Tropical Ecology **48**(1): 99-105, 2007 ISSN 0564-3295

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Germination behaviour of some leguminous and actinorhizal plants of Himalaya: Effect of temperature and medium

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Abstract: In the present study seed germination behaviour of some common nitrogen fixing species have been investigated under different temperature regimes and medium. Of the six selected species three were actinorrhizal **(***Alnus nepalensis*, *Coriaria nepalensis* and *Myrica esculenta*) and three were legumes (*Lespedeza gerardiana*, *Vicia rigidula* and *Casia floribunda***)**. Seeds of selected species were collected and kept under four temperature regimes. The two medium used were filter paper and soil + sand mixture. The germination of *L. gerardiana* seeds remained unaffected by temperature and in all conditions seed germination was 100%. However, in *A. nepalensis*, *C. nepalensis* and *C. floribunda* maximum seed germination was observed in alternate day/night temperature and in *M. esculenta* and *V. rigidula* maximum seed germination was observed at 25 °C and 35 °C temperature, respectively. In all species compared to soil + sand mixture seed germination was greater on moistened filter paper. Mean germination capacity of each species was greater than the percent mean germination value indicating that many seeds could not resume physiological activity.

Resumen: En el presente estudio se investigó el comportamiento germinativo de las semillas de algunas especies fijadoras de nitrógeno comunes bajo diferentes regímenes de temperatura y en diferentes medios. De las seis especies seleccionadas, tres fueron actinorrícicas (*Alnus nepalensis*, *Coriaria nepalensis* y *Myrica esculenta*) y tres fueron leguminosas (*Lespedeza gerardiana*, *Vicia rigidula* y *Casia floribunda*). Las semillas de las especies seleccionadas fueron recolectadas y conservadas bajo cuatro regímenes de temperatura. Los dos medios utilizados fueron papel filtro y una mezcla de suelo y arena. La germinación de semillas de *L. gerardiana* no fue afectada por la temperatura y en todas las condiciones fue de 100%. Sin embargo, en *A. nepalensis*, *C. nepalensis* y *C. floribunda* la máxima germinación fue observada bajo una temperatura alternante día/noche, mientras que en *M. esculenta* y *V. rigidula* la máxima germinación de semillas se observó a temperaturas constantes de 25 ⁰C y 35 ⁰C, respectivamente. En todas las especies, la germinación de semillas en la mezcla de suelo y arena fue mayor en comparación con la obtenida en papel filtro humedecido. La capacidad promedio de germinación de cada especie fue mayor que el valor porcentual promedio de germinación, lo que indica que muchas semillas no pudieron reiniciar la actividad.

Resumo: Neste estudo foi analisado o comportamento da germinação de sementes de algumas espécies fixadoras de azoto sujeitas a diferentes regimes de temperaturas e meios. Das seis espécies seleccionadas três eram ectinomicorrízicas (*Alnus nepalensis, Coriaria nepalensis* e *Myrica esulenta*) e três eram leguminosas (*Lespedeza gerardiana*, *Vicia rigidula* e *Casia floribunda*). As sementes das espécies seleccionadas foram colectadas e mantidas sob quatro regimes de temperatura. Os dois meios usados foram o papel de filtro e solo e uma mistura de areia. A germinação das sementes de *L. gerardiana* não foram afectadas pela temperatura e em

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todas as condições a germinação das sementes foi de 100%. Contudo, para a *A. nepalensis*, *C. nepalensis* e *C. floribunda* a germinação máxima das sementes foi observada para uma temperatura alternada dia/noite enquanto que para a *M. esculenta* e *V. rigidula* a germinação máxima foi observada a uma temperatura constante de 25 ºC e 35 ºC, respectivamente. Para todas as espécies a comparação entre solo e mistura de areia verificou-se que a germinação foi maior no papel de filtro húmido. A capacidade de germinação média de cada espécie foi maior do que a percentagem média de germinação indicando que muitas sementes não foram capazes de continuar a actividade fisiológica.

