

Seed Germination and Seedling Growth in *Pinus kesiya* Royle ex Gord. I. Influence of Imbibition, Substrate pH and Moisture Level

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Imbibition of *P. kesiya* seeds at varying temperatures showed better seed germination and seedling growth at 5°C. A 24-hr duration was found most favourable. At pH 6.0 and 6.4, early germination was recorded; however total germination was higher at pH 4.8. The water potential of -2.5 bars stimulated seed germination and seedling growth. Higher water potentials were found inhibitory.

Key Words: *Pinus kesiya*, Seed germination, Imbibition, Water potential, pH

Introduction

Substrate moisture level (Satoo 1966, Larson & Sehubert 1967) and pH (Vaartaja 1957, Maull 1963) are two important soil factors which play an important role during germination and early seedling survival. The stratification by soaking the seeds at low temperatures influences the germination in many pines (Barnett 1971). The present investigation is aimed at understanding the requirement of imbibition, substrate moisture level and pH during germination and early seedling establishment in *P. kesiya*, — an early successional species in the sub-tropics of Khasi Hills, growing at an altitude of 1311 m (25° 40'N latitude and 91° 35' E longitude).

Material and Methods

Mature cones of *Pinus kesiya* Royle ex Gord. were collected from university campus, Shillong during February-March, 1982. The seeds were extracted by air drying and stored in sealed plastic bags at room temperature in dark.

Seeds were washed thoroughly with tap water. The surface sterilization with 0.1% mercuric chloride for 30 min was found satisfactory. The seeds were then washed repeatedly with sterilized distilled water to remove the disinfectant. Viability of the seeds was tested with 0.1%, 2, 3, 5-triphenyl tetrazolium chloride after puncturing the seed coat with the help of a needle.

Seeds were imbibed at different temperatures viz., 5, 15, 25, and 35° C for 24 hr. In another set of experiment, the seeds were imbibed at 5°C for varying duration from 0-96 hr in dark. Unimbibed seeds were treated as control. For pH studies, petridishes with unimbibed sterilized seeds were supplied with acidic water (pH 4.8-6.8). In all the above mentioned treatments, the seeds were germinated at $25 \pm 2^\circ\text{C}$ in dark.

For water stress studies, water potential from 0 to -15.0 bars was created with sucrose solutions and seeds were germinated at 20°C under 25 lux of cool fluorescent light for 16 hr-days.

Each petridish (9 cm diameter) containing sterilized filter paper was supplied with 2.0 ml of respective solutions at 2-d interval. For all treatments four petridishes with 20 seeds each were used and the experiments were repeated twice. Seeds were considered as germinated when radicle protruded about 1 mm beyond the seed coat. Germination was recorded from 6th to 20th day at 2-d interval. The germination percentage was calculated as seeds germinated $\times 100$ /potentially germinable seeds and the data were analysed after angular transformation. Germination rate and days to peak germination were calculated by the formulae of Campbell and Sorensen (1979) as given below.

$$\text{Germination rate} = \frac{\sum n}{\sum nd}$$

$$\text{Days to peak value} = \frac{\sqrt{\mu^2 + 8\delta^2} - \mu}{4\delta^2}$$

where n is the number of germinated seeds; d , the days to germination from initial counting; μ , the rate mean; and δ , the rate standard deviation.

Growth parameters like length and biomass were recorded on 20th day of seedling growth. Biomass was estimated by oven drying the materials for 48 hr at 55-60°C. Statistical

analyses were performed for standard deviation and analysis of variance following the method of Snedecor (1961).

Results

The seeds imbibed at 5°C showed better germination percentage and rate as compared to control and seeds imbibed at higher temperatures. The imbibition of seeds at 5°C also resulted in maximum length of seedling parts and their biomass (table 1). Imbibition for various periods at 5°C indicated that 24 hr duration was optimum for germination percentage and rate. However, the growth of seedling was almost equal in seeds imbibed for 12 and 24 hr at 5°C except radicle length (table 2).

Maximum percentage germination resulted in seeds subjected to pH 4.8 (table 3). However, the pH of 6.0 and 6.4 resulted in early germination. As compared to control, the length of various seedling parts decreased at pH 4.8 treatment and showed a slight increase at pH 6.0 and above. The biomass variations of said seedling parts also showed similar pattern.

A water potential of -2.5 bars resulted in maximum germination and seedling growth (table 4). As compared to control, at -10.0 bars water potential, approximately decreases of five-fold in germination percentage and twice in rate were observed. Higher water potentials were inhibitory to germination and growth.

