

Variability in cone, seed and seedling characteristics of *Pinus kesiya* Royle ex. Gordon

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Abstract Investigations were made to determine the nature and extent of variations present for 10 morphological characters of cone, seed and seedlings of 17 plus trees (PTs) of *Pinus kesiya* growing in eastern Himalayas (Meghalaya state) of India. The significant variations has been observed in cone weight, cone length, cone diameter, seed length, seed diameter, seed weight, number of seeds per cone, seed germination, seedling height and seedling collar diameter among different PTs of the species. Significant positive correlation ($p < 0.01$) between seed weight, cone length and cone diameter showed that seed weight in *P. kesiya* depend more on the cone size. Seed germination is found positively correlated ($p < 0.01$) with seed weight, seed diameter, cone size and number of seeds per cone in the study. Among geographical factors, latitude and altitude are found positively correlated ($p < 0.05$) with cone weight and seed diameter, respectively. The genetic parameters for seed, cone and seedlings of PTs of *P. kesiya* also showed a wide range of variability in terms of variance, coefficient of variability, broad sense heritability, genetic advance and genetic gain. Seed weight, seeds per cone and seedling diameter showed high heritability values (>75) coupled with

maximum genetic gain. Traits with such values indicate the presence of good amount of heritable additive components and are under strong genetic control. The findings of the study revealed that PTs expressed both phenotypic and genotypic differences in the seed, cone and seedling characters, which may be attributed to the differences in genetic makeup of various PTs and environmental factors i.e. genotypic and environmental interaction. The presence of high variability in *P. kesiya* PTs for different characters provide further opportunities to improve the population in subsequent generations so as to establish this species as an important timber tree to be grown on less fertile and degraded soils in its distributional ranges.

Keywords Plus trees · Seed traits · Heritability · Genetic gain · Improvement

Introduction

Pinus kesiya (Khasipine) is widely distributed between 30°N and 12°N in South East Asia and occurs in Myanmar, India, Tibet, Laos, Vietnam, Thailand, the Philippines and the People's Republic of China. In India, the species occurs in Khasi and Jaintia Hills of Meghalaya, Arunachal Pradesh, Nagaland and Manipur. The tree grows at altitudes between 350 and 3,000 m above sea level, though the species is concentrated at levels between 700 to 1,200 m. This is the only tropical pine growing in the eastern Himalayas and is a pioneer species and colonizes fire-killed areas and sites degraded by shifting cultivation (Troup 1921). As the tree grows in a variety of soils and is tolerant to various pests and climatic conditions (wind, frost and drought), it is possible to grow the tree widely in sub-tropical and tropical areas. The production rate of the

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species is moderately high due its rapid growth rate, vigorous germination and propagation in favourable conditions (Anon 2002). *P. kesiya* is an important wood producer, particularly in southern Africa and other tropical regions; however, poor stem form and branching pattern have militated against the acceptance of this pine as a plantation species. If the problems associated with the species can be iron out through breeding programmes and other means, the tree will become an important timber tree especially on less fertile and degraded soils in its distributional ranges.

The scattered wide distribution pattern of *P. kesiya* suggests the presence of high degree of ecotypic variation and development of divergence local races subsequently, as has been documented in a number of studies. International provenance trials and other researches on this pine suggest the presence of enormous genetic variability with significant differences in quantitative and qualitative traits (Hansen et al. 2003). The geographic variation in individual tree species may reveal an underlying pattern of genetic variation shaped by natural selection under specific geoclimatic conditions and other evolutionary processes. The pattern of genetic variation is typically expressed by correlations between environmental variables and genetic source response. The assessment of genetic variability is a key to progress in tree improvement and is a useful tool in determining the strategies for tree improvement of any important species. The plantations established from genetically uniform material are highly vulnerable to major climatic factors or epidemic particularly for insects and diseases (Aradhya and Philips 1993). Plant species possess substantial genetic variation in several growth and adaptive traits and the use of selective genotypes can enhance site productivity and reduce the risk of mal-adaptation (Hammann et al. 2000).

