

Racial Hybridization and Inbreeding Depression in *Cedrus deodara* (Roxb.) G. Don.

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Abstract

Aim of the study: This study aims to establish the inter-racial hybridization in *Cedrus deodara* from Garhwal Himalaya, India.

Material and methods: Racial hybridization was conducted successfully in *Cedrus deodara* taking pollen (male gamete) from three different races/populations, i.e. Dhanoulti, Pauri and Harsil (locality-specific) at Dandachali population where female/ovulate cones were selected as mother trees.

Main results: The results revealed that cone and seed set percentages in the selected races varied from 58.8 to 68.2 % and 79.0 – 86.2 %, respectively. Manual pollination resulted 0.972 and 0.953 inbreeding depression for cone and seed set, respectively.

Highlights: *C. deodara* proclaimed high inbreeding depression at embryonic stage also manifests high compatibility for developing hybrids in its natural range of growth in Himalaya.

Keywords: Ovulate Cone, Reproductive Success, Receptivity, Deodar, Pollen, Himalaya

Cedrus deodara'da (Roxb.) G. Don. İrk Hibridizasyonu ve Akrabalı Çiftleşme Depresyonu

Öz

Çalışmanın amacı: Bu çalışma, Hindistan, Garhwal Himalaya'dan *Cedrus deodara*'da ırklar arası melezleşmeyi kurmayı amaçlamaktadır.

Materyal ve yöntem: Dişi/yumurtlama konilerinin ana ağaç olarak seçildiği Dandachali popülasyonunda Dhanoulti, Pauri ve Harsil (yere özgü) olmak üzere üç farklı ırk/popülasyondan polen (erkek gamet) alarak *Cedrus deodara*'da ırksal hibridizasyon başarıyla gerçekleştirilmiştir.

Temel sonuçlar: Sonuçlar, seçilen ırklarda kozalak ve tohum set yüzdelerinin sırasıyla %58,8 ila %68,2 ve %79,0–86,2 arasında değiştiğini ortaya koydu. Manuel tozlaşma, sırasıyla koni ve tohum seti için 0.972 ve 0.953 akrabalı yetiştirme depresyonu ile sonuçlandı.

Araştırma vurguları: *C. deodara*'nın embriyonik aşamadaki yüksek akrabalı yetiştirme depresyonu, Himalaya'daki doğal büyüme aralığında melezler geliştirmek için yüksek uyumluluk gösterir.

Anahtar Kelimeler: Yumurtlama Konisi, Üreme Başarısı, Alıcılık, Deodar, Polen, Himalaya

Introduction

The genus *Cedrus* consists of four species, i.e. *Cedrus atlantica* Manetti, *Cedrus brevifolia* Henry, *Cedrus deodara* (Roxb.) G. Don and *Cedrus libani* Barrel (Vidakovic, 1991). *C. atlantica*, the Atlas cedar is native to Morocco and Algeria that grows at altitudes between 1000 and 2000 m, in the Atlas and Riff Mountains. It flowers in September or October and the seeds maturation takes place in 18 months. *Cedrus libani*, the cedar of Lebanon is native to the

mountains of Asia Minor, Syria and Lebanon. It occurs naturally between 1300 m and 2100 m elevation (Farjon, 2012). *Cedrus brevifolia* Cyprus cedar is native to Troodos mountain range of Cyprus that grows naturally at an elevation between 900 and 1400 m. It is endemic to Cyprus and its forests are of priority habitat type. Flowering in *C. brevifolia* occurs in September to October and seeds mature take about one year (Farjon, 2012). *C. deodara*, the Himalayan cedar/ deodar, is an important



