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EXPLORING INTRASPECIFIC PROVENANCE VARIATION IN SEED MORPHOLOGICAL TRAITS OF *ALBIZIA PROCERA* IN MID-HIMALAYAN REGION OF INDIA

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Albizia procera is a multipurpose leguminous tree that not only fixes atmospheric nitrogen but also provides wood for a variety of purposes, nutritious fodder for livestock, traditional medicine and shade for tea plantations. The leaves are used to cure ulcers and have insecticidal effects, and the bark contains tannins and a reddish gum. Consequently, it can be considered a good choice for reforestation projects and agroforestry systems. However, insufficient supply of high quality seeds is a major hurdle that comes in the way of producing healthy nursery planting stock of this tree species. Therefore, there is a need to identify quality seed source and provenances for successful tree improvement and forest regeneration program. Keeping this in view, the study was planned to investigate the provenance variation with respect to pod, seed and germination behavior of *Albizia procera*. Pods along with seeds were collected from different provenances of mid-Himalayan region. Measurements were made with respect to morphological characters of pods as well as seeds and germination behavior. The data thus collected were subjected to appropriate statistical analysis i.e. analysis of variance technique and genetic variability studies. Results revealed that there occur considerable morphological and physiological variations among provenances for pod and seed characters as well as for germination response of *Albizia procera*. Pods and seeds from the Forest Research Institute (FRI), Dehradun seed source were superior as compared to other provenances for all the traits, and therefore, can be considered for plantation and afforestation programs.

Keywords: *Albizia procera*, seed source, analysis of variance, genotypic and phenotypic variation

INTRODUCTION

Multitudes of leguminous trees are found in the forest ecosystems. Leguminous nitrogen fixing tree species play a major role in improving the productivity of degraded forest soils and wasteland reclamation programs. The role of nodulating trees in improving soil fertility has been documented by many researchers as these tree species stabilise sandy and eroded soil and exploit deep underground water by virtue of their extensive root systems (Cruz & Valdes 1990, Galciana et al. 1996, Rasanen & Lindstrom 2003). *Albizia procera,* commonly known as 'white siris' is a component of the tropical and subtropical moist and wet forest types where rainfall is 1000–5000 mm year^{-1}. Taxonomically, this tree species belongs to domain: Eukaryota, kingdom: Plantae, phylum: Spermatophyta, subphylum: Angiospermae, class: Dicotyledonae, order: Fabales, family: Fabaceae, subfamily: Mimosidaeae and genus: *Albizia* (Arce et al. 2008). It is a component of tropical and subtropical moist forest types and homesteads at an elevation of about 900 m and above mean sea level. The native range of *A. procera* is South and Southeast Asia between 30˚ N to 15˚ S latitudes. The tree occurs naturally in India, Nepal, Bangladesh, Andaman Island, Myanmar, Southern China, Laos, Thailand, Cambodia, Vietnam, Malaysia, Philippines, Indonesia and northern Australia. In India, the species is found in the sub-Himalayan tracts from Yamuna eastwards to West Bengal, Satpura range, Gujarat and Andamans (Dhuria & Tiwari 2015). White siris is drought tolerant and susceptible to frost (Troup 1921, Djogo 1992). This tree species is highly valued for supplying nutritious feed for animals, providing timber for a variety of uses, shade for tea plantations,

and nitrogen fixation. Variations in seed and pod morphological characters and seedling growth among different provenances for many forest trees have been reported by several researchers for tree improvement program (Arthanari et al. 2013, Masoodi et al. 2014, Kant & Kumari 2016, Tiwari & Dhuria 2018). Although *A. procera* is commonly propagated through seeds, frequent damage by bruchids and seed dormancy due to the presence of hard seed coat inhibits the seed germination (Ganguli et al. 2000). Thus, poor adaptability and slow growth reduce the germination and regeneration of this tree legume. Therefore, it is necessary to raise uniform, healthy and vigorously growing planting stock of *A. procera* in the nursery. However, since little is known about the variability and its significance in *A. procera,* the present study was conducted with the following objectives: (i) to identify the best seed source for producing healthy nursery planting stock and (ii) to evaluate the morphological and physiological variations in pod and seed traits of selected seed sources. Achieving these objectives will allow the development of seed transfer guidelines for productivity improvement in the mid-Himalayan region, and thereby, improve afforestation and breeding programs.

