1995) Ficus species (Rai *et al.*, 1988), *Prunus cerasoides* (Tewari, 2005), *Mallotus phillipensis* (Tewari *et al.*, 2016), *Q. leucotrichophora* (Tewari *et al.*, 2017; Tamta and Singh, 2018) and *Q. semecarpifolia* (Tewari *et al.*, 2019).

Moisture plays a critical role in maturation and change in fruit/seed moisture content are strong manifestations that ripeness is progressing (Rediske, 1961). Loss of moisture during maturity is a more inherent phase of seed development than is implied by passive concept of seed drying. Decline in moisture content in maturing seeds is closely related to seed maturity (Shah *et al.*, 2010). In the present study seed moisture content also emerged as a good indicator of seed maturity. In *P. roxburghii* the seed moisture was 21 and 23% coincided with maximum germination. As for fleshy fruits, increase in moisture is accompanied with ripening, while maturing dry fruits lose moisture (Mittal *et al.*, 2017). According to Edward (1980) pine attained maturity is generally reached when moisture content of cone is below 50%. Tewari *et al.* (2011) also reported seed moisture content between 29.8±1.35 and 34.13±1.50% as maturity indicator in *P. cerasoides*. Shah *et al.* (2006) stated that moisture content of 23.4-36.1% can be associated with optimum germination of *Pyracantha crenulata*. Edwards (1980), Shah (2005), Tewari (2005) reported a significant correlation between maturity and physical parameters, but there are also many unsuccessful attempts. Decline in fresh weight moisture content from maturity seed is closely related to seed maturity (Pandit *et al.*, 2002). It can be concluded from the present study that best germination of *P. roxburghii* seeds occur when the seed moisture content is between 21.67 and 31.67%. The change in cone colour from yellowish brown to dark brown is also a useful indicator of cone maturity. The germination of seeds and natural regeneration of this species is good but the fire is the main cause of death of seedlings. In such cases knowledge of the exact time of cone/seed maturation is essential for future multiplication of the species in the Himalayan region.

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