

Altitudinal variation in morphometric traits of pod, seed, and seedling growth of *Bauhinia variegata* L. in Garhwal Himalaya

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ABSTRACT *Bauhinia variegata* L. is a fast growing tree that has the ability to fix atmospheric nitrogen. The aims of the present study was to estimate the variability in pod, seed and seedling growth and biomass production in 10 populations of *B. variegata* in Garhwal Himalaya. Significant variations were observed in pod, seed traits, seed germination, seedling growth and biomass production among populations. Among morphological traits, the seed weight was one of the most variable characters. Among seedling attributes, the seedlings vigour index, roots length, leaves dry weight and roots dry weight were found more variable characters. Geographical variables (altitude, latitude, longitude, temperature and rainfall) significantly ($p < 0.05$) influenced the pod size, seed morphological traits, seed germination, seedling growth characters and allocation of biomass. Broad-sense heritability (h^2) resulted moderate to high for pod characters, seed morphological traits and all the seedling growth parameters (except the leaves dry weight and survival percentage). These variations might be geographically structured and genetically controlled.

KEYWORDS: *Bauhinia variegata* L., biomass, genetic gain, heritability, seedling, population.

Introduction

The genus *Bauhinia* has 300 species spreading all over the world with 30 species occurs in India. *Bauhinia variegata* L. belongs to the family Fabaceae. It is commonly known as Guriyal in Hindi and mountain-ebony in English (Gaur 1999, Mali and Dhake 2009). *B. variegata* is a medium sized, fast-growing, moderate light demanding deciduous tree with good coppicing ability. The species is native to South-eastern Asia and widely distributed in India. It grows well in tropical and sub-tropical climate with hot and dry summers and mild winters (Gautam 2012) and can withstand ranges of 0°C - 37.5°C mean annual temperature and 500-2,500 mm mean annual rainfall. The tree grows well in areas with full sun or partial shade and it is fairly resistant to drought, but susceptible to fire. Frost kills leaves of seedlings and saplings in winters but they recover during summers. The tree grows in all types of soil, preferring a fertile, moisture-retentive, but well-drained soil. *B. variegata* restores fertility to acidic and degraded soils because of its nitrogen fixing ability (Acharya and Kafle 2009).

The phenophases; senescence commence on November-December and the tree remain leafless up to March. The large pink to purple or white flowers appears from February to April, chiefly on the upper leafless branches and the lower branches often being still in leaves. The flower buds of *B. variegata* are sold in market for making pickles and vegetables that generates cash and enhances the income of resource-poor rural farmers. Being a good fodder species, it is considered as a green protein banks

during lean period. Flower buds, flowers, stem, roots, stem bark, seeds and leaves of *B. variegata* contains diverse photochemicals i.e. flavonoids, tannins, kaempferol, terpenoids, saponins, cardiac glycosides and quercetin which are very important and useful in improving the health of peoples. Thus, this species gains the recognition as a traditional medicinal tree, used for the treatment of different diseases (Gautam 2012, Al-Snafi 2013, Sharma et al. 2021). The wood of *B. variegata* is used for making agricultural implements, low-grade constructions and packing cases.

B. variegata is the most preferable tree species to afforestation in urban areas, agroforestry and degraded lands due to its wide adaptability, resistance to strong winds, frost, drought and high aesthetic value for its ornamental flowers (Bhatt and Verma 2002). The production of seeds in *B. variegata* is affected severely due to the fact that the species is heavily lopped for fodder during lean period and the tree becomes necked which influence the formation of flowers in the trees, as the flower formation takes place in one year old shoot in leafless phase. To overcome this, the farmers used to retain few shoots on the tree for the production of flowers. Moreover, the flowers are also harvested for making vegetable and pickles, ultimately hamper the production of seeds. The seeds of *B. variegata* mature before rainy season and a large proportion of seeds fail to germinate as the seeds could not bury in the soil properly due to its large size, leading be eaten by rodents, rates, monkeys. The seedlings also grazed by the animals and damaged by fire. Little attention was given to the production of quality seedlings for different ar-

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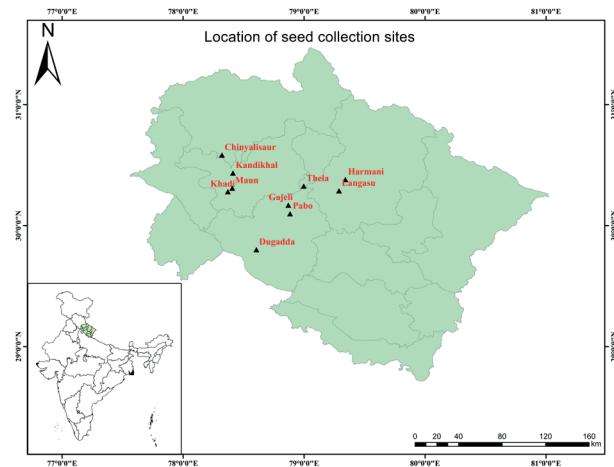
tificial regeneration programmes. Selection of suitable ideotypes, collection of pods/seeds and their morphological variations among the provenances/altitudes are useful to produce quality planting materials and tools for tree improvement programmes. A little work had been carried out to investigate the provenance variation in *B. variegata*. Therefore, the present study was framed to understand the population variation in pod, seed and seedling characteristics of *B. variegata* in Garhwal Himalaya, India, with an attempt to answer the following questions: (i) Do the geographically distinct populations cause variation in pods and seeds traits of *B. variegata*? (ii) Does this variation affect seed germination and seedling growth of selected populations of *B. variegata*? (iii) Do the pod, seed, seed germination and seedling growth parameters was influenced by the geographic coordinates and climatic factors of different studied populations?