MATERIALS AND METHODS

Data description

Site survey and collection of samples

An extensive survey was conducted across five different agro climatic zones of Uttrakhand to explore the natural population of *A. procera* and geographic coordinates were recorded (Table 1 & Figure 1). On the basis of plant height (m) and plant diameter (cm), 20 to 30 year old superior ideotypes of *A. procera* were randomly marked at each locality for the collection of pods. Fully matured and ripened pods of *A. procera* were randomly plucked and collected from a wide altitudinal range within their natural distribution from the marked trees (Figure 3a). Harvested pods from each seed source were brought to the laboratory, sun dried separately for 2–3 days and thereafter, the seeds were extracted (Figure 3b).

Table 1 Geographic coordinate information of seed sources

Name of location	District	Altitude (amsl)	Latitude (N)	Longitude (E)
Forest Research Institute (FRI) Dehradun	Dehradun	671	30° 20’ 38.89”	77° 59’ 50.19”
Indo Tibetan Border Police (ITBP) Campus, Dehradun	Dehradun	626	30° 18’ 33.90”	77° 59’ 37.54”
Pant Nagar University	Udham Singh Nagar	229	29°1’ 9.99”	79° 29’ 17.93”
Ram Nagar	Dehradun	558	30° 10’ 34.81”	78° 13’ 5.49”
Lal Kuan Forest Range	Nainital	261	29° 4’ 1.10”	79° 31’ 19.05”

Figure 1 Natural population distribution of *Albizia procera* in Uttrakhand

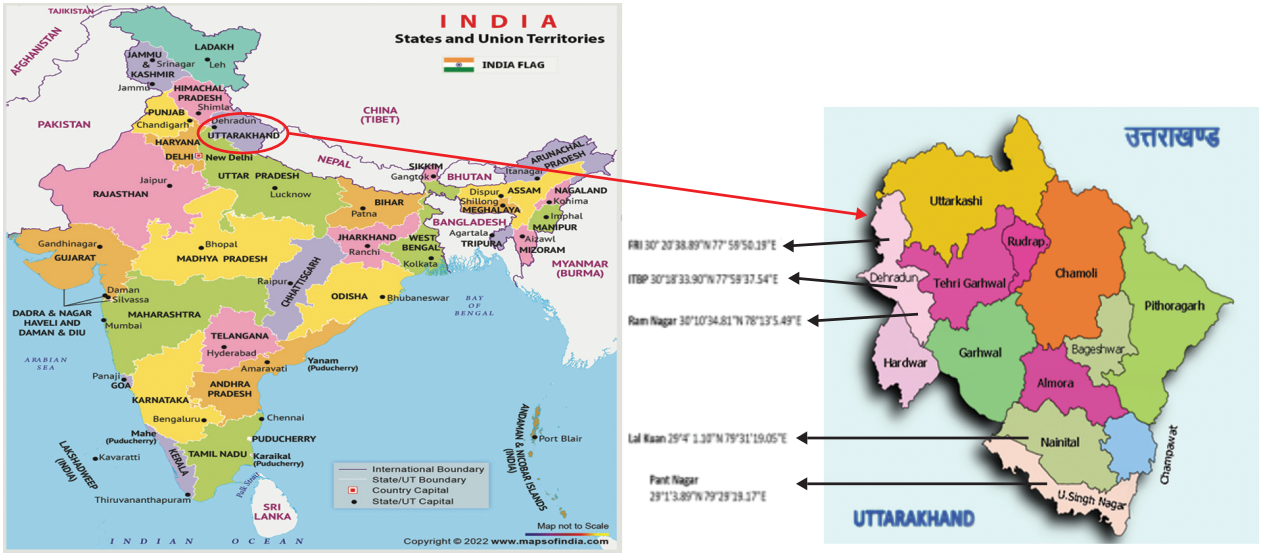

Figure 2 Optical representation of sampling sites of Uttrakhand

Figure 3 Pods (a) and extracted seeds (b) of Albizia procera collected from different provenances of Uttrakhand

