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Response of rhizobial inoculation to seed germination with respect to various seed sources of *Albizia procera* collected from Uttarakhand

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Novelty statement
Germination of a seed is the primary basic conditions in afforestation by regular or artificial means. Seed gives the normal source to multiplication and conservation of vegetation. Forest tree improvement programs begin with the exploration of easy to get to variety in the whole scope of species spreading and delimitation of seed sources. The present study is helpful in selecting a good source of superior seed quality and germination method for healthy seedlings.

Abstract
Albizia procera commonly known as 'Safed siris' is an important agroforestry tree distributed transversely in India. Tree belongs to family Fabaceae, sub-family Mimosoideae and is important biological nitrogen fixing tree. Delayed and irregular germination is a serious constraint in the large scale propagation of *Albizia.* Leguminous trees and plants help to increase the soil fertility through nitrogen fixation and thereby increase the yield. *Rhizobium* is the microorganism that lives in a symbiotic relationship with leguminous plants and trees. It is distinctive in fixing the atmospheric nitrogen in the root nodules of plants and makes it available to the plants. The present analysis was conducted to explicate outcome of rhizobial inoculation on germination of seeds and seedling vigour index of *Albizia procera* collected from five different sites of Uttarakhand viz., FRI, ITBP, Ram Nagar, Pantnagar and Lal Kuan. The trial was carried out in laboratory using paper towel method. The seeds collected from different sites were inoculated with Rhizobium isolated from *Albizia procera* soil samples. The results showed that the seeds inoculated with Rhizobium performed better than the control. Seed emergence, root, shoot length of seeds collected from FRI showed maximum seedling germination percentage (84%), seedling vigor(784.42), shoot length (7.89 cm), root length (5.23 cm) and dry weight of seedling (10.965 g/seedling) followed by ITBP. Stunted growth of *Albizia procera-Rhizobium* was observed in Lal Kaun rhizobial strain. It can be inferred from the results that inoculation with rhizobia has incredible prospective in improving afforestration of *Albizia procera* plants. This result may be helpful in obtaining superior seedling of *Albizia procera* for nursery planting.

Keywords: *Albizia procera*, seedling vigor, shoot length, root length and total dry weight

Introduction
During the couple of years research on biofertilizer has gained attention. Researchers had made efforts to study the impact of *Rhizobium* on the plant growth for the production of quality seedling (Khan and Uniyal 1999) [21]. The importance of *Rhizobium* as biological nitrogen fixer and make the nutrients available to the plant is well documented in the literature. *Albizia procera* is commonly found on alluvial soil and in moist even muddy places. It is widespread in low lying moist Savannahs and having good compliance for growing in moist as well as drought conditions. Due to its wide distributional range, varying climatic conditions, its long evolutionary history, a large dissimilarity within and among variety is likely to occur which may be replicated in genetic establishment of its diversity. Their implication site/seed source variation study in tree upgradion is well recognized (Callaham 1964, Wright 1976, Suri 1984) [11, 33, 30]. These studies are necessary for scanning the available genetic variation, to utilize the most excellent matter for obtaining highest yield for further breeding work (Shiv Kumar & Banerjee 1986) [28] and also help in analyzing and comparing superior characters which have immense significance in breeding and/or tree up gradation programmes besides preserving these variations intact for future research programme (Devgiri 1997) [15]. Discrepancy in germination of seeds, growth of seedling and their morphological characteristics among sites already reported for several forest trees (Dangasuk *et al*., 1997) [13].

Divergence between the sites might be due to variation of different sites to varied ecological surroundings, genetic differences and soil types (Ginwal *et al*., 2005; Elmagboul *et al*., 2014) [20, 17].

Tree improvement programmes accomplishment depends upon the calculation of the amount, type and cause of genetic variability within a species. Proper planning and designing of provenance research enables quick and economical use of those provenances, which yield well adapted and productive forests. Provenance testing is essential to screen the naturally available genetic variation and to prefer the best accessible type for further increasing effective tree improvement and breeding effort. For tree improvement programme, it is important to attain information about the best provenance for the given site. Thus the purpose of this experiment was to assess the difference in location of *Albizia procera* based on germination, seed morphological and early seedling growth characteristics, in order to obtain the most suitable seed source for the production of quality seedlings (planting materials) for mass afforestation and agroforestry systems.