Key words: Germination capacity, germination value, mean daily germination, response breadth, temperature.

Introduction

Natural regeneration of species through seeds would depend primarily upon production and germination capacity of seeds and successful establishment of seedlings. Each species has its own characteristic set of germination requirements. Whether or not viable seeds of a given species will germinate and the time at which they do so depend on a number of factors, including those present in the seed's environment (Bewley & Black 1994). Like many other physiological processes, seed germination is temperature dependent and the optimal temperature varies considerably between species (Teketay 1997). The outcome of germination in nature depends to great extent upon the duration of optimal conditions of the above factors. Therefore, a proper understanding of the potential for natural regeneration requires knowledge of the influence of these factors on germination of species involved and the suitable period during which suitable condition exist in nature (Sah *et al.* 1989).

The young and rising Himalayan Mountains are particularly vulnerable to landslides and erosion. The hill slopes are subjected to severe biotic stresses in form of over grazing, lopping, recurring fires, defective road construction etc. All these factors have enhanced the rate of landslides to an alarming level. The surface of landslide-damaged sites is an inhospitable environment characterized by shortage of soil, soil moisture and nutrients (Chaudhry 1989). Reclamation of such sites thus requires suitable species that not only hasten the process of recovery, but could also serve significantly in the overall nitrogen economy of region by replenishing nitrogen-poor habitats. This not only reflects the argument previously produced for the importance of nitrogen fixing species in land reclamation, but also extends it to the subtleties of what may be involved in practice.

In the present study seed germination behaviour of some common nitrogen fixing plant species of Kumaun Himalayan region have been studied at four different temperature regimes and two varying mediums. The main objective of present study was to evaluate suitable species which can be used for revegetation of degraded lands having different soil temperature regimes.

Material and methods

The seeds of six nitrogen fixing species were collected from different sites at Nainital and adjacent area during 2002-2003 from monospecific patches of plant species growing naturally. The species were: *Alnus nepalensis* D.Don, *Coriaria nepalensis* Wall, *Myrica esculenta* Buch-Ham ex D.Don, *Lespedeza gerardiana* Grah. ex Baker, *Vicia rigidula* Royal, *Casia floribunda* Cav. Descr. Of these, former three are actinorhizal and later three are legumes. Distribution and habitat characteristics of these species are given in Table 1. Before seed collection, an extensive survey of the area was made and mature healthy plants were marked for seed collection. Plants exhibiting poor growth, abnormalities or disease were avoided. Mature disease and insect free fruits (pods and cones) were collected and brought to the laboratory. Fruits were air dried and separation of seed was done manually. Thus, collected seeds

Table 1. Distribution and habitat characteristics of the species studied.

Species/ Family	Life form	Distribution altitude, m	Habitat
Alnus nepalensis D.Don (Betulaceae)	Tree	1200-2500	Near streams forms gregarious patches
Coriaia nepalensis Wall. (Coriariaceae)	Large deciduous shrub	1200-2600	On rocky and eroded slopes
Myrica esculenta Ham. (Myricaceae)	Tree	1500-2200	Common in *Quercus leucotrichophora* and mixed oak forests
Lespedeza gerardiana Grah. Ex Baker (Papilionaceae)	Under shrub	1500-2000	Open grassy localities
Vicia rigidula Royle (Papilionaceae)	Herb	2000-2800	Forest edges, scrub jungles and roadsides
Cassia floribunda Cav. Descr (Cesalpiniaceae)	Shrubby herb	Up to 1800	Throughout the hills in waste lands

were cleaned and packed in polyethylene bags for the seed germination studies.

Germination tests of each species were done by taking 5 replicates of 20 healthy seeds. Only seeds that sank to the bottom when immersed in water were used in these experiments. Seeds were sterilised in 0.1% $HgCl_2$ solution and kept under different tests conditions.

