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Reassessment on Germination Requirements of *Pinus kesiya* Royle ex Gord. Seeds under Controlled Laboratory Conditions

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ABSTRACT Seeds of *Pinus kesiya* Royle collected in February, 1998, were incubated at 20, 25 and 30° C temperatures on different substrata, namely, sand, top-of-paper and between rolled towel paper. For sand and top-of-paper, complete dark and alternate (16 hrs dark and 8 hrs light) treatment were given; seeds in between rolled towel paper experienced only dark treatment. For seeds on sand and top-of-paper observations were recorded on every alternate day for radicle and needle-shoot emergence, whereas for those in between rolled towel paper daily observations were recorded. From the data collected value of different indices, namely, Maguire Germination Rate Index (GRI), Peak Value and Czabator Germination Value (GV) were calculated separately for radicle and needle-shoot emergence. It was observed that of all combinations of temperature and dark/dark-light treatment, the most favourable combination for radicle emergence was that of 25°C on sand with dark-light treatment, which showed the highest GRI, Peak Value and GV, being 23.20, 17.33 and 72.01, respectively. For needle-shoot emergence, the same combination of treatment as for radicle emergence, showed the highest GRI and GV, being 6.27 and 9.33, respectively; the Peak Value failed to differentiate the most favourable environment from less favourable ones. From the final radicle emergence, it was evident that all the temperatures were favourable for sand substratum with dark-light combination, whereas the final needle-shoot emergence was most favoured by sand substratum with dark-light combination at 25°C; as a compromise 25°C incubation temperature, sand with dark-light combination may be recommended for germination test under laboratory conditions for seeds of *Pinus kesiya* should evaluation be done on the 21st day of incubation.

Key words : Germination, GRI, GV, *Pinus kesiya*, temperature

For optimal germination, it is an established fact that oxygen, temperature and moisture are the most important factors [1, 2]. In laboratory germination test, besides the environmental factors, substratum on which the seeds are placed, is equally important for germination of seeds of many species [3]. Providing adequate requirements in the laboratory ensures all vigourous seeds to germinate to the maximum. Among agricultural crops requirements vary among species of the same genus [4].

In the laboratory light may be an additional requirement for certain species but in others it may be indispensable; seeds of *Muntingia calibra* at optimum temperature of 25°C failed to germinate in dark but germinated under constant white fluorescent light [5]. Seeds of some species required specific temperature for optimum germination as in *Canavalia brasiliensis, Leuccaena leucocephala, Clitoria ternatea* and *Clopogonium muconoides* being 28°C [6], whereas seeds of three species of *Coronilla* required different temperatures [7]; seeds of other species like *Cichorium intybus* showed their adaptability over a wide range of temperatures of 27-33°C [8] and those of *Lupinus havardii* ranged from 24-29°C [9]. Sometimes, alternating temperatures are recommended for certain species, for instance, three ash species exhibited rapid and complete germination at alternating temperatures

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of 25/5°C [10]. Chipped seeds of *Leucaena leucephala* showed an adaptation over a wide range of temperatures between 10-35°C in addition to recommended alternating temperatures of 30/20°C [11]. The interaction of temperature, substratum and light combinations have been noted in many species. A significant interaction between temperature and substrata for shoot and root length in dark and light conditions, respectively, was observed for *Briza subaristata* [12].

Pinus kesiya Royle is a timber yielding species of economic importance of North-eastern India, particularly in Meghalaya. Unfortunately, the unplanned extraction has rendered this species vulnerable as its distribution is restricted only to reserved forest in higher altitudes. Keeping in view of the importance of the species and inability to obtain desired results from seeds under laboratory conditions on either sand or top-of-paper in dark and light at 30° C, as prescribed by ISTA [13, 3], it was considered that it would be worthwhile to reassess its germination requirements under controlled laboratory conditions, using different substrata, light and temperature regimes.

MATERIALS AND METHODS

Seeds of *Pinus kesiya* Royle were extracted from the cones collected in February, 1998, by drying them in the sun. Surface sterilized seeds with 0.1 per cent $HgCl_2$ solution for 1 minute, followed by repeated washings with sterile distilled water were put for germination by placing them on the sand, top of layers of Whatman No. 1 filter paper and in between rolled towel paper. Seeds in rolled towel paper experienced only dark period in a cycle of 24 hours, whereas seeds placed on sand and on top of filter paper experienced both continuous dark period and also 8 hours light alternating with 16 hours of dark period at each incubation temperature regime. The incubation temperatures of 20°, 25° and 30° C were selected. There were fifteen treatments, each with 150 seeds in 3 replicates. Radicle length of 2mm was taken as a score of germinated seeds, whereas for needle-shoot emergence seedlings with over 1cm were considered as emerged.