Objectives

1. Study the disparity in seed and pod distinctiveness of *Albizia procera* collected from different location of Uttarakhand
2. Study the outcome of rhizobial inoculation on various seed germination parameters of *Albizia procera* by paper towel method

Materials and methods

Collection of seeds

Seeds of *Albizia procera* were collected, from 15-20 years old mature and healthy trees from five different geographic locations of Uttarakhand *viz*., FRI, ITBP, Lalkaun, Ram Nagar and Pant Nagar. Collected seeds were sun dried before experiment.

Albizia procera *Albizia procera seeds*

Fig 1: Isolation, authentication and screening of rhizobia

Isolation from the Soil

For the isolation of bacteria, soil dilution and pour plate techniques were employed using 10gm of dried soil and serial dilutions were made up to 10^{-6}. The suspension was streaked on YEMA medium and kept for 2-5 days at 28±2°C. The colony were picked up and transferred to YEMA slant for further characterization.

Purification and preservation of rhizobia

Isolates obtained from soil samples of *Albizia procera* were purified on YEMA medium by streak plate method. Various authentication test (Congo red test, Bromothymol blue test, Hofer's alkaline test, Ketolactose agar test) were performed in order to confirm that the isolates were rhizobia. The purified isolates were preserved on slants for further studies.

Seed Sterilization and Seed germination

A study was conducted to elucidate the pattern of germination behaviour of *Albizia procera* seeds towards rhizobial inoculation among different seed sources. Germination response of seeds was studied in Dept. Of Seed Sc. &[2]Technology, College of Horticulture, UHF- Nauni, Solan, HP. The healthy and uniform seeds of *A. procera* were sterilized using 70% alcohol by dipping in a beaker for one minute followed by 0.2% HgCl solution for 50 sec and finally washed 5 times with sterile distilled water. Culture broth of rhizobial isolate was prepared and seeds were inoculated overnight with rhizobial broth. Then the rhizobia inoculated seeds were germinated by placing them between two layers of papers and rolled by towel. The rolled towels were placed in seed germination chamber in upright position. The relative humidity was maintained at 90-95%. Daily germination counts were recorded for two weeks or till it become constant for consecutive days. Different germination parameters observed are following:

a) Germination percentage: It is the percent of germinated seeds at the completion of germination.

b) Germination value: calculated by the method of Czabactor (1962) and defined as index combining rate and totality of seed germination.

Germination value = Peak Value × Mean daily germination

c) Germination energy index (GEI): Germination energy index was calculated by the method of Grouse and Zimmer (1958).

$$GEI = \frac{A1+(A1+A2)+(A1+A2+A3)\ldots\ldots\ldots\ldots+(A1+A2+An)}{(N+n)}$$

Where

A1, A2, A3…..

An = No. of seeds newly germinated up to n days.

N = Total number of seeds for experiment.

n = Number of days of observation.

d) Germination period: Number of days takes to complete germination. It will be recorded for each replication.

e) Vigour index: evaluated by the method of Abdul-Baki and Anderson, 1973.

Seedling vigour index-I = seedling length (cm) x Germination (%)

Seedling vigour index-II = seedling dry weight (mg) x Germination (%)

Seedling length (cm)

Length was measured by taking healthy seedlings (ten at the time) from each replication of sample and expressed in cm. Average value of replication of these seedlings was expressed.

Seedling dry weight (mg)

Seedling dry weight were calculated (ten normal seedlings per sample) by drying at 80˚C in hot air oven for 48 h. The

sample were cooled in desiccator for 30 minutes before its weight was taken. The average weight of each seedling expressed in milligrams dry weight of seedling.

Result

In the survey *Albizia procera* trees three from each site were marked at different places in Uttarakhand: FRI Dehradun, ITBP Dehradun, Pantnagar University, Rani Pokhri Rishikesh and Lal Kaun Forest Range (Table 1). The data of altitude, longitude and latitude with GPS coordinates was given in the Table 1. Root and soil samples were also collected from the marked trees.