timber tree that occurs in the western Himalayan Mountains at an elevation of 1500-2600 m (occasionally between 1200-3600 m). *C. deodara* covers large parts as pure forests and also found mixed with other coniferous and broad-leaved species. It is an anemophilous and monoecious species, pollination takes place in the autumn, and the mature cones disperse the seeds in next autumn. The age of sexual maturity in *C. deodara* reached in between 28 and 32 years and the rotation age in Himalaya is 120 years. The occurrence of multiple sexual morphs, i.e. pure male, pure female, mixed monoecious, predominantly male and predominantly female, has been identified in natural populations of *C. deodara* (Khanduri et al., 2021). The information about the gametic (male and female) production, pollen mediate gene flow and blooming synchrony/asynchrony in the population is available in *C. deodara* (Khanduri & Sharma, 2002a; 2009; 2010). The mating system and reproductive barriers in *C. deodara* has not been studied so far. However, there was no reproductive isolating barrier among three Mediterranean Cedrus species i.e., *C. libani*, *C. brevifolia* and *C. atlantica* (Fady et al., 2003). Baring to this, an attempt has been made to study the mating system and crossing between genetically different populations (i.e. races). Hybridization in conifer do not generally support compatibility and is regarded as important natural evolutionary processes which can be investigated successfully using controlled pollination experiments. Mating between races bestow birth to new hybrids and in nature, natural hybrids form automatically through speciation (Arnold, 1997). In nature, it occurs when compatibility occurs between two distinct genotypes which are difficult to differentiate morphologically (e.g. in *Picea rubens* and *Picea mariana*, Bobola et al., 1996). The studies of mating system also facilitate to estimate the level of outcrossing in the population. Inbreeding depression is a ubiquitous phenomena present in plants, which preferably be estimated from the seed set data achieved from manual crosses (del Castillo and Trujillo, 2008). Little attention

was paid to this important source in mating systems studies.

The outcrossed forests trees species are usually characterized as high genetic diversity within populations, supports gene flow, promote heterozygosity, aids gametic linkage equilibrium and have good adaptive characters (Hamrick and Godt, 1989; Kremer, 1994; Notivol et al., 2007; del Castillo and Trujillo, 2008). Maintaining genetic diversity costs high mutation load (Scofield and Schultz, 2006; Petit and Hampe, 2006) that can only be eliminated whenever inbreeding takes place (Ferriol et al., 2011). The probability of inbreeding is more when there is random mating and small size of population (O'Connell et al., 2004). Inbreeding occurs due to presence of less number of recessive genes and embryonic lethal allele which causes detrimental effect on fitness, i.e. Seed abortion (Keller and Waller, 2002), retards seed germination (Sorensen, 2001), enhances infantile death (Koelewijn et al., 1999), reduces growth (Sorensen and Miles, 1982; Bower and Aitken, 2007), leads to morphometrics defect during plant developmental phase (Wilcox, 1983), devitalizes fertility and seed production during early age of trees (Kärkkäinen and Savolainen, 1993; Durel et al., 1996), and enhance the probability of diseases in a population (Frankham, 2002; Altizer et al., 2003). Inbreeding depression supports outcrossing and retards breeding system to evolve towards self-fertilization (Lande and Schemske, 1985; Byers and Waller, 1999). Large numbers of studies have been concentrated on shorter-lived plants on mating system and inbreeding depression as compared to that of long-lived outcross species (Charlesworth and Charlesworth, 1987; White et al., 2007; Ahlinder et al., 2021). Therefore, this study has been made in *Cedrus deodara*, a highly valuable tree species of western Himalaya because of its multifarious uses, i. e. furniture making, constructions of bridges, boat houses and buildings because of its light-weight, rot-resistant and high durability characters (Tewari, 1994). The essential oil (cedarwood oil) obtained from its heartwood is used as insect repellent, anti-fungal ointment and several beauty products, viz.

soap, shampoo etc (Tewari, 1994. It is also recognized as a medicinal tree used for curing of several ailments in ayurveda and unani system of medicine (Chaudhary et al., 2011). The aim of this study were to (i) evaluate inter-racial crossing compatibility in three populations/races of *C. deodara*, and (ii) estimate outcrossing and inbreeding depression in *C. deodara* from western Himalaya, India.

Material and Methods

Study Site

This study was conducted in a natural population of *C. deodara* at Dandachali (Tehri Garhwal) during the flowering season from September 2014 to November 2015. The study site is located between 30° 18' N and 78° 24' E with an elevation between 1800 and 2200m. Deodar was the main species in the canopy layer (pure forest). The other selected populations/races were Dhanolti (30° 25' N and 78° 40' E; altitude 2200 – 2300 m), Pauri (30° 09' N and 78° 48'E; altitude 1900 m) and Harshil (31° 02' N and 78° 45' E; altitude 2500 m) which were more than 30 km (Dhanolti), and 100 km away from Dandachali population.

