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The effect of seed coat removal on seed germination of *Pinus gerardiana* Wallich ex D. Don. *Chilgoza* pine

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Pinus gerardiana is known as "*Chilgoza* pine" which yields small edible seeds. The *chilgoza* pine is a small to medium sized tree occasionally attaining a height of 24m and a girth of 3.5m. The pine is confined to the eastern Afghanistan parts of Pakistan and India. In our studies we have investigated the effect of seed coat removal on seed germination of *P. gerardiana* and have determined the optimum germination temperature. Seed coat was found to have inhibitory role as the removal of seed coat promoted both rate and final germination. When germinated under various constant temperatures the optimum temperature for germination was found to be 20 °C, matching the average spring temperature prevailing in its habitat.

Keywords: *Chilgoza* , germination, *Pinus gerardiana,* seeds.

Introduction

The seeds of *Pinus gerardiana* are used as dry fruit and have high commercial value. The seed harvest has been a chief source of income for the indigenous people in the Suleiman Mountains of Pakistan. The seeds are considerably large about 21 mm in length and wingless (rudimentary wing which usually adheres to adjacent scale) with an average seed weight of 350 mg. The seeds have very thin seed coat which is hard and can easily be peeled. The *chilgoza* pine is a small to medium sized tree occasionally attaining a height of 24m and a girth of 3.5m.

The distribution of *Pinus gerardiana* is very restricted. It is found in eastern Afghanistan, parts of Pakistan and India where it is found in scattered dry inner valleys of North-Western Himalayas (Singh 1993. Critchfeild & Little 1966, Champion et al. 1965). It grows on the elevation of 1600 m to 3400 m above the sea level. *Chilgoza* forest is spread over about 200sq-km area in Balochistan.

The species carries substantial economic importance as it is exploited for its seeds and logged for fuel and construction which puts huge strain on the species. With the exception of very limited forestry related research work, mainly performed in India and a small fraction in Pakistan (e.g. Sharma & Yadev 1996; Singh et al. 1992; and Siddique et al.1983), there is no scientific work regarding seed germination of *Pinus gerardiana.* The present study is an effort to understand the germination behaviour of the species.

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Materials and Methods.

The Plant Material

Seeds were extracted from cones of *P. gerardiana* collected from *chilgoza* pine forest located in District Zhobe, Balochistan, Pakistan from 47 randomly selected trees during the month of September. Cones were air-dried for one week before they were kept in oven at 35°C for 24 hours to allow them to open. Seeds were extracted by knocking cones against hard surface and then shaking the dried cones. Empty, fungal infected, and pest infected seeds were discarded.

Germination Conditions

For the germination experiment 5 replications of 20 seeds were imbibed in petri dishes of 9 cm diameter lined with 2 layers of filter paper and moistened with 8 ml of distilled water. Dark germination was achieved by keeping seeds in lightproof metal boxes throughout the course of germination. The examination of dark incubated seeds was carried out under green safe light.

The seed was considered germinated on the visible radicle protrusion. The germinated seeds were removed from petri dishes. Measurements were ended when there was no additional seed germination.

In addition to the final germination T_{50} was also used to determine the rate of germination. T_{50} is the time needed for 50% of the final germination value, and it is calculated by linear interpolation from the two germination values closest to median germination.

Germination experiments were conducted under constant temperatures and under alternating temperature and light conditions using growth chamber Heraeus (Bk. 5060 EL, W.C. Hanau, Germany). The incubator is equipped with white light source consisting of fluorescent tubes (Philips TLD 18W/33) and incandescent tubes (Philips Philinea 6276 × 60W) (Thanos et al. 1991). In all cases temperature inside the chambers was kept constant within ±1 °C.