Experiment 1

Petri dishes and filter paper were sterilised in an autoclave at 120 °C for half an hour. The seeds were placed in the dishes between moistened filter papers and kept at room temperature and in three incubators at 15 °C, 25 °C and 35 °C constant temperature. Before keeping Petri dishes the incubators were sterilised by keeping the temperature on maximum for 2 h and then wiping thoroughly with alcohol. The papers were regularly moistened and observed daily up to 5 weeks (in case of *M. esculenta* up to 10 weeks).

Experiment 2

Sieved forest soil and fine commercial sand were mixed in 1:3 ratio, sterilized at 110 °C in an oven and filled in earthen pots. Seeds were placed in the soil, watered regularly with distilled water and observed daily up to 5 weeks.

In the Petri dishes the seeds were considered germinated if the radical exceeded 3 mm in length and in soil these were considered germinated when the shoot appeared above the soil surface. At the end of each experiment, ungerminated seeds were classed either as sound viable or dead. Seeds that collapsed when pinched gently with forceps and had a yellow or brown embryo were considered dead while seeds that did not collapse when pinched and had a firm, white embryo were considered sound viable. Results were expressed as:

(i) Germination capacity, GC, the percentage of seeds that had germinated at the end of the test, calculated following Paul (1972) as:

$$GC\ (\%) = \frac{\text{Total germinated seeds + total ungerminated sound seeds}}{\text{Total seeds tested}} \times 100$$

(ii) Germination value, GV, calculated according to Czabator (1962) as:

$$GV = MDG \times PV$$

where, germination value, GV, combines germination speed (represented by peak value) and completeness (mean daily germination) into a single index, in which MDG = mean daily germination, the accumulated number of germinant at the end of the test divided by the number of days in the test, and PV = peak value, the maximal quotient obtained by dividing the accumulated number of germinant by the corresponding number of days.

Niche breadth for each species was calculated using Levins's (1968) equation (see Bargali & Singh 1995):

$$B = \frac{1}{(\sum_{i=1}^{s} Pi^2)\ S}$$

where, B is niche breadth; Pi is the proportional response of species P in the i^{th} temperature regime and S is the number of temperature regimes. The measure ranges from 0 to 1 with 1 being a perfectly even distribution of response.

Results

Analysis of variance showed a three-way interaction ($p<0.05$) between species, temperature regime and medium. In each species percent germination of seeds increased with increasing time; however, time taken for the initiation of germination varied from species to species (Fig 1). In *A.nepalensis, C. nepalensis* and *C. floribunda* maximum seed germination was observed in alternate day/night temperate regime and germination decreased from 15 °C to 35 °C temperature. Germination value and germination capacity of these species showed similar trends