Materials and methods

Seed collection

The seeds of *B. variegata* were collected in ten geographically isolated natural habitats/populations in Garhwal Himalaya. The places of collection are more than 30 km air distance far from each other, which ranged between $29^{\circ}48' 14''$ N to $30^{\circ} 61'51''$ N and $78^{\circ} 22' 01''$ E to $79^{\circ} 25' 82''$ E with the altitudinal ranges from 668 to 1,875 m a.s.l. (Fig. 1).

Figure 1 - Seed collection sites map.



In each place of seed collection, five phenotypically superior trees were selected in March 2015 (time of flowering). The superior trees were selected on the basis of criteria, *i.e.* medium size, straight bole, highly branched and disease free. The selected trees were 100 to 300 m apart from each other to avoid narrowing down the variation in sample trees due to relatedness or inbreeding (Schmidt 2000). Temperature and rainfall were recorded from the nearby place of seed collection sites (Tab. 1).

The mature pods were harvested from five selected trees of ten different populations by direct climbed

Table 1 - Geographic locations of the different populations of *B. variegata* Garhwal Himalaya, India.

Seed sources	District	Altitude (m asl)	Latitude (N)	Longitude (E)	Temperature 0C	Rainfall (mm/year)
Dugadda	Pauri	668	$29^{\circ} 48' 14''$	$78^{\circ} 36'08''$	25.2	756
Langasu	Chamoli	815	$30^{\circ} 22'23''$	$79^{\circ} 19'73''$	25.3	751
Khadi	Tehri Garhwal	898	$30^{\circ} 16'15''$	$78^{\circ} 22'01''$	23.0	742
Gajeli	Pauri	960	$30^{\circ} 14'08''$	$78^{\circ} 34'40''$	24.0	712
Chinyalisaur	Uttarkashi	1,033	$30^{\circ} 61'51''$	$78^{\circ} 32'10''$	21.0	810
Harmani	Chamoli	1,151	$30^{\circ} 06'45''$	$79^{\circ} 25' 82''$	22.5	875
Tela	Rudraprayag	1,254	$30^{\circ} 22' 59''$	$78^{\circ} 53'12''$	23.8	852
Kandikhal	Tehri Garhwal	1,556	$30^{\circ} 20' 40''$	$78^{\circ} 38'05''$	16.2	823
Pabo	Pauri	1,620	$30^{\circ} 15' 38''$	$79^{\circ} 01'25''$	18.0	838
Maun	Tehri Garhwal	1,875	$30^{\circ} 18'55''$	$78^{\circ} 24'67''$	15.5	948

ing method. The pods were ripened from the end of May to the beginning of June (Singh et al. 2016). The harvested pods were put in cotton bags in open place to allow for an adequate air circulation. Prior to dry, five replicates with 20 pods were selected randomly from each population for measuring the pod length and breadth (with the help of meter scale in cm), number of seeds per pod (counted manually), and pod weight taken with an electronic balance. Further, the pods were dried in natural sunlight condi-

tions to favour dehiscence. Seeds were winnowed to separate the husk. Finally, one kg seeds (each from 2,150 to 3,000 seeds) was collected from each population and it was stored at room temperature for further study.

For measuring the morphological parameters of seeds, five replicates of 20 seeds each from every population were selected randomly from one kg seeds and their length, breadth and thickness were measured with the help of digital calliper (Mitutoyo

Absolute). The seed weight (g) (eight replicates with 100 seeds from each population) was taken using an electronic balance. Five randomly selected samples of five replicates (20 seeds each from 2,150 to 3,000 seeds) in a population were measured for fresh weight by placing the samples in metal dish and placed in hot air oven at 104°C for 24 hrs for drying and moisture contents was calculated.

Greenhouse experiment

The seedling growth parameter was carried out in the experimental block of silviculture nursery at College of Forestry, Ranichauri, Tehri Garhwal (30° 15' N, 78° 30' E; 1,900 m a.s.l.) under mid hills of Uttarakhand, India. The experimental nursery was situated in temperate climates with chilled winters. The mean monthly maximum and minimum temperature during the study period (June 2015 - 2016) were 20.5 and 7.2°C, respectively with the average annual rainfall of 875 mm during the experimental periods. Major portion of annual rainfall usually occurs during August. Winter rains and snowfall occurs from December-February.