Racial Hybridization

The experiment was conducted at Dandachali forest where mother trees were selected to perform racial hybridization. Six mother trees were chosen randomly in the population. The trees were healthy and bearing branches about 2.5 m above from the ground, which facilitates us to climb to the trees easily and perform pollination experiments manually. 360 ovulate cons in all the selected mother trees were isolated during its developmental phase before receptivity. 120 ovulate cones were selected for each race/population in which pollen grains were applied from that race. Pollination experiment was conducted during October 2014. Pollen from each race was collected at the time of pollen cone dehiscence (Fig. 1A) from five different candidate trees growing 100 m apart in that population. Collected pollen grains were mixed and taken in an air tight vial and stored in a refrigerator at 2°C till pollination experiment performed. At the time of

receptivity of ovulate cones (Fig. 1B), the collected pollen grains from each race/population were applied/ blown to the isolated ovulate cones (pollination bags) on the mother trees through a syringe from four sides and top of the pollination bag and the pollination bag was sealed with a piece of tape to warrant coverage of ovulate cones (Khanduri and Sharma, 2002b). Labelling for different races on pollinated ovulate cones at different branches was done so that the cone development for different races can be seen distally. Before application, the viability of collected pollen grains was tested through acetocarmine methods. The receptivity of ovulate cones of *C. deodara* lasts for 4 to 5 days (Khanduri and Sharma, 2010). The pollination bags from the isolated ovulate cones were removed after 20 - 25 days of manual pollination (Fig. 1C). The cone development was monitored after every three months till maturity. The cone and seed set data were recorded at maturity (converted into percentile) in the second fortnight of October 2015. The ovule number was estimated at the time of peak receptivity (leaving the rudimentary ovules at the base and top of the cones) from 60 ovulate cones from the selected mother trees (10 from each mother tree) and the average value was used as a threshold value to estimate fruit set percentage.

Outcrossing

In another experiment, self (geitonogamy) and cross pollination test was done by choosing 100 ovulate cones; 50 for each test. The isolation of ovulate cones and pollination was done similarly as described above. For the test of geitonogamy (inter flower selfing), pollen were taken from the same tree in which ovulate cones were bagged and applied to ovulate cones as per the method explained above and labelling was done. For cross pollination, the pollen grains were collected from different individuals (other than the selected mother trees) in the population at Dandachali forest growing 100 m apart and applied to the bagged isolated ovulate cones as done for racial pollination experiment. The data for cone and seed set were taken at maturity. The cone and seed set data were also recorded for

open pollination for which 100 ovulate cones were marked at the time of pollination and conversion to cones and seeds were recorded at maturity. To compare cone set among manually selfed and crossed treatments, and among open pollinated and manually crossed treatments, a Chi-square analysis was carried out (Zar, 1999) to know the pattern of variability between these treatments.

Inbreeding Depression

Inbreeding depression was calculated for cone and seed set following the formula; $\delta = 1 - W_s/W_o$ Where δ = inbreeding depression, W_s = the performance of progeny resulted from self-pollination and W_o = the performance of progeny estimated from cross-pollination (Lande & Schemske, 1985). A delineation inbreeding depression value of $\delta = 0.5$, signified that the selfing is preferred below the value and beyond which outcrossing is favoured (Lande & Schemske, 1985).