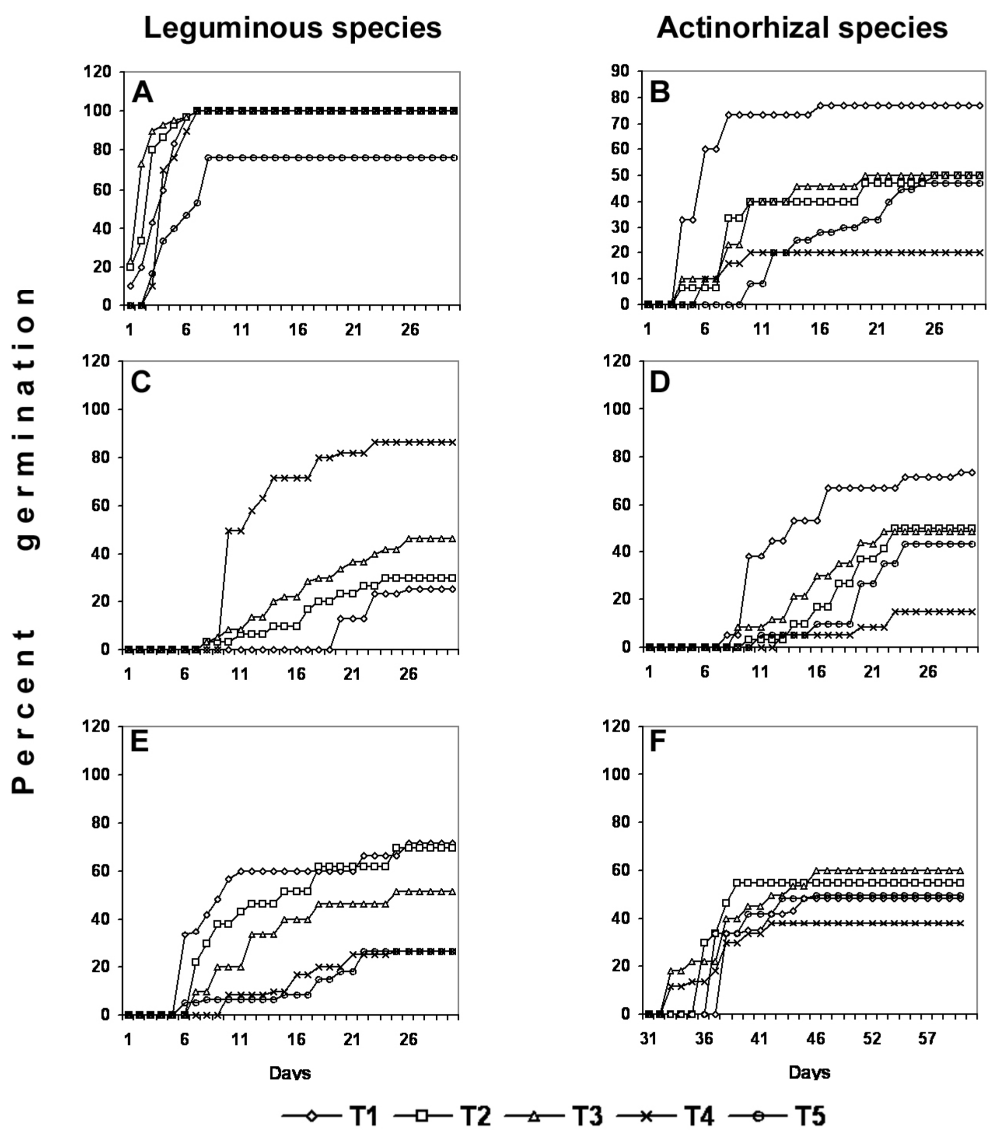

Fig. 1. Germination behaviour of *Lespedeza gerardiana* (A), *Alnus nepalensis* (B), *Vicia regidula* (C), *Coriaria nepalensis* (D), *Cassia floribunda* (E) and *Myrica esculenta* (F) as affected by temperature regimes and mediums. T_1 = Alternate day/ night temperature; T_2 =15 °C; T_3 = 25 °C; T_4 = 35 °C constant temperature and T_5 = soil + sand mixture.

(Table 2). In *V. rigidula* germination of seeds increased with increase in temperature and maximum germination was observed at 35 °C temperature regime. In case of *L. gerardiana* seed germination was not affected by temperature regime and in all conditions seed germination was 100% (Table 2). In *M. esculenta* seed germination started after 30 days and was maximum at 25 °C temperature in incubator. In most of the species germination capacity was high in comparison to the actual germination value indicating that seed could not resume physiological activity.

In all species, seeds on filter paper had higher ($p<0.05$) germination than seeds in soil (Table 2). *L. gerardiana* and *M. esculenta* showed wider response breadth as compared to other species while *V .regidula* showed narrow response breadth (Table 3). Optimal temperature condition for each species is also given in Table 3.

Discussion

Temperature regimes regulate seed germination by affecting enzymatic activities, reaction rates and changes in the physical state of cellular components. Low temperature may inhibit the catabolic activity and temperatures higher than the optimum tend to bring about the disruption of metabolic processes by inactivating some enzymes and denaturing proteins (Maguire 1973). Observations recorded in the present experiment indicate that alternate day/night

Table 2. Germination parameters of different species as affected by temperature.