The seed is the most important stage in the life cycle of plants (Baskin and Baskin 2001), and seed traits, including mass, dormancy and dispersal, are central components of plant life histories (Thompson 1987), whose importance to plant fitness is widely appreciated (Moles et al. 2007; Turnbull et al. 2012). Traditionally, seed mass within species was considered to be a remarkably constant characteristic (Bu et al. 2007). The objective of the present investigation was to understand the nature, extent and pattern of genetic variation existing in different superior phenotypes (PTs) of *P. kesiya* in respect of cone, seed and seedling characters and the degree of genetic control on characters of cone and seed morphology and whether these are related with seed germination and seedling characteristics. Such an investigation is needed to establish a strong genetic base for selection of some prominent characters to be considered in restoration or re-vegetation programmes of the species.

Materials and methods

Seeds were sampled from 17 superior PTs grown in natural distribution ranges of the species in the state of Meghalaya. These PTs were selected following standard methodology of point grading and are at least 100 m apart from each other (Singh and Mahanta 2013). The geographical positions of different PTs of *P. kesiya* used in the experimentation are given in Table 1.

The mature cones (50 in number) of *P. kesiya* were collected from PTs during the month of January from branches all around the tree to avoid the effect of cone position on the seed traits studied. The cones were brought to seed laboratory at Rain Forest Research Institute, Jorhat (Assam). After appropriate drying the cones in mild sun, seeds were extracted manually from the cones.

Cone and seed characteristics

Cone and seed parameters of all plus trees were measured following International Seed Testing Association (ISTA 1993) rules. Measurements were recorded for cone length, cone diameter and fresh weight of cone using twenty randomly drawn mature cones of each tree. In this case, five cones were considered as a replication. Seeds per cone were also counted and recorded. The seed dimensions i.e. seed length and seed diameter of 25 seeds in four replicates was recorded in millimeters using scale and electronic caliper. Seed weight was taken of the pure seed fraction in 8 replications and expressed as the 100 seed weight.

The seeds were soaked in distilled water for 24 h before germination tests, after that seeds were disinfected with 0.05 % of mercuric chloride solution for 5 min and washed three times with distilled water before putting them for germination test. The seeds of different PTs (100 seeds in four replications) were evenly placed on Whatman #1 filter paper in sterile Petri dishes (100 mm × 25 mm) and these dishes were placed inside the germination chamber, with a light intensity of 750 lux and photoperiod of 16 h light at constant temperature of 30 °C. Germination test was run for 21 days and germination count was recorded daily and quantified as per cent germination according to ISTA (1993) rules.

Seedlings traits (Nursery)

Nursery studies were conducted by raising the seedlings of *P. kesiya* in forest nursery at Barapani, Umiam in the state of Meghalaya (91°53'N, 25°34'E and 950 m altitude). Seeds of PTs were sown during the month of February in polythene begs (size 22 cm × 15 cm) filled with soil mixture of soil, sand and FYM in the ratio of 1:1:1. The seedlings were grown and maintained up to July next year.

Table 1 Geographical location of plus trees of *Pinus kesiya*

Plus tree no.	Location/forest	Longitude (°E)	Latitude (°N)	Altitude (m)	Mean annual temperature (°C)		Mean annual rainfall (mm)
					Maximum	Minimum	
3	RietKhwan	25.35	91.54	1,150	25.74	14.42	2,205.8
5	RietKhwan	25.35	91.54	1,150	25.74	14.42	2,205.8
6	RietKhwan	25.35	91.54	1,150	25.74	14.42	2,205.8
7	RietKhwan	25.35	91.54	1,150	25.74	14.42	2,205.8
8	RietKhwan	25.35	91.54	1,085	25.74	14.42	2,205.8
9	RietKhwan	25.35	91.54	1,085	25.74	14.42	2,205.8
15	Barapani	25.34	91.53	950	25.74	14.42	2,205.8
18	Barapani	25.34	91.53	950	25.74	14.42	2,205.8
21	Malki	25.50	91.80	1,650	21.05	12.26	2,159.3
22	Malki	25.50	91.80	1,650	21.05	12.26	2,159.3
23	Motinagar	25.40	91.75	1,450	21.05	12.26	2,159.3
24	Motinagar	25.40	91.75	1,450	21.05	12.26	2,159.3
25	Motinagar	25.40	91.75	1,430	21.05	12.26	2,159.3
26	Motinagar	25.40	91.75	1,430	21.05	12.26	2,159.3
27	RietLeban	25.45	91.78	1,350	21.05	12.26	2,159.3
28	RietLeban	25.45	91.78	1,350	21.05	12.26	2,159.3
29	RietLeban	25.45	91.78	1,350	21.05	12.26	2,159.3

After about 1 year of growth and at the time of planting out in July, height and collar diameter of 40 seedlings of each plus tree were recorded using scale and electronic caliper. Here 10 seedlings were treated as one replication.