Extracted seeds were again sun dried to reduce moisture. Discolored, stained and damaged seeds were removed and the remaining healthy seeds were used for further experiments.

Morphological characteristics of pods and seeds

Morphological characteristics viz., seed length (cm), seed weight of 100 seeds (gm), seed width (cm), seed thickness (cm), pod length (cm), number of seeds per pod, pod weight of 100 pods (gm), pod width (cm) and pod thickness (cm) of randomly extracted seeds and pods were recorded for each provenance for screening the best seed source. Length, width, weight and thickness of pods and seeds were measured as per standard, ISTA (1996). Seed density in cubic centimeters (cc) was determined by water displacement method (Pandey & Pandey 1991).

Germination behavior of seeds

Germination behaviour of seeds from different seed sources of *A. procera* was also studied to develop a selection criterion for future tree improvement programs. For this experiment, seeds from different provenances were soaked in 500 ml distilled water in a beaker and then surface sterilised with 0.1% $HgCl_2$. Thereafter, the seeds were washed twice with distilled water

before subjecting them to germination test. Three replicates from each source were placed on double layered Whatman No.1 filter paper moistened with distilled water in petri dishes (9 cm diameter). Thereafter, the petri plates with seeds were placed in germination cabinets at 25 ± 1 °C for 21 days (Figure4). Observations were recorded on a daily basis for 21 days and the moisture content was checked regularly to ensure that the seeds did not dry. Germination of seeds was considered once they attained a radical length of approximately 1 cm. Daily germination counts were recorded and germination value (GV) was calculated according to Czabator (1962). The speed of germination was expressed in terms of germination value.

Germination percentage

The seeds that germinated normally till the end were counted and germination percentage was calculated as:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Germination value (GV)

Germination value (GV) is an index combining speed and completeness of seed germination. The GV was calculated according to Czabator (1962):

$$GV = PV \times MDG$$

where, MDG (mean daily germination) = total number of germinated seeds/ total number of days, PV (peak value) = highest seed germinated/ number of days.

Germination energy index (GEI)

Germination energy index was calculated according to Grouse and Zimmer (1958):

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

where,

$A_1, A_2, A_3 \ldots An$ = number of seeds newly germinated up to n days respectively.

N = total number of seeds for experiment.

n = number of days of observation.

Speed of germination

To determine the speed of germination, the number of days taken to complete germination was recorded for each replication.

Statistical methods

ANOVA (analysis of variance technique)

One-way classification of data using analysis of variance (ANOVA) was performed to detect the difference among provenances. Once the difference among provenances was found significant, pairwise comparison was performed using least significant difference (lsd). The analysis was performed in R open software by using the R function:

Anova < -aov(characters ~seed source)

Figure 4 Germination of *Albizia procera* seeds in petri dishes

Genetic estimates

Genetic estimates, i.e., phenotypic variance (Vp), genotypic variance (Vg), environment variance (Ve), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), environment coefficient of variation (ECV), broad sense heritability, genetic advance and genetic gain, were computed for each character to study the variation among provenances. Genotypic and phenotypic correlation describes the degree of relationship between characters, which may facilitate a further criterion for selection in breeding program. The improvement program should pay attention to correlated quantitative characters because improving one character can lead to simultaneous correlated changes in the other.

$$\text{Genotypic variance (Vg)} = \frac{\text{MSg} - \text{Mse}}{\text{r}}$$

where MSg = mean sum of squares due to seed source, MSe = mean sum of squares due to error and r = replication.