Discussion

Seed germination and seedling growth of *P. kesiya* were greatly improved by imbibing the seeds in water at 5°C. To improve seed germination, the soaking of seeds has been tried by many workers. Barnett (1971) reported that imbibition at 5°C stimulated seed germination in southern pines. Paul et al. (1973) observed induction of seed

Table 1 *Effect of imbibition at different temperatures on seed germination and seedling growth in Pinus kesiya*

| Temperature (°C) | Germination | | | Growth/seedling or seedling part | | | | | | | |
|------------------|-------------|-----------------|-------------------|----------------------------------|---------|-----------|--------------|---------|-----------|-------|----------|
| | % | Rate (unit/day) | Day to peak value | Length (mm) | | | Biomass (mg) | | | | |
| | | | | Hypocotyl | Radicle | Cotyledon | Hypocotyl | Radicle | Cotyledon | Shoot | Seedling |
| Control | 57.00 | 0.217±0.116 | 3.28 | 50.15 | 18.26 | 16.90 | 2.92 | 0.60 | 0.34 | 5.60 | 6.20 |
| 5 | 92.70 | 0.541±0.087 | 1.76 | 53.82 | 21.35 | 19.65 | 3.31 | 0.71 | 0.41 | 6.16 | 6.87 |
| 15 | 85.15 | 0.430±0.187 | 1.80 | 51.08 | 19.35 | 17.76 | 3.23 | 0.62 | 0.36 | 5.76 | 6.38 |
| 25 | 75.93 | 0.332±0.029 | 2.97 | 49.17 | 17.93 | 16.79 | 2.89 | 0.55 | 0.36 | 5.31 | 5.86 |
| 35 | 70.95 | 0.217±0.116 | 3.28 | 47.23 | 17.75 | 16.65 | 2.82 | 0.55 | 0.30 | 4.91 | 5.46 |
| L.S.D. (P=0.05) | | | | 3.86 | 2.97 | 1.97 | 0.97 | 0.16 | 0.11 | 1.21 | 0.73 |

±Standard deviation

Table 2 *Effect of imbibition period at 5°C on seed germination and seedling growth in Pinus kesiya*

| Imbibition period (hr) | Germination | | | Growth/seedling or seedling part | | | | | | | |
|------------------------|-------------|-----------------|--------------------|----------------------------------|---------|-----------|--------------|---------|-----------|-------|----------|
| | % | Rate (unit/day) | Days of peak value | Length (mm) | | | Biomass (mg) | | | | |
| | | | | Hypocotyl | Radicle | Cotyledon | Hypocotyl | Radicle | Cotyledon | Shoot | Seedling |
| 0 | 57.00 | 0.217±0.116 | 3.28 | 50.15 | 18.26 | 16.90 | 2.92 | 0.60 | 0.34 | 5.60 | 6.20 |
| 6 | 89.10 | 0.433±0.119 | 2.04 | 54.69 | 21.75 | 19.53 | 3.38 | 0.76 | 0.36 | 5.82 | 6.58 |
| 12 | 91.70 | 0.460±0.132 | 1.90 | 59.96 | 25.46 | 19.67 | 3.40 | 0.79 | 0.41 | 6.15 | 6.94 |
| 24 | 92.70 | 0.541±0.087 | 1.76 | 60.03 | 29.25 | 20.26 | 3.42 | 0.86 | 0.40 | 6.26 | 7.12 |
| 48 | 87.10 | 0.295±0.027 | 3.33 | 48.12 | 20.29 | 17.73 | 3.36 | 0.74 | 0.39 | 5.92 | 6.66 |
| 96 | 60.24 | 0.200±0.054 | 4.43 | 43.08 | 20.26 | 16.16 | 3.21 | 0.69 | 0.36 | 5.60 | 6.29 |
| L.S.D. (P=0.05) | | | | 3.16 | 2.13 | 2.78 | 0.17 | 0.19 | 0.09 | 0.09 | 0.73 |