Statistical analysis

The collected data on various aspects were analyzed statistically for variability, correlation and genetic superiority using SPSS software. The generalized linear model (GLM) was employed for analysis of variance (ANOVA) as per skeleton suggested by Sukhatme and Amble (1985).

Simple correlation coefficients (Karl Pearson's) between seed, cone, germination, seedling and geographical parameters were also calculated using SPSS software.

Genetic variability of different characters was calculated as:

Variance

The genotypic and phenotypic components of variance were calculated from ANOVA as described by Burton (1952).

$$\sigma_g^2 = (M_{sp} - M_{se})/r \tag{1}$$

where, σ_g^2 is genotypic variance, M_{sp} is plus trees mean sum of square, M_{se} is error mean sum of square, r is number of replication.

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2 \tag{2}$$

where, σ_p^2 is phenotypic variance σ_g^2 is genotypic variance, σ_e^2 is error mean sum of square.

$$G_{CV} = \sqrt{\sigma_g^2/M_e} \times 100 \tag{3}$$

where, G_{CV} is genotypic coefficient of variance, σ_g^2 is genotypic variance, M_e is population mean.

$$P_{CV} = \sqrt{\sigma_p^2/M_e} \times 100 \tag{4}$$

where, P_{CV} is phenotypic coefficient of variance, σ_p^2 is phenotypic variance, M_e is population mean.

Heritability

Broad sense heritability was calculated as per Lush (1994).

$$h^2 = \sigma_g^2/\sigma_p^2 \times 100 \tag{5}$$

where, h^2 is heritability, σ_g^2 is genotypic variance, σ_p^2 is phenotypic variance.

Genetic advance

The genetic advance was calculated as per Johnson et al. (1955).

$$G_s = K \times h^2 \times \sqrt{\sigma_p^2} \tag{6}$$

where, G_s is genetic advance, K is selection differential (2.06 at 5 % selection intensity) (Cotterill and Dean 1990), h^2 is heritability, σ_p^2 is phenotypic variance. Genetic gain:

Table 2 Cone, seed and seedling characteristics in plus trees of *Pinus kesiya*

Plus Tree	Cone fresh weight (g)	Cone length (cm)	Cone diameter (mm)	Seed length (mm)	Seed diameter (mm)	Seed weight (g)	Seeds/ cone	Seed Germination (%)	Seedling height (cm)	Seedling collar diameter (mm)
3	26.30	57.76	33.58	5.19	2.47	1.44	74.20	74.00	36.10	5.17
5	44.03	76.38	40.32	5.49	2.70	1.64	88.80	74.62	35.71	5.32
6	31.21	62.49	39.09	5.91	2.66	1.61	63.00	71.50	40.63	5.48
7	35.39	67.02	37.05	5.13	2.44	1.36	67.60	68.37	32.63	4.23
8	44.39	68.11	38.19	5.04	2.65	1.71	76.80	74.62	31.99	4.68
9	56.20	72.52	40.41	5.39	2.41	1.51	102.0	75.35	31.68	4.39
15	38.44	61.53	36.92	4.85	2.44	1.26	41.20	67.50	31.18	4.53
18	57.21	81.34	41.00	4.87	2.80	1.72	99.40	76.62	34.50	5.24
21	33.85	65.11	35.79	5.96	3.05	1.79	68.60	76.25	27.17	3.91
22	53.59	68.60	40.58	4.95	2.75	1.75	89.60	78.37	31.52	4.12
23	61.66	89.91	41.49	5.27	2.89	2.24	86.60	83.05	28.20	3.81
24	51.76	68.52	39.69	5.03	2.57	1.43	95.00	74.17	27.60	3.45
25	42.95	75.34	34.91	5.14	2.69	1.42	89.00	72.50	18.57	3.27
26	30.73	68.53	38.77	5.53	2.99	2.20	74.60	80.25	23.45	3.64
27	29.09	63.16	37.07	5.13	2.63	1.49	48.60	67.87	24.12	3.45
28	26.24	62.65	37.23	5.13	2.78	1.61	64.60	71.00	21.99	3.25
29	75.56	84.15	47.22	5.84	2.96	2.17	93.80	80.37	27.84	3.38
CV	32.42	12.43	8.04	6.63	7.06	17.46	22.92	6.00	19.06	18.14
SE	4.78	3.04	1.31	0.23	0.12	0.08	0.08	3.64	2.14	0.35
CD (0.05)	9.38	5.95	2.57	0.47	0.28	0.16	0.16	7.14	4.19	0.68