$$\text{Phenotypic variance (Vp)} = \text{Vg} + \text{Ve}$$

where Ve (environment variance) = MSe.

$$\text{Phenotypic coefficient of variation (PCV)} = \frac{\sqrt{\text{Vp}}}{\text{mean}} \times 100$$

$$\text{Genotypic coefficient of variation (GCV)} = \frac{\sqrt{\text{Vg}}}{\text{mean}} \times 100$$

$$\text{Broad sense heritability } (h^2) = \text{Vg/Vp}$$

$$\text{Genetic advance (GA)} = k \times V_p \times H^2$$

where k = selection criteria.

$$\text{Genetic gain } (GG) = \frac{\text{GA}}{\text{mean}} \times 100$$

R code used for genetic estimates of characters was:

```
# Install package:install.packages(variability);
library(variability, lib.loc = ~/R/win –
library/4.6)
#Variability parameters: gen.var(data[3:…],
data$geno, data$rep)
#Genotypic correlation: geno.corr(data[3:…],
data$geno, data$rep)
#Phenotypic correlation:pheno.
corr(data[3:…], data$geno, data$rep)
```

RESULTS

Intraspecific variation research among the provenances has set a strong base for putting off many plantation barriers in addition to enhancing plantation programs. In the present investigation, the variation in seed and pod characters of *A. procera* tree differed significantly among different seed sources of Uttrakhand.

Mean performance of pod and seed characters from different seed sources

Fresh pods and seeds of *A. procera* were reddish brown in color with elliptical shape. The mean performance of all pod and seed traits, viz., pod length, pod width, pod thickness and 100-pod weight from pods of different provenances revealed significant difference among seed sources (Table 2). The pods from FRI exhibited pod length of 13.33 cm which was significantly higher than that from other provenances. Variations in pod width and number of seeds per pod also followed the same trend as that of pod length, showing that pods from FRI (2.13 cm, 11.0 cm) were significantly larger than those from other seed sources. However, the seeds from Ram Nagar and Pant Nagar did not show significant variations for number of seeds per pod. Among all the provenances, the highest pod weight (100^{-1} pods) was recorded for FRI (57.59 gm) followed by Ram Nagar (53.17 gm) while the lowest weight of 38.87 gm was recorded for Lal Kuan. The FRI seed source also surpassed other seed sources for pod thickness (0.31 cm).

Seed characters also showed significant variation among different provenances. Seed length ranged from 0.49 to 0.83 mm while seed

width ranged from 0.46 to 0.59 mm. Similarly, the range for seed thickness, 100-seed weight and seed density were recorded from 0.14 to 0.28 mm, 3.24 to 6.13 mm and 0.99 to 1.84 mm respectively. The FRI seed source excelled while Lal Kuan seed source showed the lowest values for all seed characters under study. It is evident from the presented data that seeds with higher length and width possess higher seed weight.

Performance of seed germination from different seed sources

Germination behavior of *A. procera* seeds is presented in Table 2. The perusal of data showed significant variations in seed germination behavior among different seed sources. The seed germination ranged between 70.10 to 87.12 percent. The germination studies of Uttrakhand seed sources showed that FRI seed source exhibited the highest germination percentage (87.12%), germination value (9.09), germination energy index (8.67) and speed of germination (5.73). However, seeds from Lal Kuan seed source showed the lowest germination percentage (70.10%).

Genetic estimates

Variances, coefficient of variability and estimate of genetic component for pod and seed characters of *A. procera* are presented in Table 3. Heritability and genetic advance are important selection parameters. Heritability along with genetic advance are normally more helpful in predicting the gain under selection. As suggested by Johnson et al. (1955), more than 60% heritability is considered as high heritability and more than 20% genetic gain is considered as high genetic gain. High heritability accompanied with high genetic advance was recorded in all the seed and pod characteristics except seed width. This indicated that the heritability is due to additive gene effects and selection may be effective. Seed width showed high heritability accompanied with low genetic advance that indicated non-additive gene action and selection for this character might not be useful. Tiwari and Dhuria (2018) observed high heritability in pod length of *A. procera* in provenances of Chhattisgarh, India. Mamo et al. (2006) estimated variance components, phenotypic coefficient of variation (PCV) for seed size characters. Genotypic