Species	Temperature/Soil	Mean germination (%)	Mean daily germination	Mean germination value	Mean germination capacity (%)
A. nepalensis	Alternate day/night	76.7 ± 1.82	0.51 ± 0.01	0.94 ± 0.02	83.3 ± 1.2
	15 °C	50 ± 0	0.33 ± 0	0.28 ± 0.02	66.7 ± 2.1
	25 °C	50 ± 4.5	0.33 ± 0.04	0.29 ± 0.05	66.7 ± 4.3
	35 °C	20 ± 3.8	0.17 ± 0.03	0.09 ± 0.01	66.2 ± 1.4
	Soil	46.7 ± 5.2	0.31 ± 0.01	0.12 ± 0.02	66.7 ± 2.1
C. nepalensis	Alternate day/night	73.3 ± 1.7	0.43 ± 0.03	0.38 ± 0.03	83.3 ± 4.3
	15 °C	50.0 ± 2.8	0.33 ± 0.02	0.14 ± 0.02	66.7 ± 4.3
	25 °C	48.3 ± 3.3	0.32 ± 0.02	0.15 ± 0.02	65.0 ± 0.9
	35 °C	15.0 ± 2.9	0.10 ± 0.02	0.015 ± 0.005	48.3 ± 3.1
	Soil	43.3 ± 3.4	0.29 ± 0.022	0.10 ± 0.017	50 ± 4.1
M. esculenta	Alternate day/night	48.3 ± 1.7	0.16 ± 0.005	0.034 ± 0.001	71.7 ± 1.7
	15 °C	55 ± 0	0.24 ± 0.0	0.086 ± 0	66.7 ± 3.3
	25 °C	60.0 ± 2.9	0.27 ± 0.01	0.072 ± 0.003	75.0 ± 2.9
	35 °C	38.3 ± 1.7	0.17 ± 0.007	0.031 ± 0.004	58.3 ± 2.5
	Soil	35 ± 2.2	0.23 ± 0.01	0.035 ± 0.003	70.0 ± 3.2
L. gerardiana	Alternate day/night	100 ± 0	2.9 ± 0	9.23 ± 0.42	100 ± 0
	15 °C	100 ± 0	2.9 ± 0	16.1 ± 1.1	100 ± 0
	25 °C	100 ± 0	2.86 ± 0.0	15.6 ± 1.33	100 ± 0
	35 °C	100 ± 0	2.86 ± 0.0	10.9 ± 0.32	100 ± 0
	Soil	76.7 ± 1.7	0.51 ± 0.013	0.42 ± 0.01	83.3 ± 3.2
V. rigidula	Alternate day/night	25.0 ± 1.2	0.17 ± 0.02	0.035 ± 0.004	66.7 ± 2.4
	15 °C	30.0 ± 1.7	0.20 ± 0.02	0.052 ± 0.005	71.7 ± 5.2
	25 °C	46.7 ± 2.2	0.31 ± 0.01	0.09 ± 0.024	80 ± 3.2
	35 °C	86.7 ± 5.7	0.58 ± 0.02	0.59 ± 0.07	91.7 ± 6.3
	Soil	25.0 ± 2.1	0.17 ± 0.01	0.033 ± 0	50 ± 4.1
C. floribunda	Alternate day/night	71.7 ± 1.7	0.48 ± 0.01	0.35 ± 0.01	88.3 ± 4.4
	15 °C	70 ± 3.5	0.47 ± 0.02	0.32 ± 0.01	83.3 ± 2.2
	25 °C	51.7 ± 1.7	0.34 ± 0.01	0.27 ± 0.05	66.7 ± 2.9
	35 °C	26.7 ± 2.1	0.20 ± 0.02	0.05 ± 0.006	40.0 ± 1.7
	Soil	26.7 ± 0.01	0.18 ± 0.01	0.43 ± 0.002	43.3 ± 1.1

Table 3. Response breadth and optimal condition for seed germination of the species studied.