Table 1: Sites of *Albizia Procera* in Uttarakhand

Name of location	District	Altitude (msl)	Latitude	Longitude	Plant height (m)	Plant diameter (cm)
FRI Dehradun	Dehradun	671	30° 20'38.89"N	77° 59'50.19"E	24	28
		660	30° 20'31.43"N	77° 59' 48.92"E	22	27
		666	30° 20'29.29"N	77° 49' 23.16"E	25	31
ITBP	Dehradun	626	30°18'33.90"N	77°59'37.54"E	20.5	23.8
		619	30°18'28.74"N	77°59'51.38"E	22	26
		618	30°18'18.07"N	78° 0' 1.27"E	20	29
Pantnagar University	Udham Singh Nagar	228	29°1'3.89"N	79°29'19.17"E	19.2	26
		229	29°1'9.99"N	79°29'17.93"E	23	28
		225	29°0'29.30"N	79°29'16.69"E	25	29
Ramnagar	Dehradun	558	30°10'34.81"N	78°13'5.49"E	20.5	23.8
		556	30°10'27.18"N	78°13' 18.46"E	26	32
		557	30°10'28.24"N	78°13'18.46"E	23	30
Lal Kaun Forest Range Nanital	Nanital	261	29°4' 1.10"N	79°31'19.05"E	22	29
		256	29°3' 43.82"N	79°31'26.47"E	20	27
		257	29°3' 37.34"N	79°31'19.06"E	24	28

Isolation of rhizobia associated with *Albizia procera* rhizospheric soil of different seed sources

In the present study we isolated the nitrogen fixing bacteria from collected adjoining soil samples of *Albizia procera*. Results for isolation of rhizobia from rhizospheric soil are shown in Table 2 below. Isolation of bacterial colonies was carried out from rhizospheric soil of *A. procera* collected from selected location of Uttarakhand. The growth on nutrient agar media was counted and reported as cfu/g of soil. The maximum bacterial count was observed in soil from FRI (240 X 10^4 cfu/g soil) and minimum in Pantnagar (150 X 10^4 cfu/g soil) among different seed sources.

Table 2: Bacterial viable count in rhizospheric soil of *Albizia procera* collected from different seed sources

	Sites	Bacterial population (10^4 cfu/g soil)
Uttarakhand	FRI Dehradun	240
	ITBP	220
	Pantnagar University	150
	Ramnagar	210
	Lal Kaun Forest Range Nanital	197
CD (0.05%)		3.45

Authentication of isolated Rhizobial isolates

Among all the bacterial isolates, isolated from rhizospheric soil 21% were acid producer while percentage of alkaline producers was 79%. Also 97% of bacterial isolates showed positive results for Ketolactose test while this percentage for showing positive results for Congo red test as well as plant infection test was 76% (Plate 1 & Fig 2). So, on the basis of these authentication tests more than 90% were confirmed as Rhizoba and further they were evaluated for various plant growth promoting traits.

