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Ethnobotanical knowledge, populations, and *ex-situ* conservation trials in *Juglans regia* Linnaeus (Juglandaceae) in Sikkim

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Abstract

The English or Persian walnut (*Juglans regia* Linnaeus) is one of the highly used edible nuts in the world. In India, north-western Himalaya is known to have largest availability of the species in nature and under domestication. However, in Sikkim, lack of scientific information on the population availability and of any satisfactory cultivation practices are the constraints in expanding this highly valuable bioresource. The present study, a first of its kind for Sikkim, provides details on ethnobotanical knowledge, populations and *ex-situ* conservation on *J. regia*. Ethnobotanical knowledge and use practices offered over nine areas of importance. Four population sites exhibited a poor to moderate availability of species; density ranged between 5 ind/ha (1500 m) to 40 ind/ha (1424 m). Significant correlations were recorded among the seedling growth, seed size and days taken to final seedling emergence (FSE); seedling collar diameter strongly (negatively) correlated to days of FSE. Seed length significantly influenced to plant height and collar diameter at early stages of growth. Transplanted seedlings under different growing conditions showed varied responses; the open condition appeared most favourable in nature. Direct seed sowing in three nursery conditions resulted better seedling emergence performance over three habitat conditions in nature; under canopy microhabitat provided significantly better for the highest emergence and early onset time.

Key words: *Juglans regia*, Ethnobotany, *ex-situ* conservation, field trials, seedling emergence, seed-size

INTRODUCTION

Of the 20 recorded species of genus *Juglans* Linnaeus, the English or Persian walnut (*Juglans regia* Linnaeus) is most widely cultivated (McGranahan & Leslie 1990). As an important non-timber forest produce, *Juglans regia* (Juglandaceae; Walnut; Akhrot in Hindi) is reported to be a native from south-eastern Europe to north-western China (Xinjiang province) through Turkey, Caucasus, northern Iraq, Iran, Pakistan and India, Pamir, Nepal, Himalaya and Tibet (Leslie & McGranahan 1988). The temperate zone with 900 – 3500 m altitude is favourable climatic zone for the species. In India, *J. regia* has a wide distribution from northwestern Himalaya to eastern Himalayan ranges, extending to Darjeeling and Sikkim. In Sikkim Himalayan region, Walnut is found growing along 1200 m to 2000 m amsl. The deciduous, monoecious and heterodichogamous in nature, *J. regia*, normally grows to 20 m heigh; however, it is possible to have records of 100 – 200 years old trees (Khan *et al* 2010), and even 1,000 years old (Leslie & McGranahan 1998). The species is commercially domesticated in India, China, USA, Spain, France, Italy, etc for both timber and nut production. In nature, trees supplement the valuable food supply to wild animals, thus the species becomes important element of forest ecosystem.

The high nutritional value and excellent quality timber of Walnut offers significantly good economy to people. For containing polyunsaturated fatty acids, especially 18:2 and 18:3 and protein value (Savage *et al* 2001), and in having multi-used medicinal properties for human, species became one of the most favourite ethnobotanical plants. Also, the consumption of nuts may help providing some protection against heart diseases (Savage *et al* 2001). Roots and resinous and scented bark of the tree are anthelmentic, astringent and detergent (Chopra *et al* 1986). All parts of tree contain an aromatic compound, called, Juglone (C10H6O3; 1,4-napthaquinone, 5-hydroxy-8CI; Harborne 1988), which may vary between trees (Thakur & Chahalan 2011). Walnut is reported to have anti-carcinogenic effects (Thakur & Chahalan 2011). The high calorie level and rich nutrient composition make the fruits highly usable in food industry worldwide.