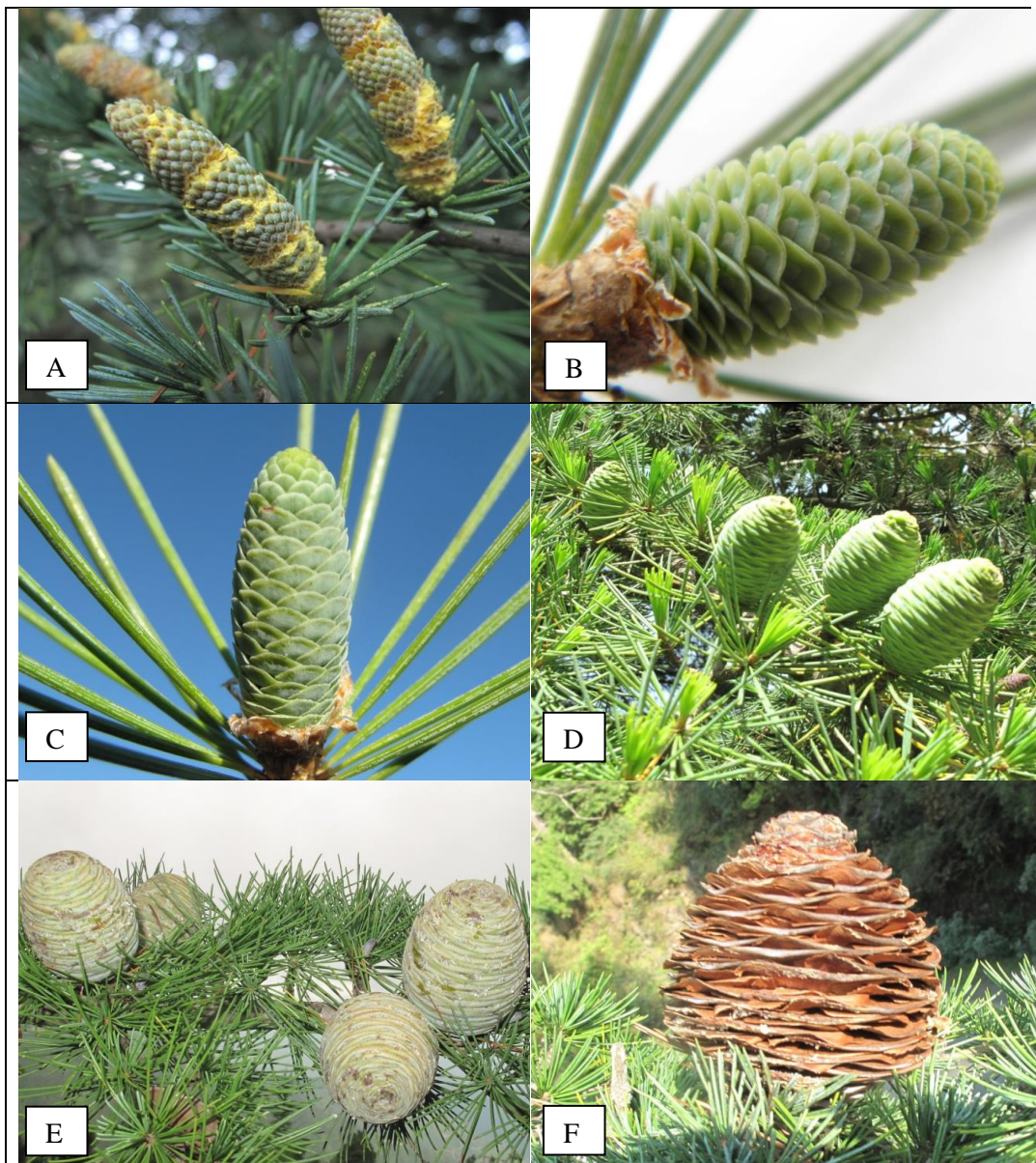


Figure. 1 A. Beginning of dehiscence in male cone (a best stage for collection of pollen grains), B-F. Different developmental stages of female cone. B. Ovulate cone at peak receptivity, C. after 20 days of pollination, D. Development after 6 months in April, E. Development after 10 months in August, F. Maturity at 12 months in October.

Results

The results of racial hybridization was very exciting with a success of 58.8 to 68.2 % cone set and 79.0 – 86.2 % seed set among three populations/races (Fig. 2A). The maximum value was recorded for Dhanolti population/race, followed by Pauri and minimum for Harsil for both cone and seed set percent success. The geitonogamy has also shown 2.0 and 4.2 percent cone and seed set, respectively (Fig. 2B). The cone and seed set was comparatively higher (70.6 and 88.4

%, respectively) in manually cross pollination as compared to that of open pollination (62.8 and 82.6 %, respectively). The estimated inbreeding depression for cone and seed set was 0.972 and 0.953, respectively. There was significant differences between manually selfed and crossed treatments ($\chi^2 = 27.28$, $p < 0.0001$) and between the open pollinated and manually cross treatments ($\chi^2 = 7.84$, $p < 0.004$).