Polybags were filled in the nursery with potting mixture made by sand, soil and FYM (Farm Yard Manure) in the ratio of 1:2:1. Hundred seeds (five replicates of twenty seeds each) of the each population were sown in the polybags in 27 June 2015. Seed emergence was recorded daily up to 28 days or until there was no further emergence for at least two weeks. The weeding and watering of polybags were done manually when needed until the end of the experiment, i.e., 12 months.

For measuring of the shoot length, collar diameter, number of leaves/plants, five replications (10 seedlings each replicate) were taken from each population. The length of shoots was measured from cut base to the shoot tip with the help of meter scale in cm. The collar diameter (mm) was measured using a digital calliper (Mitutoyo Absolute). The total number of leaves was counted manually. After measurements, the seedlings were uprooted and their root length (cm) was measured with the help of meter

scale from the cut base to the tip of the taproot. Total length of seedlings was measured by adding shoot and root length for each population. The shoot, root and leaves were weighted with the help of electronic balance (Contech make) and put in oven at 104°C for 24 hrs to obtain the dry weight.

Data analysis

Mean germination time (MGT) was calculated on the basis of equation given by Ellis and Roberts's (1981). Germination index (GI) was calculated as per formula given by Kendrick and Frankland (1969). The seedlings vigour index was calculated following the formula of Abdul-Baki and Anderson (1973). Root length was divided by the shoot length of the same seedling to determine the root/ shoot length ratio. Phenotypic, genotypic and environmental coefficient of variation was calculated with the methods suggested by Sharma (2006). Heritability was estimated with the formula of Falconer (1998). Genetic advance and genetic gain was calculated as per formula given by Shukla et al. (2006). Data were arc sine transformed and two way analysis of variance (ANOVA) and correlation coefficient (r) were calculated using the statistical software WASP version 1.0 (online software package), ICAR, GOA, India.

Results

Variation of pod characteristics in *B. variegata*

The morphological traits of pods varied significantly among the populations ($p<0.05$). The pod length breadth, thickness and the number of seeds/pod were higher in Khadi population and lowest in Harmani population (Figs. 2 and 3). Among pod traits, the pod length had the higher coefficient of variation (CV %) exhibited the more variable character. The number of seeds/pod had shown significant ($p<0.01$) positive correlations with the altitude of the populations.

Figure 2 - Population variation in pod characteristics of *B. variegata*.

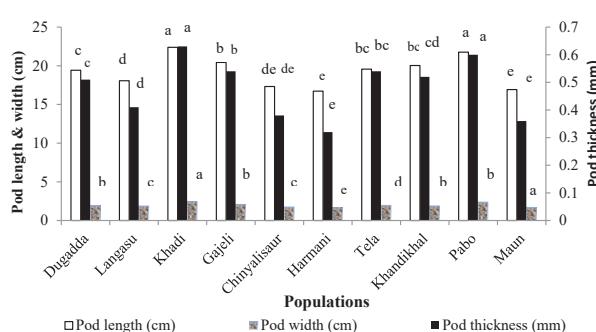
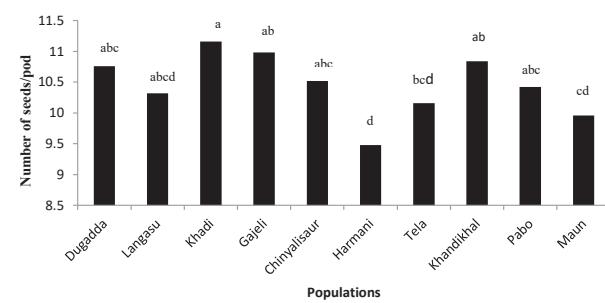


Figure 2 - Population variation in number of seeds per pod of *B. variegata*.



Variation of seed characteristics

Significant ($p < 0.05$) variation in seed length, seed breadth, seed thickness, seed weight, seed length/breadth ratio, seed weight and seed moisture percent were recorded with respect to dif-

ferent geographic populations studied (Tab. 2). Seed thickness was recognized as a most variable character, and it was recorded maximum coefficient of variation (CV %) among other seed traits (Tab. 2).

Table 2 - Population variation with respect to seed characteristics of *B. variegata* (Mean values followed by the same letter within column are not significantly ($P < 0.05$) different between population).