In *J. regia*, Khan *et al* (2010) found existing genetic diversity amongst genotypes collected from Pakistan. Similarly, genetic structure of 12 domesticated European and 3 natural and naturalized Asiatic populations exhibited genetic diversity amongst populations (Fornari *et al* 1999). In Romania, large genetic variability amongst natural hybrids of local populations and amongst populations was observed in *J. regia* (Botu *et al* 2001). There is, however, negligible published scientific works on population studies on Walnut in India. In Sikkim the situation is further bleak on studies on population structure of *J. regia*.

In *J. regia in-vitro* propagation has proved successful in some cases (Kaur *et al* 2006; Vahdati *et al* 2008), however, propagation using conventional methods is important for practical point of view especially looking at the easy to handle demand of the farmers and land managers. Hardwood cuttings of Walnut have also been tried for its propagation (Günes 1999). However, the conventional methods using seeds asexually is the best way aiming the conservation of genetic diversity in plants (Pradhan & Badola 2011a). In *J. regia*, some conventional seed germination studies are available elsewhere (Memmedov 1976; Frutos 2009); however, the Sikkim genetic stock has hardly been tested so far. To ascertain successful *ex-situ* conservation and enriching agro-forestry system, it would be important to have assessment and monitoring of the plants transplanted in nature, comparing to those growing in nursery conditions. This is much more important under the present global warming and climate change, as the understanding of adaptive behaviour of transplanted seedlings will help better conservation planning of targeted taxa (Pradhan & Badola 2010a). In Sikkim, such studies on *J. regia* are missing as the north-eastern region as a whole is far behind in cultivation practices than the western Himalaya. Understanding on ethnobotanical knowledge, populations and *ex-situ* conservation are imperative for planning conservation and management of any high value plant species (Badola & Aitken 2010; Badola & Butola 2005).

The present study on *Juglans regia* highlights, (i) ethnobotanical knowledge and traditional use practices; (ii) status of populations of species in mixed forests where people use biological resources from the sites; (iii) ex-situ propagation and field trials under nursery and in nature, using conventional approaches. The propagation trial in nature is one of the innovative aspects of the present study.

MATERIALS AND METHODS

For ethnobotanical knowledge, local folks were interacted in parts of Sikkim where *Juglans regia* Linnaeus (Juglandaceae) found growing. In addition, two villages in west Sikkim, viz. Martam and Sapong were targeted by using unstructured interviews. Over 80 individuals were categorically asked for their specific responses to structured knowledge and use practices, and data were analyzed. To get data on *J. regia* populations, four represented sites in Sikkim, where the species is a prominent component of the mixed forests and the sites have been offering bioresources to community people, were studied. At each site, ten quadrates of 10 x 10 m were randomly laid and all woody taxa were quantified (adults and young) and their girth measurements taken at breast height using measuring tape. Topography, aspect and altitude, etc were also recorded. Data were computed and analyzed for plant density, relative density, frequency, relative frequency and basal area, following standard methods (Curtis & McIntosh 1950; Misra 1968; Smith 1980).

For *ex-situ* conservation, a tri-experimental approach was adopted. For the first two experiments, mature fruits of *J. regia* were collected from trees growing at 1500 m amsl in Bermoik, west Sikkim in October 2008. In laboratory, the seeds were removed and screened out by discarding impurities and damaged or insect bitten ones, and room dried for 20 days before experiments initiated. For either experiment, conducted in polyhouse during January 2009, raised beds (25 cm above surface) were prepared (using forest humus, sand and garden soil in ratio of 2:1:2 by volume). In first experiment, 100 seeds were used; their morphological variables were recorded viz., seed length (mm) and seed diameter (mm). Each seed was numbered and accordingly sown in rows (in polyhouse, maintaining distance of 15 x 15 cm between them) in such a way that each seedling emergence and growth data could be recorded individually. Watering and weeding was done as per the requirement. Daily observation was made for the seedlings emergence from each individual marked seed, till maximum emergence achieved. Data on periodical growth measurements of seedling were correlated with seed traits and the days taken to emergence for each seed, employing Pearson correlation coefficients.