±Standard deviation

Table 3 Effect of pH on seed germination and seedling growth in Pinus kesiya

| pH | Germination | | | Growth/seedling or seedling part | | | | | | | |
|--------------------|-------------|--------------------|-----------------------------|----------------------------------|---------|----------------|----------------|---------|----------------|-------|----------|
| | % | Rate (unit/day) | Days to peak value | Length (mm) | | | Biomass (mg) | | | | |
| | | | | Hypo- cotyl | Radicle | Cotyle- don | Hypoco- tyl | Radicle | Cotyle- don | Shoot | Seedling |
| Control (7.0) | 79.24 | 0.217±0.116 | 3.28 | 50.15 | 18.26 | 16.90 | 2.92 | 0.60 | 0.34 | 5.60 | 6.20 |
| 4.8 | 90.30 | 0.345±0.101 | 2.52 | 41.17 | 13.48 | 14.66 | 2.60 | 0.39 | 0.31 | 4.70 | 5.09 |
| 5.2 | 84.10 | 0.401±0.051 | 2.52 | 43.07 | 15.29 | 14.77 | 2.60 | 0.43 | 0.34 | 5.15 | 5.58 |
| 5.6 | 82.20 | 0.412±0.092 | 2.21 | 43.67 | 16.99 | 15.84 | 2.77 | 0.43 | 0.35 | 5.15 | 5.58 |
| 6.0 | 80.10 | 0.416±0.139 | 2.02 | 46.08 | 17.14 | 16.10 | 2.79 | 0.47 | 0.35 | 5.15 | 5.72 |
| 6.4 | 80.52 | 0.432±0.127 | 2.01 | 46.61 | 17.82 | 16.10 | 2.70 | 0.47 | 0.30 | 4.31 | 4.78 |
| 6.8 | 80.45 | 0.388±0.120 | 2.20 | 48.65 | 18.09 | 16.82 | 2.32 | 0.54 | 0.31 | 4.74 | 5.28 |
| L.S.D. (P=0.05) | | | | 3.97 | 3.16 | 1.67 | 0.36 | 0.039 | 0.06 | 0.63 | 0.83 |

± Standard deviation

Table 4 Effect of water stress on seed germination and seedling growth in Pinus kesiya

| Water potential (-bars) | Germination | | | Growth/seedling or seedling part | | | | | | | |
|----------------------------|-------------|--------------------|-----------------------------|----------------------------------|---------|----------------|----------------|---------|----------------|-------|----------|
| | % | Rate (Unit/day) | Days to peak value | Length (mm) | | | Biomass (mg) | | | | |
| | | | | Hypoco- tyl | Radicle | Cotyle- don | Hypoco- tyl | Radicle | Cotyle- don | Shoot | Seedling |
| 0 | 90.00 | 0.355±0.078 | 2.59 | 30.89 | 13.82 | 14.43 | 2.36 | 0.65 | 0.29 | 4.25 | 4.90 |
| 2.50 | 95.00 | 0.316±0.037 | 1.55 | 38.89 | 33.44 | 18.36 | 3.18 | 1.62 | 0.40 | 5.87 | 7.49 |
| 3.75 | 79.10 | 0.290±0.033 | 3.41 | 30.76 | 29.28 | 11.22 | 2.84 | 1.49 | 0.39 | 5.40 | 6.89 |
| 5.00 | 79.10 | 0.209±0.036 | 4.80 | 17.86 | 22.59 | 7.03 | 2.35 | 1.43 | 0.20 | 4.12 | 5.55 |
| 10.00 | 15.80 | 0.168±0.049 | 5.21 | — | — | — | — | — | — | — | — |
| 15.00 | 5.50 | 0.085±0.007 | 11.73 | — | — | — | — | — | — | — | — |
| L.S.D. (P=0.05) | | | | 10.16 | 9.67 | 4.13 | 0.39 | 0.47 | 0.22 | 0.42 | 1.46 |

± Standard deviation, — Non-measurable

germination in loblolly pine which was due to leaching of inhibitors and increase in endogenous levels of growth regulators during the process of stratification and germination. Recently, Bevington and Hoyle (1981) noted that chilling treatment at 3°C or red light initiated the phytochrome system in *Betula* resulting in higher germination. Ghosh et al. (1974) mentioned that *P. patula* seeds soaked in water for 18 hr showed better germination. On the other hand, *P. elliottii* required 10 days for hastening the germination (Foot 1967). In the present studies, imbibition of *P. kesiya* seeds for 12 and 24 hr resulted in higher germination and growth, whereas radicle growth was much higher in seeds imbibed at 24 hr. Imbibition at higher durations resulted in delayed germination and slow growth probably due to higher moisture contents of the seeds. It has been reported in southern pines that high moisture content of seeds inhibited the total germination and delayed the peak day (Campbell 1982).

Maull (1963) in case of *P. rigida* and Vaartaja (1957) in *P. banksiana* reported that no significant relationship existed between pH and seed germination. During the present investigation, *P. kesiya* seeds

showed highest total germination at pH 4.8 whereas a better rate of germination and growth resulted at pH 6.0 and 6.4 amongst the various pH treatments.

Soil moisture availability determines the germination and early seedling survival. In the present study, water potential of -2.5 bars resulted in higher seed germination and seedling growth probably due to nutrient availability. However, increased water potentials were found inhibitory. Satoo (1966) reported that soil moisture content if decreased from field capacity resulted in poor germination of *P. densiflora*, *P. thunbergii* and *Chamaecyparis obtusa* seeds. A similar decrease in *P. ponderosa* seed germination under moisture stress was reported (Larson & Schubert 1969).

Thus, it can be concluded that imbibition of *P. kesiya* seeds results in early germination and growth which were decreased by increasing osmotic stress and impeding water-uptake.

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