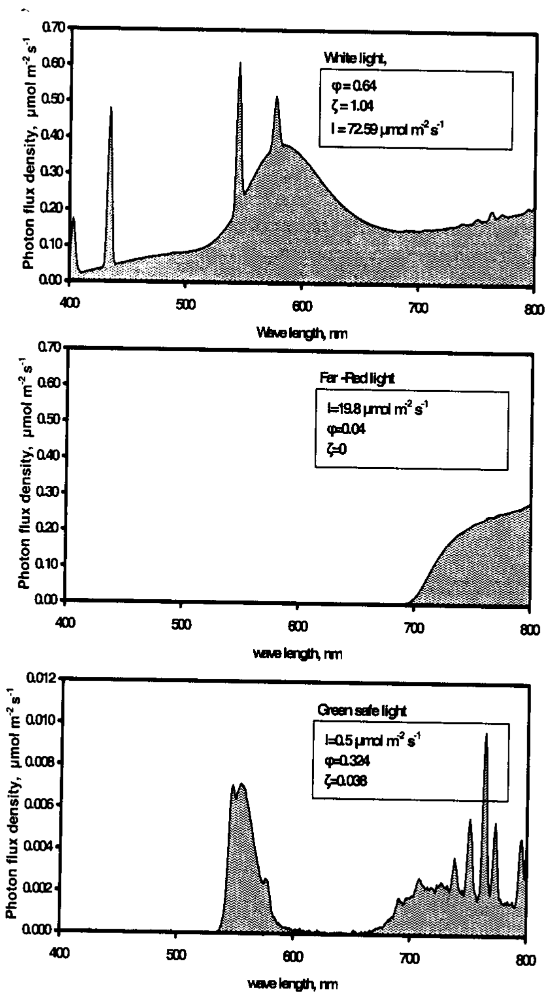

Fig. 1. Spectral distribution curves for white, far-red and green safe light regimes. Photon flux density in μmol m^{-2} s^{-1}

The far-red broad band irradiations were produced by lights filtered through a combination of two blue 627, and one red 501, Plexiglas sheets (each 3 mm thick, Rohm GmbH, Germany). The dim green safe light (0.05 μmol m-2 s-1) was achieved by one green fluorescent tube F 15T8.G.6, 15 W Green-Photo, General Electric, USA and two Plexiglas sheets (one red-orange, 478, and one green, 700) (Thanos & Dousi 1995). Photon flux rates, values of φ ([Pfr]/ [Ptot]) and ζ (660/730 nm photon ratio) were measured by Licor 1800 Portable Spectroradiometer. For spectral distribution curves see Fig. 1.

Results and Discussion

Decoating (i.e. removal of seed coat and exposing the mega gametophyte) resulted in promotion of rate and final germination of *P. gerardiana*. Fresh seeds of *P. gerardiana* when decoated, germinated to their full capacity. Time course of germination of decoated seeds of *P. gerardiana* was obtained by imbibing them under three light regimes, namely white light, far-red light (simulating "under canopy" light environment), and under darkness (Fig. 2B). The seeds were incubated at constant temperature of 20 °C under continuous light. The seed germination was optimal under all three light regimes and there was no suppression of germination by far-red light. The rate of germination was very fast and final germination was achieved within two weeks. Final germination under white light and far-red light was slightly less than 80%, while it was more than 80% under darkness. These results were compared with the germination of intact seeds which were incubated under similar light and temperature conditions (Fig. 2A) and it was found that both in light and darkness the final germination did not exceed more than 40 % and that the rate of germination was also slow as compared to decoated seeds.

Dark germination of decoated seeds as a function of temperature took place at a range of 15 to 25 °C (Fig. 2C). The seeds germinate optimally at 20 °C with final germination more than 75%, although the speed of germination was slower (T50 value 8.5d). The final germination at both 15 and 25 °C was 55%; however the seeds in 25 °C were quick to germinate with T_{50} value 5.3d. The seeds initially incubated at 5 °C failed to germinate; however on subsequent transfer to 20 °C after 30d they regain their germinability under darkness. Although their germination was not restored to full capacity and only 35% final germination is achieved.

Table 1. Final germination (% ± standard error) of P. gerardiana.

Germination conditions	Final germination, % ± standard error.	
	White light	Darkness
Germination under constant temperature of 20 °C and continuous white light	35 .0± 10.0	37.0 ± 7.5

Table 2. Final germination (% ± standard error) of decoated seeds of *P. gerardiana.*

Germination conditions	Final germination, % ± standard error.		
	White light	Darkness	Far-red light
Germination under constant temperature of 20 °C and continuous white light	75.0 ± 2.7	88.0 ± 2.5	77.0 ± 5.8

Table 3. Dark germination (% ± standard error) of decoated seeds of *P. gerardiana* as a function of temperature.