Seed sources	Altitude (m asl)	Seed length (cm)	Seed breadth (cm)	Seed thickness (cm)	Seed length/breadth ratio	Moisture content (%)	Seed weight (g/100 seeds)
Dugadda	668	1.71 ^{ab}	1.41 ^{bc}	0.24 ^{de}	1.21 ^{ab}	35.94 ^{cd}	47.45 ^c
Langasu	815	1.48 ^d	1.29 ^{ef}	0.25 ^{cde}	1.15 ^{cd}	33.76 ^{def}	44.52 ^d
Khadi	898	1.71 ^{ab}	1.45 ^{ab}	0.26 ^{bcd}	1.18 ^{cd}	37.43 ^{bc}	50.70 ^a
Gajeli	960	1.62 ^c	1.31 ^{ef}	0.28 ^{ab}	1.24 ^a	39.69 ^{ab}	48.98 ^b
Chinalisaur	1,033	1.51 ^d	1.27 ^f	0.25 ^{cde}	1.18 ^{bc}	31.62 ^{ef}	37.94 ^e
Harmani	1,151	1.40 ^e	1.29 ^{ef}	0.23 ^e	1.07 ^e	31.01 ^f	33.72 ^g
Tela	1,254	1.50 ^d	1.31 ^{ef}	0.27 ^{abc}	1.15 ^{cd}	40.47 ^{ab}	46.8c
Khandikhal	1,556	1.77 ^a	1.48 ^a	0.28 ^{ab}	1.20 ^{bc}	39.68 ^{ab}	49.66 ^{ab}
Pabo	1,620	1.68 ^{bc}	1.37 ^{cd}	0.29 ^a	1.22 ^{ab}	41.68 ^a	46.78 ^c
Maun	1,875	1.45 ^{de}	1.34 ^{de}	0.27 ^{abcd}	1.08 ^e	34.49 ^{cde}	35.54 ^f
CV		8.21	5.39	7.37	2.73	10.37	13.96

Variation in germination percent, germination index, and seedling vigour index

Significant ($p < 0.05$) variation was recorded for seed germination percent, mean germination time, germination index and seedling vigour index among different populations (Tab. 3). The seed germina-

tion percent and seedling vigour index among different populations were recorded maximum in Gajeli population and minimum germination percent and seedling vigour index was recorded in Chinalisaur population (Tab. 3).

Table 3 - Population variation with respect to seed characteristics of *B. variegata* (Mean values followed by the same letter within column are not significantly ($P < 0.05$) different between population).

Seed sources	Altitude (m asl)	Germination (%)	Mean germination time (days)	Germination Index	Seedling vigour index
Dugadda	668	82.0 ^{bcd}	11.41 ^d	0.31 ^a	5,783.35 ^{bc}
Langasu	815	84.0 ^{ab}	11.81 ^c	0.30 ^a	5,041.01 ^d
Khadi	898	81.0 ^{cd}	11.68 ^d	0.31 ^a	5,970.67 ^{bc}
Gajeli	960	86.0 ^e	11.92 ^{bc}	0.31 ^a	6,760.40 ^a
Chinalisaur	1,033	77.0 ^e	12.01 ^b	0.30 ^a	4,382.93 ^e
Harmani	1,151	79.0 ^e	12.30 ^a	0.27 ^b	4,479.73 ^e
Tela	1,254	83.0 ^{abc}	11.55 ^d	0.30 ^a	5,870.20 ^{bc}
Khandikhal	1,556	85.0 ^a	11.77 ^c	0.31 ^a	6,286.10 ^{ab}
Pabo	1,620	80.0 ^d	12.07 ^b	0.30 ^a	5,724.32 ^{bc}
Maun	1,875	78.0 ^e	11.83 ^c	0.30 ^a	4,457.57 ^e
CV		7.86	13.28	9.97	26.37 597.23

Periodic growth of shoot and root length and biomass attributes

Significant ($p < 0.05$) variation was recorded for different growth parameters of seedlings (after 12 months of growth), i.e. survival, shoot length, collar diameters, number of leaves and root length, among different populations (Tab. 4). Shoot length, collar diameter and number of leaves were higher for Gajeli and lowest for Harmani populations. The mean shoot dry weight, leaves dry weight, root dry weight

and total dry weight of seedling were significant ($p < 0.05$) among the populations (Tab. 5).

Correlation with design variables (altitude, latitude, longitude, temperature and rainfall)

A positive significant relationship was recorded for seed thickness, moisture percent, root dry weight and root shoot dry weight ratio with the altitude of populations ($p < 0.01$ and $p < 0.05$). Whereas number of seeds per pod, seed weight, seed length/breadth ra-

tio, seed germination, shoot length, shoot dry weight, leaves dry weight, total dry weight revealed negative significant relationship with the altitude. Latitude showed negative significant correlation with pod morphology, seed weight, seed moisture, germination, shoot length, collar diameter, number of leaves, root length, total seedling length, shoot dry weight, root dry weight, and total dry weight ($p < 0.01$ and $p < 0.05$). Longitude had significant positive relationship with root-shoot dry weight ratio ($p < 0.01$ and $p <$

0.05). Number of seeds/pod, seed breadth, collar diameter, root length and root/shoot length ratio were found negatively correlated with longitude. Temperature revealed positive significant relationship with all the pod, seed, growth and biomass parameters except seed thickness and root/shoot dry weight ratio ($P < 0.01$ and $P < 0.05$). Rainfall had significant negative correlation with all the studied parameters except seed breadth, seed thickness and root length ($p < 0.01$ and $p < 0.05$) (Tab. 6a).

Table 4 - Periodic growth of shoot and root length (cm) of *B. variegata* at nursery stage (Mean values followed by the same letter within column are not significantly ($P < 0.05$) different between population).