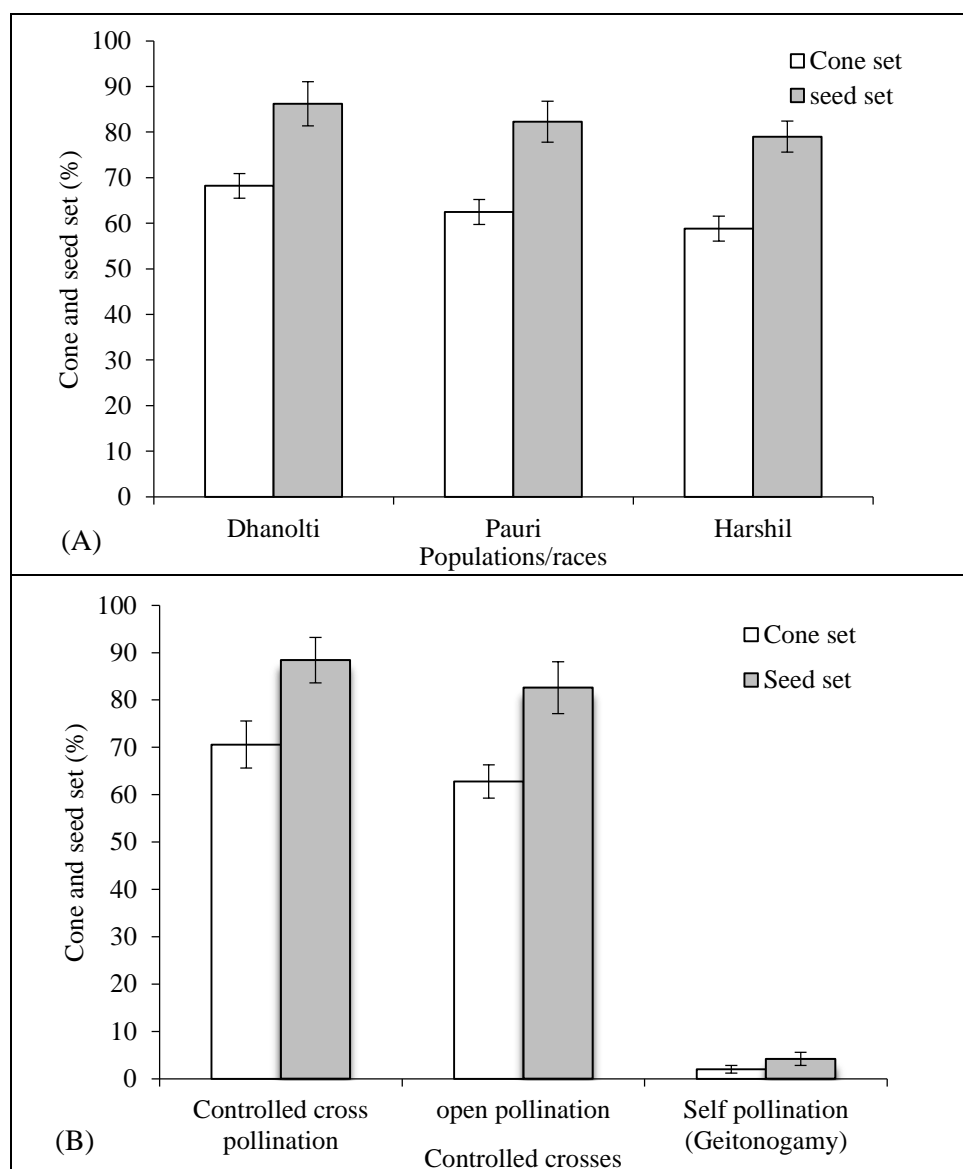


Figure 2. Cone and seed set following (A) racial hybridization, (B) controlled pollination (Bar representing standard error (SE) of the mean).

The different developmental phases of ovulate cones, i. e. Ovulate cone exposing

the bract scales to reach the air blown pollen on ovules, i. e at peak receptivity (Fig. 1B),

Ovulate cone sealing the bract scales after 20 days of pollination (Fig. 1C), development of female cones after 6 months of pollination in April (Fig. 1D), developed female cones after 10 months in August (Fig. 1E), and female cones exposing its scale at maturity in October to release the seeds (Fig. 1F), has also been illustrated.

Discussion

C. deodara has strong compatibility to mate with distinct genotype in the population which supports that the species does not have reproductive isolation barrier as reported for other three *Cedrus* species. The inter species hybridization between *C. atlantica* (as ovulate cone) and *C. brevifolia* (as pollen) proclaimed 46% successful hybridization. However, *C. atlantica* (as female) and *C. libani* (as male) accounted 78% hybridization success (Fady et al., 2003). The inbreeding depression revealed that *C. deodara* has high level of outcrossing, i.e. 0.962 clearly indicating that the species is self incompatible. The average outcrossing rate of 0.905 has also been recorded for *C. atlantica* forest of Luberon, South Eastern France (Ferriol et al., 2011). Estimated outcrossing rate in other conifer species, viz. *Pinus* are also high, averaging 0.9 (Delgado et al., 2002). In different *Pinus* species, outcrossing is recorded in the range of 0.85–0.92 for *P. caribaea*, 0.81–0.96 for *P. oocarpa* (Matheson et al., 1989), 0.68–0.97 for *P. kesiya* (Boyle et al., 1991), 0.817 - 0.980 for *P. sibirica* (Krutovskii et al., 1995), 0.93 – 0.99 for *Pinus sylvestris* (Kärkkäinen et al., 1996), and 0.73 – 0.93 for *P. albicaulis* (Krakowski et al., 2003; Bower and Aitken, 2007). Our results are at par with the studies made on other species of Pinaceae, i. e. 0.82-0.91 for *Pinus taeda* (Bishir and Namkoong, 1987), 0.71 – 0.82 for *Pinus sylvestris* (Kormutak et al., 2005), 0.76 to 0.90 for *Picea glauca* (Coles and Fowler, 1976), 0.66 - 0.78 for *Picea abies* (Skröppa and Tho, 1990), 0.905 (average value) for *Cedrus atlantica* (Ferriol et al 2011), showing high level of inbreeding depression.