In second experiment, 300 seeds were directly sown in the beds, maintaining 15 cm distance between them. Watering and weeding was done as per the requirement. Daily observation was made for the seedlings emergence till the constant reading obtained. After 12 weeks of maximum emergence, randomly, 100 healthy seedlings were carefully uprooted and divided in to four sets. All seedlings were measured for their growth parameters, treated as initial readings before transplantation. Three sets was transplanted in three habitat plots in nature at Pangthang, a broadleaf mixed forest site (ca 2000 m amsl), *viz*. Open slope, under tree canopy and marshy sites; and one set transplanted in open nursery condition, maintaining a distance of 45 x 45 cm between two plants. Initially, watering was done for each plant; after establishing over four weeks, the plants were left for natural weather conditions, except for nursery plants. For each growing condition, 12 plants were tagged to monitor and record their periodical growth and survival over 98 weeks after transplantation. Data were analyzed using one way ANOVA.

For third experiment, targeting the effect of different natural and nursery growing conditions on seedling emergence, the fruits were collected during October 2010 from Hee-kyangbari, west Sikkim (1900 m altitude) and seeds were processed in the same way as above, and stored in sealed plastic bags at 4^0C until experimented. In February end, 2011, healthy seeds were directly sown in six growing conditions (3 each in nursery and in nature). In nursery (three conditions: green house, net shade and open nursery), the beds were thoroughly prepared and provided with substrate combination of garden soil + forest humus + sand in a ratio of 2:1:2 by volume, maintaining 3 replicates of 20 seeds each, randomly. In nature, seeds were directly sown maintaining sub-plots, as 3 replicates (20 seeds each) in each 3 habitats, viz. under canopy, tree gap and grassy slope. Here, the soil was not amended by any external substrate but the natural substrate maintained. For each growing condition, a distance of 15 x 15 cm between seeds was maintained. Watering and weeding was done as per requirements; however, in nature, the watering was done only during early spell of seeds sowing and the conditions were left for nature care. Daily observation was made for the seedlings emergence till the constant reading obtained. The data were analyzed for mean seedling emergence, onset time, mean emergence time, etc. Mean emergence time (MET) was calculated following Nichols & Heydecker (1968). The least significant differences estimated following Snedecor & Cochran (1967).

RESULTS AND DISCUSSION

Ethnobotanical knowledge and traditional use practices

Ethnobotanical use of bioresources and industrial sub-sectors are highly related ventures (Pradhan & Badola 2008; Badola & Aitken 2010). In Sikkim, *Juglans regia* is highly used as very durable timber for making furniture, and fruits (nuts) are considered nutritious for having pharmacological properties by the local people, besides it has other ethnobotanical uses (Fig. 1). The fruits have high demand in local market for consumption as edible dry fruits. The edible pericarp is used to make '*Chatni*', by crushing and after adding chilies, salt, etc. New leaves and young shoots are used to poison the fishes during seasonal catch in springs/ rivers; the paste is mixed in the spring/ river water at specific locations where fishes are accessible to gather, which appeared as common ethnobotanical knowledge in two study villages (Fig. 1). Young shoots and flowers are considered poisonous by majority of respondents, if taken by animals including humans. Villagers consider the green bark of young stem during February to April as poisonous. For apprehension of poisonous nature, people do not prefer dry leaves either for bedding the cattle or as fodder (100% respondents for either villages (Fig. 1). Almost all the villagers responded that they do not eat even an edible mushroom if found growing on Walnut tree, for considering it poisonous. The outer cover of unripe fruits is used to make vegetable dye giving dark green to somewhat brownish colour, but this knowledge is limited to few respondents only (Fig. 1). The timber is considered as immensely durable and quality product by 100% respondents and used for making high class and expensive furniture. The timber is sold in local market at the rate of Rs. 40 per one square feet. The wood is also used as fuel in villages; however, due to restriction on harvesting of this timber, only a fewer number of villagers acknowledged the same. People also use the stony part of the seeds, which is treated as good fuel starter, as over half of the targeted villagers in general responded that (Fig. 1).