Seed sources	Altitude (m asl)	Survival %	Shoot length (cm)	Collar diameter (mm)	Number of leaves/seedling	Root length (cm)	Root/shoot length ratio	Total length (cm)
Dugadda	668	77.0 ^{de}	51.14 ^b	4.54 ^a	7.02 ^{cd}	19.38 ^{de}	0.38 ^e	70.52 ^c
Langasu	815	76.0 ^e	43.32 ^c	3.95 ^c	6.68 ^{de}	16.65 ^f	0.39 ^e	59.97 ^d
Khadi	898	80.0 ^a	52.44 ^b	4.28 ^b	7.34 ^{bc}	21.26 ^{bc}	0.41 ^{de}	73.70 ^b
Gajeli	960	78.0 ^{cd}	55.02 ^a	4.68 ^a	7.88 ^a	23.62 ^a	0.43 ^d	78.64 ^a
Chinalisaur	1,033	81.0 ^a	38.30 ^d	3.92 ^c	6.22 ^{fg}	18.74 ^e	0.49 ^c	57.04 ^e
Harmani	1,151	75.0 ^e	36.02 ^e	3.72 ^c	6.12 ^g	20.72 ^{bcd}	0.58 ^a	56.74 ^e
Tela	1,254	76.0 ^e	50.84 ^b	4.52 ^{ab}	6.92 ^{de}	19.90 ^{cde}	0.39 ^e	70.74 ^c
Khandikhal	1,556	79.0 ^{bc}	51.82 ^b	4.60 ^a	7.72 ^{ab}	22.20 ^{ab}	0.43 ^d	74.02 ^b
Pabo	1,620	79.0 ^{bc}	50.92 ^b	4.28 ^b	7.06 ^{ed}	20.66 ^{bcd}	0.41 ^{de}	71.58 ^{bc}
Maun	1,875	79.0 ^{bc}	37.42 ^e	3.96 ^c	6.58 ^{ef}	19.72 ^{cde}	0.53 ^b	57.14 ^e
CV		1.28	2.91	4.25	5.93	6.93	6.18	3.04

Table 5 - Biomass attributes (dry weight basis) (g/plant \pm S.D.) of *B. variegata* at nursery stage after one year (Mean values followed by the same letter within column are not significantly ($P < 0.05$) different between population).

Seed sources	Altitude (m asl)	Biomass (g)				
		Shoot dry weight (g)	Leaves dry weight (g)	Root dry weight (g)	Root/ shoot dry weight (g)	Total dry weight (g)
Dugadda	668	2.98 ^{abc}	1.85 ^{bc}	2.11 ^{bc}	0.74 ^{bc}	6.94 ^{ab}
Langasu	815	2.64 ^c	1.81 ^{bc}	1.85 ^{bcd}	0.72 ^{bcd}	6.29 ^{bc}
Khadi	898	3.34 ^a	2.10 ^a	2.20 ^b	0.67 ^{cd}	7.64 ^a
Gajeli	960	3.10 ^{ab}	1.93 ^{ab}	2.07 ^{bc}	0.69 ^{cd}	7.10 ^{ab}
Chinalisaur	1,033	2.58 ^c	1.89 ^b	1.71 ^{cde}	0.68 ^{cd}	6.17 ^{bc}
Harmani	1,151	2.86 ^{bc}	1.77 ^c	1.79 ^{bcd}	0.66 ^{de}	6.41 ^{bc}
Tela	1,254	2.62 ^c	1.67 ^{cd}	1.47 ^{de}	0.57 ^e	5.75 ^{cd}
Khandikhal	1,556	3.27 ^{ab}	1.66 ^d	2.70 ^a	0.84 ^a	7.43 ^a
Pabo	1,620	2.84 ^{bc}	1.90 ^{ab}	2.19 ^b	0.80 ^{ab}	6.92 ^{ab}
Maun	1,875	2.12 ^d	1.51 ^d	1.35 ^e	0.64 ^{de}	4.98 ^d
CV		12.23	24.91	18.53	19.91	11.17

Table 6a - Correlation coefficient between geographical variable and seed and seedling traits of *B. variegata*.