The primary data of germinants (radicle or seedling emergence) were collected on every alternate day for seeds on sand and on top of filter paper whereas those in between towel paper daily observations were made for 21 days. The secondary data, namely, Germination Rate Index, GRI[14], Peak Value and Czabator Germination Value, GV[15], were calculated using the following equations:

$$GRI = \Sigma\left(\frac{N_i}{D_i}\right),$$

Where, N_i was the number of germinants (radicles or seedlings) germinated on the day D_i, counted from the day of incubation/sowing at a particular temperature and substratum.

$$\text{Peak value} = \frac{\text{Cumulative germination (\%)}}{\text{Days after sowing } (D_i)}$$

GV = Mean Germination x Peak Value

A two-factor analysis of variance was conducted on final primary data and secondary data for both radicles and seedlings, obtained from different substrata and temperature regimes used during the experiment.

RESULTS AND DISCUSSION

For laboratory germination of seeds of *Pinus kesiya* Royle, sand substratum with dark-light treatment (Figs. 1, 2, 3 and Table 1) on the whole discriminated itself from other substrata of filter paper and rolled towel paper at all the three incubation temperatures of 20, 25 and 30° C. Seeds on sand with alternate light treatment culminated in the highest (90 to 93.33%) non-significant radicle emergence, followed by those on top-of-filter paper with dark-light combination (76.67 to 89.33%) and the lowest radicle emergence (56.67 to 70%) was observed from seeds in between towel paper at all the temperature regimes (Fig. 1). Likewise, for final needle-shoot/normal seedling development, sand substratum, dark-light treatment and 25° C incubation temperature proved to be the most favourable combination (Fig. 2) and that the top-of-paper in dark has been identified as the least favourable at all the three temperature conditions. Light is an additional factor, which enhanced the speed of radicle emergence and normal seedling

Table 1 : Indices of needle-shoot emergence, Maguire Germination Rate Index (GRI), Peak Value and Czabator Germination Value (GV)

Substratum	Treatment	GRI	Peak value	GV
Sand	20°C+D	0.23 (±0.00)	0.86 (±0.00)	0.06 (±0.00)
	20°C+D+L	4.35 (±0.16)	1.88 (±0.00)	6.80 (±0.29)
	25°C+D	4.76 (±0.36)	2.20 (±0.48)	7.56 (±1.45)
	25°C+D+L	6.27 (±0.11)	2.22 (±0.06)	9.33 (±0.29)
	30°C+D	0.0 (±0.00)	0.00 (±0.00)	0.0 (±0.00)
	30°C+D+L	4.57 (±0.70)	2.15 (±0.00)	8.13 (±1.35)
Top-of-filter Paper	20°C+D	0.50 (±0.04)	0.24 (±0.00)	0.12 (±0.01)
	20°C+D+L	1.34 (±0.21)	0.61 (±0.02)	0.70 (±0.03)
	25°C+D	0.76 (±0.03)	0.38 (±0.02)	0.18 (±0.01)
	25°C+ D+L	2.56 (±0.25)	1.13 (±0.19)	1.86 (±0.49)
	30°C+D	0.0 (±0.00)	0.0 (±0.00)	0.0 (±0.00)
	30°C+D+L	0.41 (±0.07)	0.41 (±0.07)	0.12 (±0.01)
Between Towel paper	20°C+D	1.89 (±0.27)	0.63 (±0.03)	0.97 (±0.01)
	25°C+D	5.58 (±0.09)	1.89 (±0.61)	4.47 (±1.01)
	30°C+D	4.76 (±0.43)	1.80 (±0.1 6)	4.59 (±0.13)
CD(>0.005)		0.45	0.36	2.56

D : Dark, L : Light. CD : Critical Difference

development from seeds on sand and on top-of-filter paper for a particular temperature (Figs. 1 and 2). No seedling development was observed from seeds on sand or on top-of-filter paper in dark at 30°C, however, the radicle emergence was found in both the conditions. The necessity of identifying the suitable substratum and the incubation temperature for a particular species is well documented [13, 3].