Germination Temperature under darkness °C	Final germination, % ± standard error.
5	0.0 ± 0.0
15	53.0 ± 11.0
20	74.0 ± 5.3
25	52.0 ± 3.4
(5→20)*	**36.3 ± 12**

*Incubated initially at 5 °C and subsequently transferred to 20 °C after 30d.

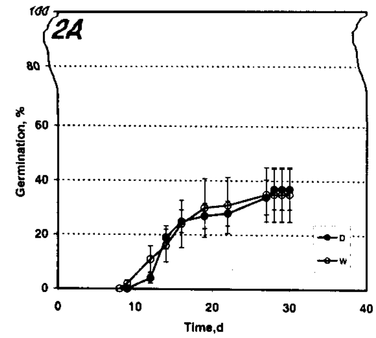

Fig. 2 A. Time course of germination of decoated seeds of *P. gerardiana* under constant temperature of 20 °C and continuous light, vertical bars represent ± standard error values. d denotes time in days. (White light: o, Darkness: •)

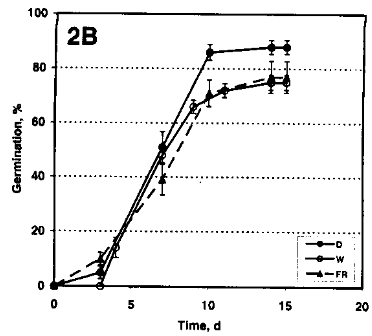

Fig. 2B. Time course of germination of decoated seeds of *P. gerardiana* under constant temperature of 20 °C and continuous light, vertical bars represent (standard error values. d denotes time in days.(White light: o, Darkness:•, Far-red light: ▲)

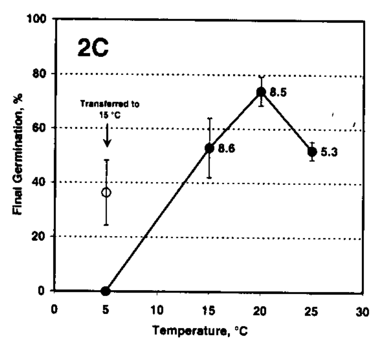

Fig. 2C. Final seed germination of *P. gerardiana*, as a function of constant temperature. Final germination levels are shown by solid circles connected with straight lines; values next to circle indicate T_{50}. Vertical bars represent ± standard error values. Open circle represents final germination of the seeds incubated at 5 °C and subsequently transferred to 20 °C after 30d

Marked effect of decoating was observed on the seed germination of *P. gerardiana*. It was observed that the rate and final germination in intact seeds of *P. gerardiana* was very slow, however remarkable increase both in rate and final germination was observed when the seeds were imbibed after seed coat was removed. The reason why this enhancement occurs is not fully understood. The seed coat of *P. gerardiana* is thin and papery and readily permeable to water, therefore there is no possibility of it to interfere with water uptake. It may act as a barrier to gaseous exchange in first few days of imbibition when seed is in 'activation' stage with increased requirement for oxygen. However once the seed is fully imbibed its seed coat is forced to crack by the force exerted by the expending embryo plus mega gametophyte and there seems no hindrance in the movement of air. Since decoated seeds were fully capable of germination in darkness the role of seed coat as an obstacle to light is also ruled out. The only possible role of seed coat that can be suggested in seed germination of *P. gerardiana* may be the presence of certain chemical inhibitors in the seed coat. Or it may act as a barrier against the leaching out of inhibitors present inside the seed. The evidence that pine seed coat has water soluble gr inhibitors was presented by some workers in the coat of *P. pinea* , they suggested that those germin inhibitors were involved in the regulation of *P. p* seed germination (Martinez et al. 1978). In the lig the possibilities discussed above there is a nee further explore this aspect of *P. gerardiana* seeds.

When the effect of white light, far-red light (simul under canopy light environment) and darkness examined on seed germination, the seeds were f slightly indifferent to far-red light, as mentioned a there is more far-red under the canopy of the tree, i be suggested that seed germination in natu designed to occur under the canopy of parent tree. is also supported by the seed dispersal behaviour c plant which, owing to its winglessness, occurs m under the canopy of the parent tree where see recruitment is eventually observed.

Keeping in view the economic and ecolo significance of the pine, it is suggested physiological studies should be conducted or germination of the species. Especially the role of and presence of germination inhibitors need fu research.

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