Variables	Pod length	Pod breadth	Pod thickness	No of seed/pod	Seed length	Seed breadth	Seed thickness	Seed weight	Seed length /breadth ratio	Moisture %	Germination %
Altitude	-0.09 ^{ns}	-0.11 ^{ns}	-0.11 ^{ns}	-0.36 ^{**}	-0.09	0.13 ^{ns}	0.59 ^{**}	-0.28 [*]	-0.30 [*]	0.27 [*]	-0.28 [*]
Latitude	-0.29 [*]	-0.26 [*]	-0.24 [*]	0.17 ^{ns}	-0.10	-0.32 [*]	-0.15 ^{ns}	-0.20 [*]	0.18 ^{ns}	-0.37 ^{**}	-0.42 [*]
Longitude	0.04 ^{ns}	0.16 ^{ns}	-0.01 ^{ns}	-0.35 [*]	-0.18	-0.30 [*]	0.10 ^{ns}	0.08 ^{ns}	0.06 ^{ns}	0.12 ^{ns}	0.19 ^{ns}
Temperature	0.46 ^{**}	0.45 ^{**}	0.51 ^{**}	0.64 ^{**}	0.54 ^{**}	0.44 ^{**}	0.18 ^{ns}	0.45 ^{**}	0.47 ^{**}	0.28 [*]	0.20 [*]
Rainfall	-0.52 ^{**}	-0.49 ^{**}	-0.53 ^{**}	-0.75 ^{**}	-0.49 ^{**}	-0.15 ^{ns}	0.05 ^{ns}	-0.68 ^{**}	-0.72 ^{**}	-0.59 ^{**}	-0.59 ^{**}
Variables	Shoot length	Collar diameter	Number of leaves	Root length	Root/shoot length ratio	Total seedling length	Shoot dry weight	Leaves dry weight	Root dry weight	Root-shoot dry weight ratio	Total dry weight
Altitude	-0.21 [*]	-0.10 ^{ns}	-0.02 ^{ns}	0.22 [*]	0.38 ^{**}	-0.13 ^{ns}	-0.40 ^{**}	-0.66 ^{**}	-0.12 ^{ns}	0.12 ^{ns}	-0.40 ^{**}
Latitude	-0.30 [*]	-0.21 [*]	-0.34 [*]	-0.59 ^{**}	-0.01 ^{ns}	-0.36 [*]	-0.30 [*]	0.08 ^{ns}	-0.21 [*]	-0.07 ^{ns}	-0.22 [*]
Longitude	-0.03 ^{ns}	-0.26 [*]	-0.18 ^{ns}	-0.50 ^{**}	-0.45 ^{**}	-0.15 ^{ns}	-0.16 ^{ns}	0.07 ^{ns}	0.03 ^{ns}	0.28 [*]	-0.04 ^{ns}
Temperature	0.50 ^{**}	0.50 ^{**}	0.55 ^{**}	0.34 [*]	-0.34 [*]	0.51 ^{**}	0.29 [*]	0.35 ^{**}	0.24 [*]	0.13 ^{ns}	0.34 [*]
Rainfall	-0.61 ^{**}	-0.46 ^{**}	-0.53 ^{**}	-0.13 ^{ns}	0.63 ^{**}	-0.56 ^{**}	-0.66 ^{**}	-0.67 ^{**}	-0.50 ^{**}	-0.23 [*]	-0.71 ^{**}

*Significant at $p < 0.05$, ** significant at $p < 0.01$, NS= non-significant.

There was significant effect of seed weight on seed moisture percent, seed germination and seedling growth and biomass attributes except leaves dry weight ($p<0.001$) (Tab. 6b).

Table 6b - Analysis of variance for seed weight vs seed moisture, germination and seedling parameters.

Source of Variation	DF	F - Value										
		Moisture %	Germination %	Shoot length	Collar diameter	No. of leaves	Root length	Total seedling length	Shoot dry weight	Leaves dry weight	Root dry weight	Total dry weight
Seed weight	1	33.19**	4.78**	322.4**	43.88**	62.18**	4.53**	155.46**	14.52**	0.73NS	8.95**	8.95**

*Significant at $p < 0.05$, ** significant at $p < 0.01$, NS= non-significant.

Genetic variability

The genotypic coefficient of variability was maximum for the seed moisture percent and phenotypic and environmental coefficient of variability was highest for the number of seeds per pod. The lowest value for the genotypic, phenotypic and environmental coefficient of variability was calculated for the seed length/breadth ratio. Broad sense heritability (h^2)

was calculated more for pod breadth, seed breadth, seed weight, root/shoot dry weight ratio, root/shoot length ratio, total seedling length and shoot length and lowest for leaves dry weight. The moderate to higher heritability with moderate genetic gain was calculated for the pod length, breadth, thickness, seed weight, seed moisture percent, total seedling height, root/shoot length and root/shoot dry weight ratio (Tab. 7).

Table 7 - Estimation of variability in different pod, seed and seedling characteristics of *B. variegata*.