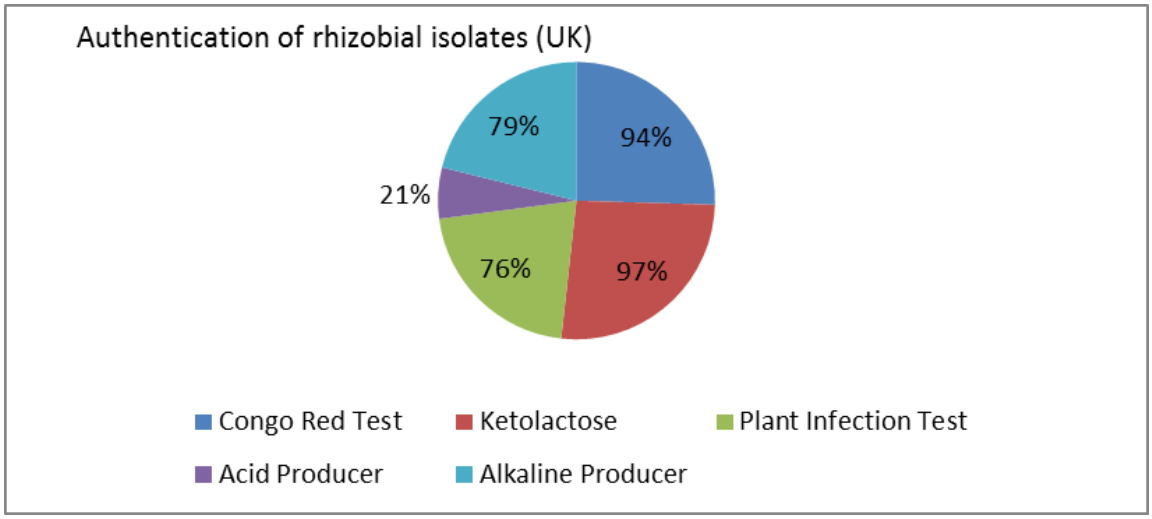
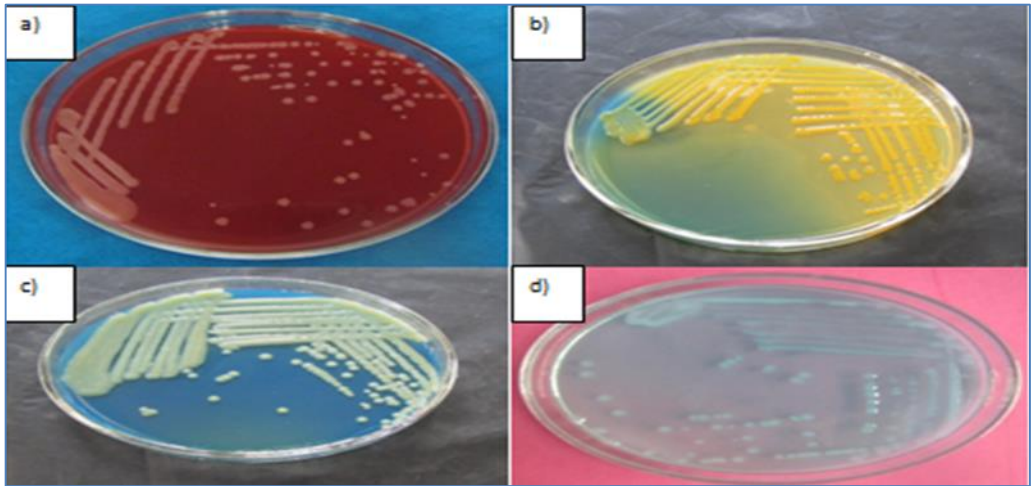


Fig 2: Authentication results of Rhizobial isolates isolated from *Albizia procera*



a) Congo red test, b) BTB test-acid producer, c) b) BTB test-alkaline producer and d) Ketolactose test

Plate 1: Various authentication test of rhizobia

Seed germination behavior of rhizobia inoculated seeds

Rhizobium isolated from test species was found to be effective for pretreatment of seed germination. After 18 days of incubation rhizobia treated *Albizia procera* seeds showed maximum seed germination comparatively to uninoculated seeds (Plate 2). Maximum seed emergence, shoot and root length of *Albizia procera* was found in seedling of FRI upon inoculation with *Rhizobium* (UF1) as compare to control. Maximum seed emergence, shoot and root length of *Albizia procera* was found in FRI-seed source with seed germination (72.4%), seedling vigor (782.64), shoot length (6.58 cm) and root length (4.23cm) followed by seeds from ITBP (Fig 3).

The germination studies of *Albizia procera* showed that FRI Dehradun showed maximum germination percent (82.36%), germination value (345) and germination energy index (4.12) followed by Ramnagar, ITBP, Lal Kaun. *Rhizobium* inoculation could bring about an increase in root and shoot formation of *Albizia procera* (Fig 3).