Table 2 Seed and pod characteristics of *Albizia procera* as influenced by various agro climatic zones of Uttrakhand

Morphological character	Provenances					lsd
	FRI	ITBP	Ram Nagar	Pant Nagar	Lal Kuan	
Pod length (cm)	13.33^{a}	10.06^{c}	11.09^{b}	9.26^{c}	7.37^{d}	0.94
Pod width (cm)	2.13^{a}	1.97^{c}	2.01^{b}	1.61^{d}	1.39^{e}	0.02
Number of seeds per pod	11.00^{a}	8.00^{c}	9.00^{b}	9.00^{b}	5.00^{d}	0.31
Pod weight (100 pods) (gm)	57.59^{a}	50.27^{c}	53.17^{b}	45.87^{d}	38.87^{e}	0.04
Pod thickness (cm)	0.31^{a}	0.21^{c}	0.26^{b}	0.11^{e}	0.16^{d}	0.04
Seed length (mm)	0.83^{a}	0.61^{c}	0.65^{b}	0.52^{d}	0.49^{e}	0.02
Seed width (mm)	0.59^{a}	0.52^{c}	0.55^{b}	0.46^{e}	0.49^{d}	0.02
Seed thickness(mm)	0.28^{a}	0.21^{c}	0.25^{b}	0.17^{d}	0.14^{e}	0.02
100-seed weight (gm)	6.13^{a}	4.48^{c}	5.22^{b}	4.46^{c}	3.24^{d}	0.04
Seed density (gm/cm^3)	1.84^{a}	1.12^{c}	1.70^{b}	1.03^{d}	0.99^{e}	0.01
Germination percentage*	87.12^{a} (9.38)	81.00^{c} (9.05)	83.89^{b} (9.21)	78.12^{d} (8.89)	70.10^{e} (8.43)	0.10
Germination value	9.09^{a}	8.45^{b}	8.50^{b}	7.06^{c}	6.87^{d}	0.10
Germination energy index	8.67^{a}	8.35^{b}	8.40^{b}	7.49^{c}	6.59^{d}	0.11
Speed of germination	5.73^{a}	4.80^{c}	5.48^{b}	4.67^{d}	4.02^{e}	0.04

Values within row with the same letter do not differ significantly ($p < 0.05$), *values in parentheses are square root transformed values, FRI = Forest Research Institute, ITBP = Indo Tibetan Border Police, lsd = least significant difference

Table 3 Variances, coefficient of variability and estimate of genetic component in *Albizia procera* for pod and seed characters

No.	Parameters	Variances		Co-efficient of variability		Genetic component	
		Vg	Vp	GCV	PCV	Heritability	Genetic advance (%)
1	Pod length (cm)	51.254	51.255	14.573	14.573	99.99	30.02
2	Pod width (cm)	4.748	5.249	21.356	22.453	90.45	41.84
3	Number of seeds per pod	4.211	7.137	24.43	31.803	59.00	38.65
4	Pod weight (100 pods) (gm)	0.096	0.097	16.993	17.02	98.96	34.95
5	Pod thickness (cm)	0.006	0.007	36.549	38.583	85.71	71.32
6	Seed length (mm)	1.083	1.345	22.108	24.642	80.52	40.85
7	Seed width (mm)	0.003	0.004	9.617	10.083	75.00	18.89
8	Seed thickness (mm)	0.003	0.004	26.513	29.547	75.00	49.01
9	100-seed weight (gm)	0.018	0.019	21.594	21.821	94.73	44.01
10	Seed density (gm/cm^3)	0.162	0.163	30.173	30.183	99.38	62.13

Vg = genotypic variance, Vp = phenotypic variance, GCV = genotypic coefficient of variation, PVC = phenotypic coefficient of variation