Some workers observe the view that favourable incubation temperature is responsible for the initial root growth and early seedling growth and successful stand establishment [16, 4]. Our observation in the present study is at variance with this view as seeds on sand with dark-light treatment at 25 and 30° C failed to produce as many as normal seedlings (Fig. 2) as emerged radicles (Fig. 1).

The Germination Rate Index (GRI) was introduced by Maguire [14], which is the sum total of quotients derived by dividing fresh daily germinants by corresponding number of days of incubation; the Peak Value is the highest quotient obtained. Czabator Germination Value (GV) was introduced by Czabator [15], which is the combination of completeness and speed of germination in pine seeds. Seeds on sand and on top-of-filter paper with dark-light combination incubated at 25°C exhibited the highest values of GRI, Peak Value and GV, for both radicle emergence (Figs. 3B, D, F), and seedling/needle-shoot development (Table 1). The applicability of GRI has also been reported by Fernandez and Johnston [17] for comparing radicle and seedling emergence from seeds of lentil, bean and chickpea in vigour testing, Villagra [18] in assessing the effect of temperature on the germination of seeds of *Prosopis argentina* and *P. alpataco*, and by Mosjidis and Zhang [4] in root growth of *Vicia* species in response to the effect of different incubation temperatures. All indices is a measure of speed at which the radicle or seedling grow, therefore, the faster is the growth the higher is the value of each index. Most of indices have been used to study the radicle emergence, however, Fernandez and Johnston [17] have also used for assessing the speed of seedling growth.

The reliability of GV as one of the indices for seed germination have also been reported by Fernandez and Johnston [17] in assessing seed vigour in lentil, bean and chickpea. The temperature effect on the germination of stratified seeds of three ash species has been assessed by GV [10] and also in germination of *Cinnamomum*

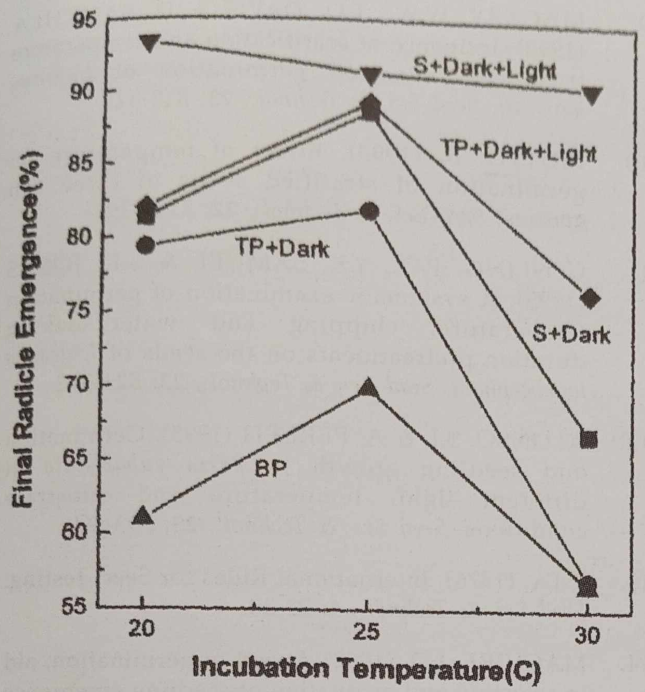

Fig. 1. Radicle emergence response curve at different incubation temperatures in dark and dark-light combination on different substrata. S : Sand; TP : Top-of-paper; BP : Between rolled towel paper. CD (>0.005) = 6.03