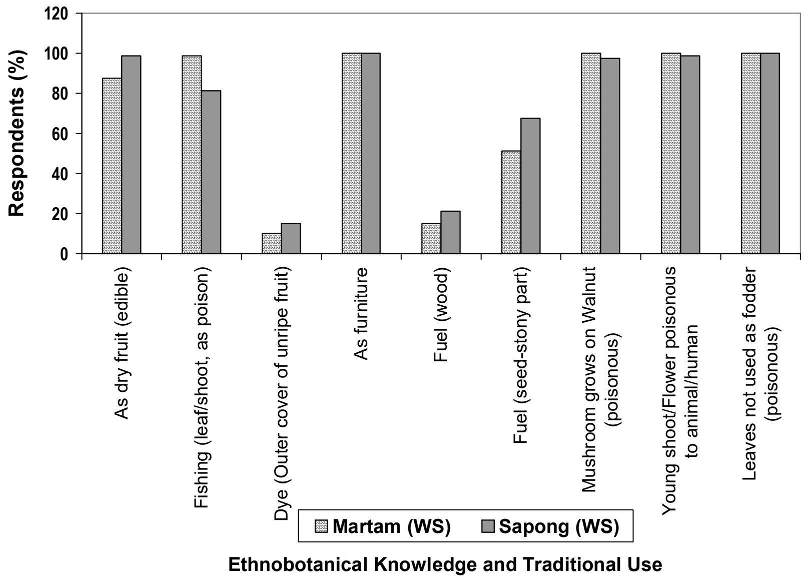

Fig. 1: Ethnobotanical knowledge and traditional use practices in *Juglans regia* in two villages in West Sikkim

Population studies

The four identified sites for *Juglans regia*, along 1424 m to 1700 m altitude at 40º to 60º slope, had tree density ranging from 5 individuals/ha (at 1500 m) to 40 individuals/ha (at 1424 m; Table 1). Similarly, frequency ranged from 20 % (1500 m) to 60 % (each at 1424 m and 1600 m). The *Michelia doltsopa* DC. and *M. velutina* DC. emerged as common associate tree species in each site (Table 1). The relative density followed more or less similar trend to that of density; however, maximum relative frequency of 20 % appeared at 1600 m for *J. regia*. Maximum basal area, i.e. 338.7 m^2/ha was calculated at 1600 m, but it was very low at 1500 m (2.03 m^2/ha). The lowest values at 1500 m, accompanied by complete absence of any saplings and seedlings, are indicative of anthropogenic pressures, as the site is very close to habitation. In general, population sites indicate poor to moderate availability of individuals of *J. regia*, which strongly suggests for appropriate species conservation management efforts in place.

Table 1. Locations, population status of trees and associated species of *Juglans regia* in study sites in Sikkim

STUDY SITE (altitude, aspect, slope)	Density (individual/ha)	Frequency (%)	Relative density (%)	Relative frequency (%)	Associated species
Hee-goan, West Sikkim (1424 m, South, 40^0)	40	60	20	17	*Alnus nepalensis, Michelia doltsopa, M. velutina, Machilus edulis, Macaranga sp, Nyassa sessiliflora,Erythrina arborescens,Engelhardtia spicata, Symplocos theifolia*
Bemoik, West Sikkim (1500 m, North, 40^o)	5	20	3	5	*Alnus nepalensis, Ficus roxburghii,Terminalia myriocarpa, Michelia doltsopa, M. velutina, Nyassa sessiliflora, Macaranga pustulata, Machilus edulis*
Chakung, West Sikkim (1600 m, North-east, 60^o)	30	60	19	20	*Castanopsis tribuloides, Michelia doltsopa, M. velutina, Symingtonia populnea, Pinus roxburghii*
Soreng, West Sikkim (1700 m, East-west, 50^o)	15	40	9	12	*Michelia doltsopa, M. Cathcartii, M. velutina ,Engelhardtia spicata,Pinus roxburghii, Castanopsis tribuloides, Nyassa sessiliflora*