Plate 2: Rhizobia inoculated seed germination by Paper towel method

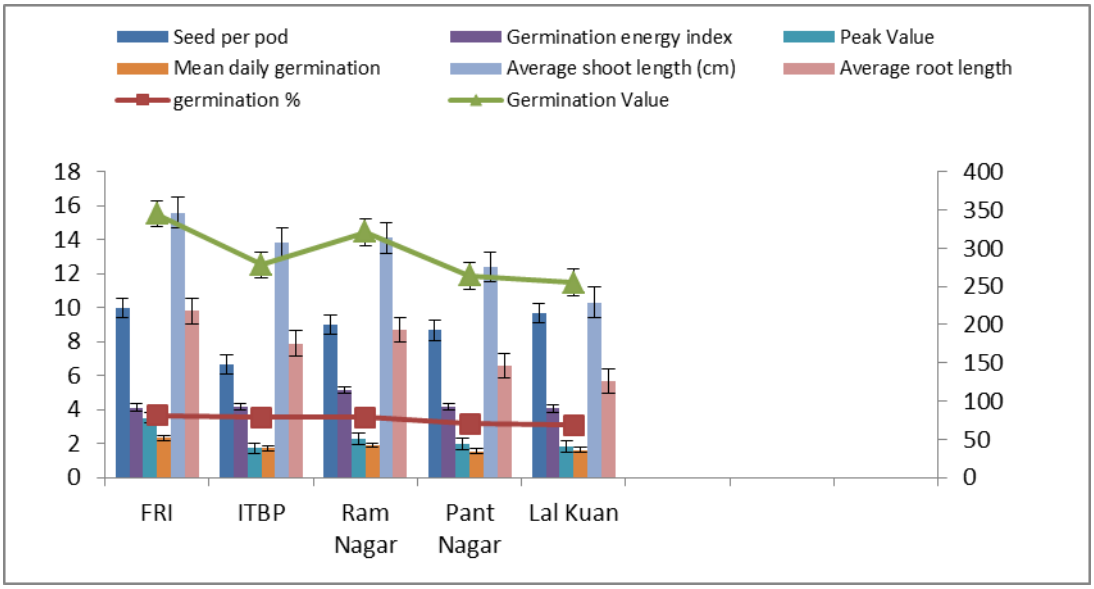


Fig 3: Germination studies of *Albizia procera* from Uttarakhand

Discussion

Seed dormancy limits the use of particular variety in nurseries for the production of superior seedlings. It is because seed dormancy can differ from stage of maturity of seed, species to species, degree of drought and consequently pretreatments (Amen 1968; Rees 1996) [3, 25]. A lot of works have been on seed germination in order to break seed dormancy and improve the germination rate by speeding up the germination (Kobmoo and Hellum 1984; Khasa 1992; Yadav 1992; Teketay 1996; Azad *et al*., 2006a, 2006b; Airi *et al.*, 2009; Azad *et al*., 2010a, 2010b, 2011a, 2011b) [23, 22, 34, 4, 6, 2, 7, 8, 9].

Studies on seed germination of important nitrogen fixing trees requires priority due to their importance in plantation program. Dormancy is an important limitation faced in hard coat species; therefore, enhancing seed germination by treating seeds is an important aspect. The time required for effective treatment differs between species and related with seed coat thickness. In the present work, effect of rhizobial inoculation on seed germination was evaluated. An increase in shoot, root length and other germination parameters was observed after inoculation of seeds with rhizobia.

The present data indicated that inoculation of seeds with native Rhizobium isolate increased the overall per cent seed germination, which indicated effective symbiotic association of native isolates with *Albizia procera*. These results are in concurrence with the earlier reports by Gaikwad (1997) [19] in horsegram. Authentication of isolated rhizobia was done as on the tests listed above. The findings of our results showed that the isolate was rhizobia only which are in similarity with the results of Agarwal *et al*. (2012) [1] who also observed that rhizobia did not absorb red colour when cultured in YEMA containing congo red dye and did not grow in Hofer's alkaline broth. They also did not utilize lactose. The results were further supported by Deshwal and Chubey (2014) and Dipta *et al* (2017) [14, 16].

The present study showed that seed germination of *A. procera* under different pre-treatments significantly increased ($p < 0.05$) over the control. Similar finding were observed by Sajeevukumar *et al*. (1995) [26] on seed dormancy and germination of *Albizia falcataria* and *A. procera* using hot water treatments (40, 60, 70 and 80°C) significantly increased germination.