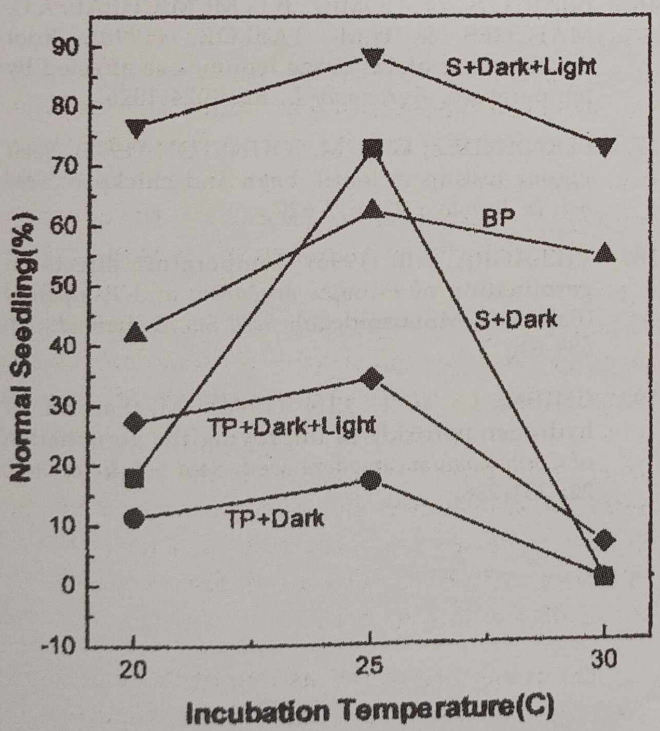

Fig. 2. Needle-shoot/normal seedling emergence response curve at different incubation temperatures in dark and dark-light combination on different substrata. S : Sand; TP : Top-of-paper; BP : Between rolled towel paper. CD(>0.005)=4.33

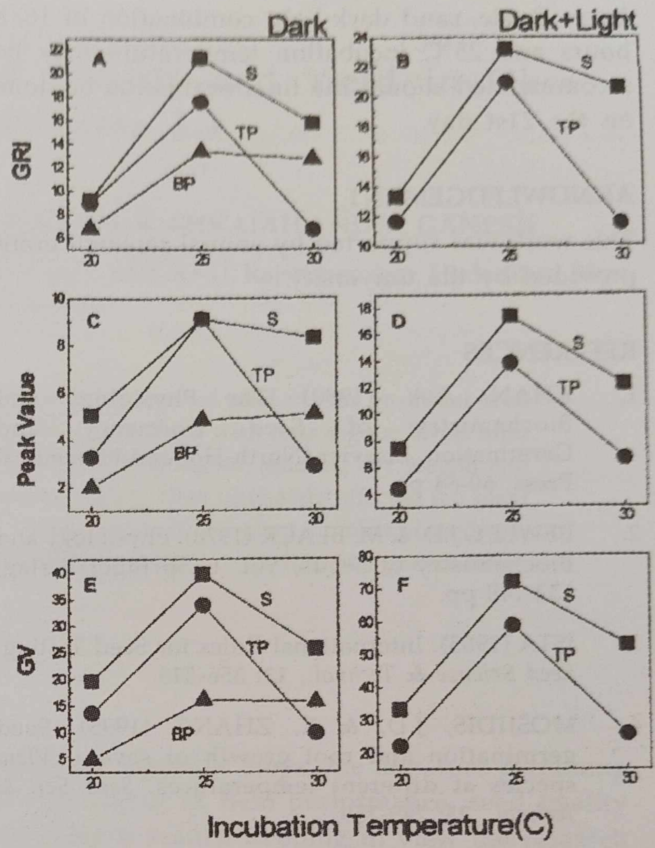

Fig. 3. Indices of radicle emergence, Maguire Germination Rate Index (GRI), Peak Value and Czabator Germination Value (GV). S : Sand, TP : Top-of-paper, BP : Between rolled towel paper.
CD (>0.005), GRI = 1.07, Peak Value = 1.90 and GV = 4.23

camphora seeds by Chien and Lin [19]. In the present study it is obvious that GV can also be used for assessing the radicle emergence and seedling growth of gymnospermous seeds. The poor seedling development from seeds incubated with dark-light treatment at 30° C, both on sand and on top-of-filter paper conforms to our observations that the seedlings fail to survive at lower altitudes, where temperature is more than 28°C. The luxuriant growth is restricted only to areas at higher altitudes, where maximum temperature never exceeds 28°C and that the natural regeneration from seeds is very high in open areas compared to that from seeds in shade. From our observations, seeds incubated at 20° C can only be assessed beyond 21 days, from the date of sowing.

Based on our observation and results obtained, for laboratory germination test of seeds of *Pinus* *kesiya* Royle, sand dark-light combination of 16/8 hours and 25°C incubation temperature may be recommended should the final evaluation be done on the 21st day.

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