Ex-situ conservation trials

Experiment I- Determining relationships amongst seed size, seedling emergence and seedling growth: The seed length (48.59 mm) and diameter (39.37 mm) showed strong relationship ($p<0.001$). Total seedling emergence (54 %) took 90.6 days average time (range: 74 to 107 days). The seed size did not show any significant relationship with the days taken to seedling emergence, thus supporting previous studies suggesting lack of relationship between seed mass and seedling emergence percent (Jones *et al* 1994; Chen *et al* 2002). After 10 days of final seedling emergence (FSE), the average stem height was 49.5 cm, which reached to 64.5 cm after 50 days of FSE. Plant height negatively but significantly correlated with days taken to FSE after 10 days of FSE (Fig. 2), but fell to a positive relationship after 50 days of FSE, indicating that the plant height after a long period will be influenced by growing environment. The plant height had a significant positive relationship with seed length until 25 day after FSE, which plunged insignificant after 50 days; however, seed diameter did not have any affect on plant height. Seedling collar diameter significantly ($p<0.001$) and negatively correlated with the days taken to FSE after 10 and 25 days of FSE, but the significant level diluted after 50 days of FSE ($p<0.1$; Fig. 2). Seed diameter had no significant effect on collar diameter; whereas, the seed length significantly correlated for the same after 10 days of FSE. The study does not fully advocate that the seed size may be effective to seedling growth in plants (Wulf 1986). In present case, collar diameter found to be significantly influenced by the days taken to FSE, as important morphological trait to seedling health which can be used in identifying vigorous seedlings for field plantation (Pradhan & Badola 2010b, 2011b).

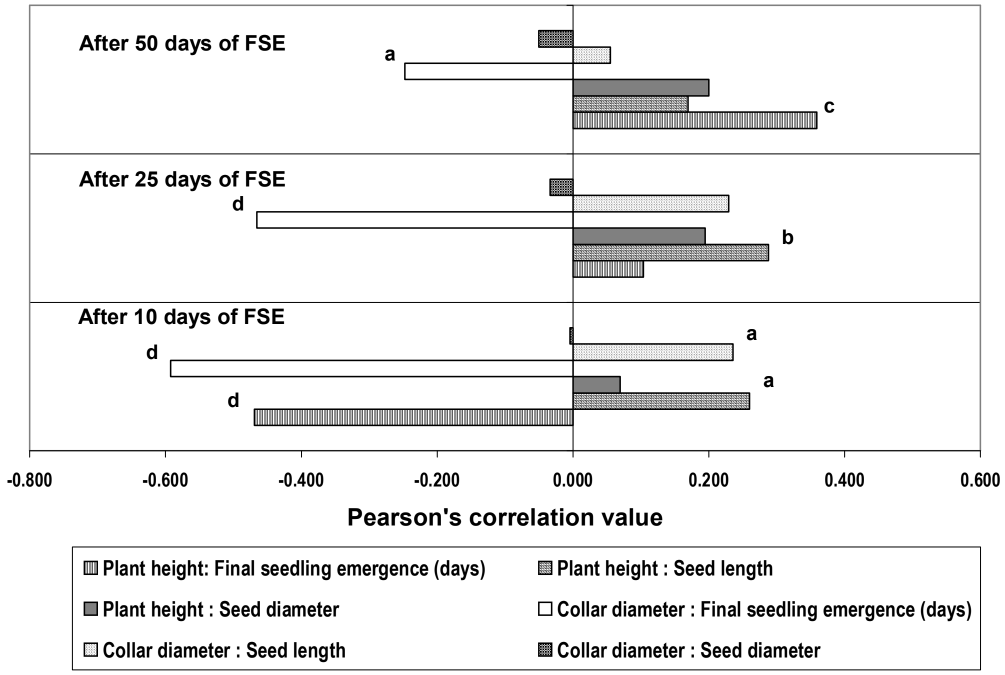

Fig. 2: Pearson correlation coefficients between seedling height and collar diameter with days taken to final seedling emergence (FSE), seed length and seed diameter, after three periods after final seedling emergence in *Juglans regia* in nursery in Sikkim (**a**: $p<0.1$; **b**: $p<0.05$;**c**: $p<0.01$; **d**: $p<0.001$)