Deviation in seed sources is important for seed germination and growth of seedling for their use in afforestation, reforestation and breeding programs. Collection of superior seeds can enhance the germination and pace up plantation programs. Similar observation have been reported by different uthors in different trees viz., Sniezko and Stewart (1989) [29] in *Acacia albida*, Vakshasya *et al*. (1992) [32] in *Dalbergia sissoo*, Nautiyal *et al*. (2000) [24] in *Quercus leucotrichophora*, Bhat and Chauhan (2002) [10] in *Albizzia lebbek*, Close and Wilson (2002) [12] in *Eucalyptus regnans*, Esen *et al*. (2007) [18] in *Prunus serotina* and Shivanna *et al*. (2007) [27] in *Pongamia pinnata*.

But the study revealed that the interaction between sources and treatment effects affected significantly ($p < 0.05$) on seed germination percentages and rates of germination. These may be due to the *Rhizobium* treatment which effect the germination of seed on different aspects of seed germination.

Conclusion

Germination is one of the most important criteria of seed quality, because it affects plant establishment and therefore the yield and the quality of the plant. Selection of superior seed source of a species for a given site region is important for optimum productivity in plantation programme of forestry and agroforestry systems. Variation in seed mass within a species may affect seed germination and germination rate. On the basis of results obtained from the study, it can be inferred that inoculation with rhizobia has tremendous potential in improving initial growth response of *Albizia procera* plants. This finding may be helpful in producing quality planting stock of *Albizia procera* for afforestration programs.

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References

1. Agrawal PK, Agrawal S, Singh U, Katiyar N, Verma SK. Phenotypic characterization of rhizobia from legumes and its application as a Bio in oculant. Journal of Agricultural Technology. 2012; 8:681-92.
2. Airi S, Bhatt ID, Bhatt A, Rawal RS, Dhar U. Variations in seed germination of *Hippophae salicifolia* with different presoaking treatments. J Forest Res. 2009; 20(1):27-30.
3. Amen RD. A model of seed dormancy. Bot Rev. 1968; 34(1):1-31.
4. Azad MS, Islam MW, Matin MA, Bari MA. Effect of pre-sowing treatment on seed germination of *Albizia lebbeck* (L.) Benth. South Asian J Agric. 2006a; 1(2):32-34.
5. Azad MS, Manik MR, Hasan MS, Matin MA. Effect of different pre-sowing treatments on seed germination percentage and growth performance of *Acacia auriculiformis.* J Forest Res. 2011a; 22(2):183-188.
6. Azad MS, Matin MA, Islam MW, Musa MZA. Effect of pre-sowing treatment on seed germination of Lohakath (*Xylia kerrii* Craib & Hutch.). Khulna Univ Stud. 2006b; 7(2):33-36.
7. Azad MS, Musa MZA, Matin MA. Effects of presowing treatments on seed germination of *Melia azedarach*. J Forest Res. 2010a; 21(2):193-196.
8. Azad MS, Paul NK, Matin MA. Do pre-sowing treatments affect seed germination in *Albizia richardiana* and *Lagerstroemia speciosa*? Front Agric China. 2010b; 4(2):181-184.
9. Azad MS, Rahman MT, Matin MA. Seed germination techniques of *Phoenix dactylifera*: a new experience from Bangladesh. Front Agric China. 2011b; 5(2):241-246.
10. Bhat GS, Chauhan PS. Provenance variation in seed and seedling traits of *Albizzia lebbek* Benth. J Tree Sci. 2002; 21:52-57.
11. Callaham, RZ. Provenance research: investigation of genetic diversity associated with, 1964.
12. Close DC, Wilson SJ. Provenance effects on pre-germination treatments for *Eucalyptus regnans* and *E. delegatensis* seed. Forest Ecol Manage. 2002; 170:299-305.
13. Dangasuk OG, Seurei P, Gudu S. Genetic variation in seed and seedling traits in 12 African provenances of *Faidherbia albida* (Del.) A. Chev. at Lodwar, Kenya. Agroforest Syst. 1997; 37:133-141.
14. Deshwal VK, Chaubey A. Isolation and characterization of *Rhizobium leguminosarum* from root nodule of *Pisum sativum* L. Journal of Academia and Industrial Research. 2014; **2**:464-67.
15. Devagiri GM. Evaluation of seed source variation in seed and seedling traits in *Dalbergia sissoo* Roxb. Ph.D.