Species	Response breadth	Maximum germination (%)	Condition for optimum germination	No. of days for maximum germination
A. nepalensis	0.865	76.7	Alternate day/night	22
C. nepalensis	0.836	73.3	Alternate day/night	29
M. esculenta	0.980	60.0	Constant 25 °C in incubator	45
L. gerardiana	1.000	100.0	In all temperature regimes	7
V. rigidula	0.791	86.7	Constant 25 °C in incubator	23
C. floribunda	0.902	71.7	Alternate day/night	26

temperature is the best for germination of most of the species. Similar observations were also made for other species by Bokhari *et al.* (1975), Rao (1986, 1992). This preference for alternate day/night temperature regime is expected, as species have to encounter marked diurnal variations in natural conditions. Thompson (1974 a, b) suggested that the diurnal fluctuations in temperature may stimulate the germination of seeds. Laboratory experiments with other species (Rao 1992) indicate that germination rates are highest with a fluctuating day and night temperature regime. In contrast to alternate temperature regime, *V. rigidula* showed maximum germination at constant 35 °C temperature in incubator indicating that all necessary physiological activities required for causing seed germination in this species have proceeded most smoothly at this temperature. Bokhari *et al.* (1975) also reported that some species apparently respond favourably and germinate better at constant temperature. *L. gerardiana* was the only species in which seed germination remains unaffected by temperature and showed 100% germination in all temperature regimes. Kozlowaski (1970) and Osmond *et al.* (1980) suggested that the species that germinate readily over a relatively wide range of temperatures should be easier to establish in the field than those with highly specific temperature requirements.

In the present study, *A. nepalensis*, *C. nepalensis*, *L. gerardiana* and *C. floribunda* showed high imbibition of water (indicated by increase in size of seeds) suggesting that these species have seed coats permeable to water. Kozlowski (1971) suggested that oxygen uptake of seeds increases soon after water is imbibed and respiratory activity generally follows the pattern of water uptake. Come & Tissaowi (1973) found that the higher the temperature in which, the seeds are placed to germinate, the smaller is the quantity of oxygen available to the embryo. This result in poor germination as obtained at 25 °C and 35 °C for most of the species. On the other hand seeds of *V. regidula* were enveloped in hard seed coats as indicated by low imbibition rates. The absorption rate is dependent upon degree of seed coat permeability as the seed coat indirectly inhibit rate of absorption by mechanically preventing seed tissue from expanding in pace with moisture uptake. The presence of an impermeable seed coat may prevent water uptake by seeds and so prevent germination, as seed do not resume physiological activity until they imbibe a certain amount of water. These seeds obtained desired hydration level when kept in constant high temperature.

Direct seeding has long been a technique for forest regeneration, advantages of which include control over stocking and genetic material, it being simpler and less expensive than planting especially when nursery facilities are limiting or non-existent (Putman & Zasada 1986). In nitrogen-fixing species the biological nitrogen fixation meets the relatively heavy demand for nitrogen in nutrient-poor degraded soils, and thus makes them useful in afforestation and reclamation of degraded and nutrient-poor lands. Each species used in the present study has capacity to germinate over a defined range of temperature. This indicates that temperature of the seed bed is a major factor in determining the germination capacity of seeds. Therefore, a preliminary study on seed germination behaviour of species over a temperature range could be useful in selection of site for establishment of a particular species. On degraded sites where original soil has deteriorated markedly, these nitrogen - fixing species can be regenerated through seeds. Once their crops become sufficiently large the return of nutrients through nutrient-rich leaf litter of nitrogen-fixing species will hasten the process of soil nutrient enrichment.

Acknowledgement

Financial support from Department of Science and Technology, New Delhi, in form of SERC Fast Track Project, is gratefully acknowledged.

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