Experiment II- Field trials- plant survival and growth in different growing conditions: After 12 weeks of maximum seedling emergence, over 98 % seedlings were survived in nursery. The transplanted seedlings under different growing conditions too showed high survival after 54 weeks, which accounted to be 100 % in both open slope (nature) and nursery, while under canopy (nature) and marshy conditions (nature) recorded over 90 % and 50 % survival, respectively. After 98 weeks of transplantation, under marshy condition, hardly any plant could be survived; hence data were analyzed only for remaining three growing conditions. In nursery and open slope (nature), 90% plants were survived, whereas, under canopy (nature) could support to 67 % plant survival (Fig. 3). Maximum increment (30.1 %) in plant height (87.5 cm) over its initial value (67.6 cm) obtained in open slope (nature), which was significantly greater ($p<0.001$) over other two growing conditions (Figure 3). Similarly, the plant collar diameter (13.5 mm) achieved highest increment (53.2 %) over initial reading (8.8 mm) in open slope (nature; $p<0.001$; Figure 4). The results reflect open (nature) condition most favourable for the sound plant growth (height and collar diameter).

Experiment III- Effect of different growing conditions on seedling emergence: In general, all nursery conditions proved very favourable to significantly higher seedling emergence with lesser emergence timings, over nature plots (Table 2). In nature (Baskin & Baskin 1998; Ceraboloni *et al* 2004; Pradhan & Badola 2010a, b) as well as in nursery (Butola & Badola 2006, 2008), variability in seed germination behaviour among growing conditions are recorded within in a species. In present study on *J. regia*, the seedling onset time (days), mean emergence time (days)

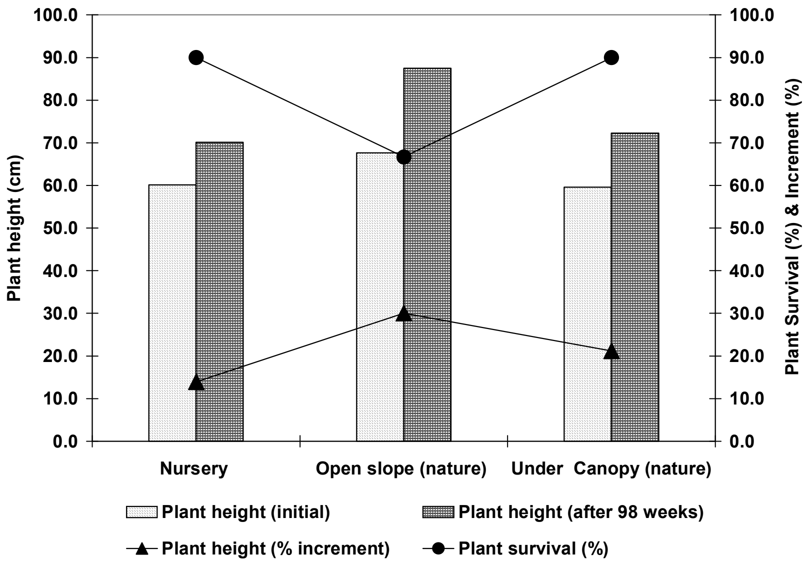

Fig. 3: Survival of plants and plant height, after 98 week of transplantation in three growing conditions, in *Juglans regia*