Thesis, Forest Research Institute University, Dehra Dun, India, 1997.
16. Dipta B, Kirti S, Bhardwaj S, Gupta S, Kaushal R. Phosphate solubilising potential of *Bacillus pumilus* for the enhancement of cauliflower (*Brassica olearacea* var. *botrytis* L.). Ecology Environment and Conservation. 2017; 23:1541-48.
17. Elmagboul H, Mahgoup S, Eldoma A. Variation in seed morphometric characteristics and germination of *Acacia tortilis* subspecies raddiana and subspecies spirocarpa among three provenances in Sudan. Global J Bio-Sci. Biotechnol. 2014; 3(2):191-196.
18. Esen D, Yildiz O, Sarginci M, Isik K. Effects of different pretreatments on germination of *Prunus serotina* seed sources. J Environ Biol. 2007; 28(1):99-104.
19. Gaikwad RT. Studies on isolation and characterization of Rhizobium and nodulation pattern in horsegram (*Dolichus biflorus* L.). M.Sc. (Agri.) Thesis submitted to M.P.K.V., Rahuri (Maharashtra). Geography. Unasylva. 1997; 18:40-50.
20. Ginwal HS, Phartyal SS, Rawat PS, Srivastava RL. Seed source variation in morphology, germination and seedling growth of *Jatropha curcas* Linn in central India. Silvae Genet. 2005; 54(2):76-79.
21. Khan SN, Uniyal K. Association of Arbuscular mycorrhizal fungi with different bamboo species at a reforested site. Ind. J For. 1999; 22:405-408.
22. Khasa PD. Scarifi cation of limba seeds with hot water, bleach and acid. Tree Plant Notes. 1992; 43(4):150-152.
23. Kobmoo B, Hellum AK. Hot water and acid improve the germination of *Cassia siamea* Britt. Seeds. Embryon. 1984; 1(1):27-33.
24. Nautiyal AR, Rawat DCS, Prasad P. Physiological aspects of seed source variation in seed germination of *Quercus leucotrichophora* A. Camus. Indian Forest. 2000; 126(3):269-273.
25. Rees M. Evolutionary ecology of seed dormancy and seed size. Phil Trans R Soc Lond B. 1996; 351:1299-1308.
26. Sajeevukumar B, Sudhakara K, Ashokan PK, Gopikumar K. Seed dormancy and germination in *Albizia falcataria* and *Albizia procera*. J Trop Forest Sci. 1995; 7(3):371-382.
27. Shivanna H, Balachandra HC, Suresh NL. Source variation in seed and seedling traits of *Pongamia pinnata*. Karnataka J Agric Sci. 2007; 20(2):438-439.
28. Shivkumar P, Banerjee AC. Provenance trail in *Acacia nilotica*. Journal of Tree Science. 1986; 5:53-56.
29. Sniezko RA, Stewart HTL. Range-wide provenance variation in growth and nutrition of *Acacia albida* seedlings propagated in Zimbabwe. Forest Ecol Manage. 1989; 27:179-197.
30. Suri SK. Analytical study of teak provenance test in North Raipur Division of Madhya Pradesh. Indian Journal of Forests. 1984; 110:345-363.
31. Teketay D. The effect of different pre-sowing seed treatments, temperature and light on the germination of five Senna species from Ethiopia. New Forest. 1996; 11:155-171.
32. Vakshasya RK, Rajora OP, Rawat MS. Seed and seedling traits of *Dalbergia sissoo* Roxb. seed source variation studies among ten sources in India. Forest Ecol Manage. 1992; 48:265-275.
33. Wright JW. Introduction of Forest Genetics. Academic Press, New Genetics. Academic Press, New York, 1976.
34. Yadav JP. Pretreatment of teak seed to enhance germination. Indian Forest. 1992; 11(2):260-264.