The results of this study suggest that *Cedrus deodara* experiences high inbreeding depression at embryonic stage. Early inbreeding depression commonly considered

as a part of ovule abortion in the family Pinaceae due to accumulation of recessive lethal (Sorensen, 1969; Koski, 1971; Charlesworth & Charlesworth, 1987), while the quantitative traits such as growth and fecundity during developmental phase of tree are affected due to moderately deleterious alleles (Sorensen, 2001). The aggregations of lethal in conifers are in part due to high mutation rates (Koski, 1971; Kärkkäinen et al., 1996). Most species of Pinaceae are usually characterized as empty seeds, which is attributed to embryo mortality (Sorensen, 1969). This is because seed development in Pinaceae takes about 12 to 24 months after pollination. Fertilization occurs very late in most species of Pinaceae and in case of *Pinus* it takes place a year after pollination. The ovules that remained unpollinated could not develop into full size seeds resultant to ovule abortion, thus empty seeds arise due to mortality of developing embryos (Sarvas, 1962). Outcrossed species at early or both early and late life stages often reveal high inbreeding depression as compared to that of selfers which usually manifests very rare in early life stages (Lande and Schemske, 1985; Husband and Schemske, 1996). The species which are capable of self-fertilization exhibits decreased inbreeding depression for purging of deleterious alleles, consequently selfers should have lower inbreeding depression and higher inbreeding depression in outcrossers (Lande & Schemske, 1985; Charlesworth & Charlesworth, 1987; Barrett & Charlesworth, 1991; Husband & Schemske, 1996; Wang et al., 1999). In a recent study, Ahlinder et al. (2021) analysed metadata of inbreeding depression of family Pinaceae using 147 estimates from 41 studies of 18 species across four life stages (i.e. embryonic, juvenile, adult growth, and reproductive life stages) and have revealed that the inbreeding depression was high at the embryonic stage in continuous populations. The observed high level of outcrossing in *C. deodara* may leads to induce good seed germination, growth survival and reproduction in western Himalaya. Also high outcrossing would suppress deleterious recessive genes due to inbreeding depression, which may leads to high genetic diversity within populations

(Hamrick and Godt, 1989) and wide adaptivity in nature (Kremer, 1994; Notivol et al., 2007). The broad-leaved species in Himalayan temperate zones are being gradually replaced by *C. deodara* forests (Sharma et al., 2018) as a result of high adaptability of *C. deodara* in its natural range.

Conclusions

Racial hybridization revealed that there is no reproductive isolating barrier in *C. deodara*, suggesting that the species hybridization would be done to develop new species of Cedrus. *C. deodara* expresses high inbreeding depression at embryonic stage. The results of breeding system of *C. deodara* of this study would be helpful for drawing management strategy of breeding populations. Early inbreeding depression will affect growth and reproductive traits in *C. deodara* which would be expository for planning future generations of tree improvement. The magnitude of inbreeding depression will indicate the extent of threat of analogous matings in breeding populations. Estimate of higher levels of inbreeding depression facilitates outcrossing, moreover, in large continuous populations random mating may also result the probability of inbred progeny. *C. deodara* exhibits a strong outcrossed mating system in Himalayan forests.

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

Conceptualization: V.P.K.; Investigation: V.P.K, M.K.R, A.S.; Material and Methodology: V.P.K, M.K.R, A.S; Supervision: C.M.S.; Visualization: C.M.S.; Writing-Original Draft: V.P.K.; Writing-review & Editing: V.P.K.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

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