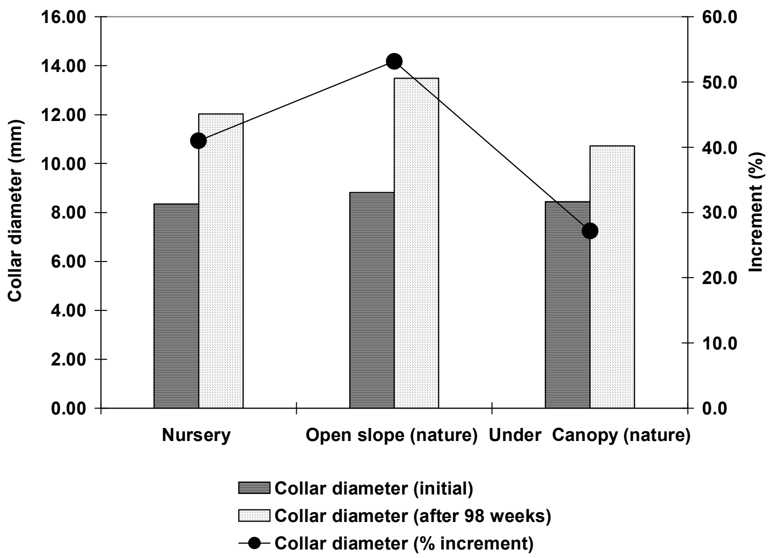

Fig. 4: Plant collar diameter, after 98 week of transplantation in three growing conditions, in *Juglans regia*

and 50 % emergence time (days) were significantly ($P<0.05$) lowest under poly house among all six growing conditions (Table 2). Among all conditions, seedling emergence was significantly ($P<0.05$) highest (58%) in net shade followed by poly house (55%; Table 2). Early onset of seedling emergence in all nursery conditions over field conditions, indicating the seeds have to wait for congenial time under relatively harsh environment in nature. Prevailing unfavourable conditions for seedling emergence in natural environment may be erratic in time and space and thus seeds develop germination strategies of encountering such fluctuations in habitats (Fenner & Thompson 2005, Norden *et al* 2008). Among nature sites, significantly ($p<0.05$) early onset, lesser mean emergence time and 50% emergence time, as well as highest germination in under canopy could be related to their inherent adaptive mechanism for survival (Pradhan & Badola 2011a).

Table 2. Effect of different growing conditions (nursery and natural) on seedling emergence behaviour in *Juglans regia* in Sikkim

	Open (nursery)	**Net-shade (nursery)**	**Polyhouse (nursery)**	**Under-canopy (nature)**	**Tree-gap (nature)**	**Grassy-slope (nature)**	**LSD (P<0.05)**
Seedling onset time (days)	50.7	45.7	41.3	87.3	94.0	95.0	4.06
Mean Emergence Time (days)	63.5	61.6	56.5	92.1	96.4	95.9	2.20
50% emergence time (days)	66.7	59.3	55.7	92.3	94.3	95.0	2.87
Seedling emergence (%)	45.0	58.3	55.0	26.7	21.7	11.7	9.52

The study on *Juglans regia*, in Sikkim, concludes and recommends, (i) the species offers very high ethnobotanical utility for people; (ii) population studies do not indicate good availability of individuals and recommend for appropriate conservation management initiatives; (iii) the seedling collar diameter negatively correlated with days to emergence and can be taken as strong indicator of healthy plant growth; (iv) in nature, open habitat is favourable recommended growing condition for plant transplantation; (v) for mass multiplication, both net-shade and polyhouse are most appropriate conditions; (vi) in nature, direct seed sowing is recommended under canopy and in tree-gaps, as favourable habitat for *in-situ* conservation and specially for the species recovery efforts by foresters and land managers, thus strengthening up-scaling technologies for the species. The study finally suggests for more field trials and technical monitoring of growth and phenology of *J. regia* under natural habitats, which may provide useful information on climate change, as yet another conventional and easy to handle tool (Badola 2010).

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