Table 4 Genotypic (G) and phenotypic (P) correlation matrix of pod, seed and germination traits in *Albizia procera*

Characters		Germination %	Germination value	GEI
Pod length (cm)				
	(G)	0.999**	0.946**	0.972**
	(P)	0.973**	0.944**	0.971**
Pod width (cm)				
	(G)	0.994**	0.928**	0.923**
	(P)	0.867**	0.866**	0.868**
Number of seeds per pod				
	(G)	0.957**	0.770**	0.888**
	(P)	0.863**	0.612**	0.715**
Pod weight (100 pods) (gm)				
	(G)	0.968**	0.981**	0.992**
	(P)	0.947**	0.976**	0.986**
Pod thickness (cm)				
	(G)	0.779**	0.922**	0.770**
	(P)	0.761**	0.904**	0.759**
Seed length (mm)				
	(G)	0.892**	0.919**	0.835**
	(P)	0.853**	0.915**	0.834**
Seed width (mm)				
	(G)	0.793**	0.926**	0.770**
	(P)	0.725**	0.892**	0.748**
Seed thickness (mm)				
	(G)	0.988**	0.972**	0.945**
	(P)	0.863**	0.909**	0.889**
100-seed weight (gm)				
	(G)	0.999**	0.891**	0.922**
	(P)	0.874**	0.782**	0.814**
Seed density (gm/cm^3)				
	(G)	0.848**	0.839**	0.767**
	(P)	0.831**	0.836**	0.764**

** Significant at 1 % level of significance, GEI = germination energy index

and phenotypic correlation of pod, seed and germination traits of *A. procera* are presented in Table 4. All the seed and pod characters were positively and significantly correlated with germination characteristics. Mamo et al. (2006) recorded a positive correlation between number of seeds, seed length, seed width and weed weight in *Juniperus procera.*

DISCUSSION

Tree production begins with the planting of seed and the planted seed quality represents the success of any plantation program. Variability studies are the prerequisites for genetic upliftment of any tree species. Variation assessment among provenance in native species is desirable to screen the available variation for higher productivity and future breeding work. The seed sources of *A. procera* exhibited considerable amount of significant variation in the morphological traits of the pods as well as seeds. Variation among seed provenances with respect to seed traits have earlier been reported in many species including *Faidherbia albida*, *Acacia karroo*, *Celtis australis*, *Dalbergia melanoxylon*, *Pinus roxbrughii* and *Jatropha curcas* (Abdelkheir et al. 2003, Singh et al. 2006, Ghildiyal et al. 2009, Sharma & Kumar 2013, Amri 2014). Tiwari and Dhuria (2018) reported significant variation in pod and seed characters of *A. procera*, except pod thickness, among different provenances. Several researchers have also reported that the seeds of single species, when collected from different locations or altitudes, varied significantly. Variation among the provenances might be attributed to genetic differences caused by the adaptation of different provenances to diverse environmental conditions and soil types (Ginwal et al. 2005, Ahlawat et al. 2007, Hela et al. 2008, Singh & Bhatt 2008, Elmagboul et al. 2014). However, Selven and Guleria (2012) reported that variation in *Acacia catechu* for seed traits did not follow any particular trend with regard to different populations.

Seed germination parameters are important characters that initiate the very beginning of tree growth. Thus, these traits help in obtaining better seedling of any plant species. It was noticed that the seed source with better seed traits had high germination performance. Different provenances have displayed significant differences in germination parameters. The presence of such differences among populations has probably been produced by different intensities of natural selection acting upon these traits in their natural habitats. In most plant species, seeds vary in their degree of germination between and within populations and between and within trees (Gera et al., 2001, Mkonda et al. 2003). Variation in germination behavior that occurs among different populations within the same species had been widely reported (Singh and Bhatt 2008, Rawat and Uniyal 2011, Shukla and Charkravarty 2011, Jagadish et al. 2014, Thakur & Dhuppe 2015). Kant and Kumari (2016) studied the variation in germination in *Tecomella undulate* seeds and concluded that germination ranged from 58.2–69.0% in different sources.