Characters	Coefficient of variance			Broad sense heritability (%)	Genetic advance (%)	Genetic gain (%)
	Genotypic	Phenotypic	Environmental			
Pod length (cm)	4.39	4.70	1.67	87.29	3.96	20.57
Pod breadth (cm)	4.23	4.32	0.88	95.88	1.22	62.45
Pod thickness (mm)	1.51	1.58	0.56	91.67	0.22	45.09
Pod length/breadth ratio	1.98	3.28	2.61	36.45	1.27	12.74
Seed length	1.04	1.10	0.36	89.47	0.27	16.95
Seed breadth	1.38	1.40	0.27	96.24	0.33	24.44
Seed thickness	0.83	0.92	0.15	81.82	0.09	33.65
Number of seed/ pod	12.09	24.15	20.78	25.92	8.04	76.90
Seed length/breadth ratio	0.45	0.54	0.29	70.59	0.10	8.65
Seed weight	9.26	9.42	1.72	96.66	12.70	28.73
Seed moisture	22.32	23.45	4.03	91.83	16.74	45.77
Germination %	0.47	1.48	1.41	10.06	0.87	1.07
Survival (%)	2.72	5.61	4.90	23.53	34.62	2.72
Shoot length (cm)	15.33	15.60	2.90	96.53	198.85	4.26
Root length (cm)	9.04	10.82	5.93	69.91	316.18	15.58
Total seedling length (cm)	12.40	12.52	1.80	97.94	1,692.95	25.26
Collar diameter (mm)	7.80	9.11	4.70	73.33	58.50	13.76
No. of leaves/seedling	8.14	9.21	4.32	78.04	102.94	14.81
Shoot dry weight (g)	11.68	16.89	12.20	47.83	47.25	16.64
Leaves dry weight (g)	0.56	24.35	24.98	5.26	4.72	2.64
Root dry weight (g)	18.59	26.28	18.58	50.0	52.51	13.15
R/S length ratio	4.55	4.55	22.72	99.80	29.13	66.20
R/S dry weight ratio	20.20	19.98	20.20	99.96	29.13	41.61

Analysis of variance

Pod length was significantly ($p<0.05$) varied among the populations while the pod thickness, seed

length, seed weight, seedling vigour index, seedling length and root/shoot length ratio were significantly ($p<0.01$) different among the populations (Tab. 8).

Table 8 - Analysis of variance (ANOVA) for different morphological traits of pod, seed and seedlings characteristics.

Source of variation	DF	Pod length	Pod breadth	Pod thickness	Seed length	Seed breadth	Seed thickness	Seed weight	Germination %	
Treatment	9	34.78*	17.27 ^{ns}	39.76**	35.99**	17.27 ^{ns}	4.0 ^{ns}	145.32**	1.17 ^{ns}	
Replication	4	0.20 ^{ns}	1.60 ^{ns}	0.23 ^{ns}	3.15 ^{ns}	1.60 ^{ns}	1.25 ^{ns}	1.04 ^{ns}	11.75 ^{ns}	
Source of variation	Seedling vigour Index	Shoot length	Collar diameter	No. of leaves	Root length	Total length	Root/shoot length ratio	Shoot dry weight	Leaves dry weight	Root dry weight
Treatment	27.76**	140.25**	15.6 ^{ns}	18.3 ^{ns}	12.64 ^{ns}	84.06 ^{ns}	30.27**	5.52 ^{ns}	0.97 ^{ns}	6.07 ^{ns}
Replication	3.89 ^{ns}	1.66 ^{ns}	5.01 ^{ns}	1.04 ^{ns}	1.45 ^{ns}	0.16 ^{ns}	3.72 ^{ns}	7.18 ^{ns}	1.24 ^{n_{ns}}	5.63 ^{ns}

*Significant at $p < 0.05$, ** significant at $p < 0.01$, NS= non-significant.

Discussion

Morphological variation in seeds traits in between or within populations of *B. variegata* was due to evolutionary responses of plant to maximize the potential fitness by producing a larger number of seeds and increase the chance of establishment of seedlings through immense allocation of maternal resources to individual seeds (Zhang 1998). In present study, populations located at lower to middle altitude was produced heavier seed, maximum germination per cent, high shoot length and higher biomass. This could probably be due to environmental factors which might have played a significant role in phenology, particularly in flower initiation. The earlier production of the flower might have played an important role in the development of heavier seeds and their associated parameters. Similar variation in seed length, breadth and thickness of different populations was recorded in *Celtis australis* L. (Singh et al. 2006) and *Dalbergia sissoo* Roxb (Singh and Bhatt 2008a).

It is evident from the results that the seeds of Gajeli population were superior with respect to seed germination while the seed germination of *B. variegata* was significantly ($P < 0.05$) varied among the populations. The populations situated at lower altitude (i.e. Dugadda, Langasu, Khadi, Gajeli) and those in middle to higher altitude (i.e. Tela, Khandikhal and Pabo) was resulted more than 80 percent germination. Seed germination negatively significantly correlated with the populations indicating that the lower altitude exhibited higher germination. Seed germination percent, is a physiological phenomenon strongly influenced by the environmental conditions such as temperature, light and soil moisture (Abdelbasit et al. 2014). Various researchers were reported that the pods/seeds of a species collected from different sources or altitudes differ in pod size, seed morphology, viability, germination, seed dormancy, seedling growth and biomass production. In fact, long lived tree species like *B variegata* grows over a wide range of climatic conditions, altitudes and soil conditions which create variation in morphology of pods, seeds, seed germination, growth and biomass (Singh et al. 2010, Gairola et al. 2011, Ahirwar 2012, Selvan and Guleria 2012, Arthanari et al. 2013, Kumar 2014, Amri 2014, Palani et al. 2015, Fredrick et al. 2015, Nyoka et al. 2015, Freigoun et al. 2017, El Guemri et al. 2019,

Iwaizumi et al. 2019, Lawin et al. 2021). The sharp decrease in germination index (GI) values with the increased mean germination time was observed in *B. variegata* (Tab. 3). Similar trend was also observed in *Quercus leucotrichophora* A. Camus (Saklani et al. 2012), *Trichoderma asperellum* (de Souza et al. 2021) and *Phyllostachys edulis* (Emamverdian et al. 2021).

The seed maturation time of *B. variegata* is between May and June, and the seeds are dispersed by gravitational force and when come with the right moisture conditions they germinate quickly. Seedlings can survive in dry periods. This might be causing morphological variation in pods, seeds, and seed germination among the populations. Raddad (2007) suggested that the tree species which seeds germinate and survive in dry periods are expected to show variation in morphological and physiological traits that may arise due to the evolution of certain potentiality and adaptive genetic correlation between germination and post-germination traits.

Significant variation ($p < 0.05$) was observed in seedling parameters, among the populations. Among the studied populations, six had > 50.0 cm and 4.0 mm shoot length and collar diameter after twelve months of growth, respectively. Similarly, five populations exhibited > 20.0 cm root length. After twelve months of growth, Gajeli population exhibited > 75.0 cm total length of seedlings; five populations (Khandikhal, Khadi, Pabo, Tela and Dugadda) had the total seedling length of > 70.0 cm. However, four populations (Langasu, Chinalisaur, Maun and Harmani) exhibited poor growth as compared to that of other populations studied. These variations in growth characteristics of *B.variegata* from different populations could be attributed to adaptation to climatic conditions in which they grow, because the seedlings from all the populations were raised under similar conditions (Singh and Pokhriyal 2001). Further, the variation in growth parameters in the nursery among the populations of origin is related to climatic condition and/or geographic location of the population (Weber et al. 2015, Bayala et al. 2017). In this study, different seed and seedling traits (seed breadth, seed weight, germination, seed moisture, shoot length, root length and total seedling length) showed great variation within populations. Significant variation in

morphological traits and biomass production was also observed among different populations (Himachal Pradesh, Uttarakhand, Haryana, Jammu and Kashmir) of *B. variegata* (Wani and Wani 2014). In another study, collar diameter, plant height and leaf area of *B. variegata* varied among the genotypes (Anand and Dwivedi 2014). These results are in line with the results of present study.

Out of 22 parameters of pods, seeds, seedling growth and biomass in *B. variegata*, 21 parameters were significantly correlated with geographical variables (altitude, latitude, longitude). Latitude was inversely correlated with pod length, pod breadth, pod thickness, seed breadth, seed weight, seed moisture, seed germination, shoot length, collar diameter, number of leaves, root length, total seedling length, shoot dry weight, and total dry weight, which indicating southern trend of these parameters. The longitude exhibited positive correlations with root/shoot dry weight, indicating that this trait is increasing towards eastern extremes. The inverse correlation of longitude with number of seeds/pods, seed breadth, collar diameter, root length and root/shoot length ratio indicates that these traits are increasing toward western extremes. Similar trend of relationship was found in *Dalbergia sissoo* (Singh and Bhatt 2008). The seed weight of *B. variegata* significantly improved the germination and seedling growth attributes, indicating that seed weight could be a valuable indicator of the survival and growth potential of the seedlings (Singh et al. 2006).

Moderate to high heritability with moderate genetic gain was recorded maximum for pod breadth, seed breadth, seed thickness, seed weight and seed moisture, total seedling length, root/shoot length and root/shoot dry weight ratio, indicating that these characters are highly genetic origin. Genetic advance was also highest in total seed length as compared to other seed traits. High heritability values accompanied by high genetic gain have earlier been reported for seed weight in *Celtis australis* (Singh et al. 2008b), *Anthocephalus cadamba* Mig. (Sudrataj 2016), *Madhuca latifolia* (Roxb.) A. Chev (Nayak and Sahoo 2020), and *Emblica officinalis* L (Naithani et al. 2020). In present study high heritability with moderate genetic gain was recorded for seed weight.

Conclusion

The present study was carried out to investigate the population variation in respect to pod, seed and seedling traits of *B. variegata* to select the suitable population for production of high-quality planting material. Significant differences in pod, seed, and seedling traits among the populations are due to wide distribution of *B. variegata* in Garhwal Himalayan region. Seed weight was highly significantly

correlated with all other seed, seedling and biomass allocation traits. The pod and seed moisture percentages were variable among the ten populations indicating that these populations are facing different environmental conditions, leading to variation in morphometric characters of pods and seeds. Further morphological variation in pods and seeds influenced the seedling growth and biomass production in nursery condition. Among the studied populations, Khadi, Gajeli, Dugadda and Khandikhal exhibited higher seed weight and seedling growths and these populations are recommended for the collection of seeds for the production of quality planting materials in nursery. Further, the seeds of *B. variegata* should be collected in the month of June and should be sown in polybags after one week of sun drying for obtaining maximum germination.

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