Understanding the nature and cause of variability to exploit the natural variability for higher production is necessary. The data obtained for each character under study were subjected to analysis of variance, which was further used to divide the phenotypic variability into components due to genetic and non–genetic factors. Estimates of the variance components for pod and seed characters revealed that genotypic and phenotypic variances were almost close to each other for all characters except number of seeds per pod and germination percentage (Table 3). Among pod characters, the lowest genotypic as well as phenotypic coefficient of variation was found in pod length followed by pod weight, which indicated that these characters were more consistent as compared to other pod characters under study. Among seed characters, the lowest genotypic as well as phenotypic coefficient of variation was found in seed width. Pod thickness and seed density had high genetic advance coupled with high heritability, which indicated the presence of additive genes in these characters and suggested improvement program through selection of these characters. A trait possessing high heritability and genetic gain indicates that the given trait is governed by additive gene action, therefore provides the most effective condition for selection (Tazeen et al. 2009, Ndukauba et al. 2015). Mahmood et al. (2003) reported that heritability estimate is important in tree improvement program.

For a successful breeding program, correlation studies among the seed, pod and germination parameters are necessary. Associated quantitative attributes are of a significant interest in an improvement program,

as the improvement of one aspect might cause concurrent corresponding changes in different characters. Correlation studies showed that genotypic correlation was higher than their phenotypic coefficient of correlations indicating an inherent association between the characters. The genotypic correlation indicates genotypic association among the traits, and is therefore a more reliable estimate value for examining the degree of relationship between the characters under study (Johnson et al. 1955). Genotypic and phenotypic correlations are presented in Table 4. The perusal of table indicated that the genotypic as well as phenotypic correlation of germination characters was positively and significantly aligned with the pod and seed characters. Therefore, it can be interpreted that germination is directly associated with the seed and pod characters and improvement in seed and pod characters will lead to better germination. Previous studies on *Pongamia pinnata*, *Aquilaria malaccensis*, *Dalbergia sissoo* and *A. chinensis* lend support to the present findings (Dhanai et al., 2003, Devagiri et al. 2004, Palanikumaran et al. 2016, Dubey et al. 2020). However, Jagadish et al. (2014) also studied the effect of provenance variation on the germination behavior of *Vateria indica* and reported that there was no significant difference with respect to speed of germination and time taken to complete germination among different provenances.

CONCLUSION

Significance of provenance testing in native species is desirable for screening the available variation for higher productivity and future breeding work. Studying genetic variability is very important in improving this species in future selections. The present study reports the findings of morphological variations in pod and seed characteristics of *A. procera*. On the basis of the present study, it can be concluded that significant differences exist for morphological characters and germination behavior among all the provenances considered for the study. These variations might be attributed to the mutual interactions of geo-climatic and genetic makeup of these seed sources. This study identifies FRI seed source as superior to other seed sources under study with respect to germination behavior, pod and seed characters. The study suggests that among all the seed sources, seeds from FRI could potential serve as the frame work for producing healthy nursery stock for planting. The study will also ensure provision of planting materials for farmers. Hence, seed source screening provides a great opportunity for success of afforestation program. Further, it was also observed that all germination characters under study were positively and significantly correlated with pod and seed characters, thus indicating a strong association of these characters with germination. However, for a better understanding, more provenances should be explored to study variations among seed and pod characters. The quality seed production of *A. procera* is the need of the hour as the species is a good alternative for regeneration and agroforestry system. Further, the best seed source is required for the production of quality seeds of this species. Although several studies have been conducted over the past few years on performance of seed and pod characteristics of the species, little literature is available on variation studies in different provenances with respect to seed and pod characters. Therefore, the present study was conducted with the motivation of studying the variation among provenances and identifying the best seed source of *A. procera* in mid-Himalayan region of India.

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