CV Coefficient of variation, SE standard error, CD critical difference

The expected genetic gain, in percent of mean, was calculated following (Burton and Devane 1993).

$$G_g = G_s \times 100/M_e \quad (7)$$

where, G_g is genetic gain, G_s is genetic advance, M_e is population mean.

Results and discussion

Seed/cone/seedling variability

The present study has conclusively established significant variations in superior PTs of *P. kesiya* for seed, cone and seedling characters (Table 2). All the ten characters studied revealed significant differences among 17 PTs at 5 % level of significance. The highest coefficient of variation was recorded for cone fresh weight (32.42) followed by number of seeds per cone (22.92) and seed weight (17.46). Seed weight and seed diameter varies from 2.24 to 1.26 g and 3.05 to 2.41 mm, respectively, among different PTs of the species in this study. Large variation in seed size was observed within populations (Simons and Johnston 2000) and seedlings of larger seeds tend to have greater survival

and improved performance under a wide range of environmental conditions (Manning et al. 2009). Seedling height and collar diameter at nursery level also showed sufficient amount of variation among different PTs of *P. kesiya* in this study. Such variations in relation to habitat have also been reported in *Pinus wallichiana* (Singh and Thapliyal 2012) and in *P. bungeana* (Wang et al. 1998). Mamo et al. (2006) reported the variation in seed and germination characteristics among *Juniperous procera* populations in Ethiopia. Wang et al. (2014) also examined intra and inter-specific variation in seed mass and morphology in relation to altitude, habitat, plant height, and phylogeny among forty-two species of *Rhododendron* on the Tibetan plateau.

The differences recorded for various traits in PTs may be in response to different intensities of natural selection pressure upon these traits in their natural habitat and such differences have an adaptive advantage in local edapho-climatic conditions. Since the seeds were collected from different locations, differences observed in cone, seed and seedling parameters may be attributed to both internal (maternal, hereditary) and external (environmental) conditions operating at the time of seed development (Harper et al. 1970). In a leguminous species the pod, seed and

Table 3 Correlation coefficients (r values) of cone, seed, seedling and geographical parameters of *Pinus kesiya*

Characters	Cone weight	Cone length	Cone diameter	Seed length	Seed diameter	Seed weight	Seeds/cone	Seed germination	Seedling length	Seedling diameter	Latitude	Altitude
Cone weight	1	0.829**	0.836**	-0.381	0.208	0.437	0.719**	0.612**	0.046	-0.111	0.587*	0.040
Cone length		1	0.708**	-0.184	0.428	0.612**	0.673**	0.678**	-0.107	-0.132	0.410	0.079
Cone diameter			1	-0.086	0.360	0.610**	0.523*	0.583*	0.133	-0.074	0.697*	0.027
Seed length				1	0.423	0.258	-0.157	0.079	-0.034	-0.028	-0.010	0.293
Seed diameter					1	0.828**	0.175	0.669**	-0.363	-0.335	0.340	0.592*
Seed weight						1	0.329	0.874**	-0.163	-0.223	0.440	0.406
Seeds/Cone							1	0.661**	0.045	0.010	0.236	0.123
Seed germination								1	-0.055	-0.110	0.339	0.412
Seedling length									1	0.915**	-0.055	0.021
Seedling diameter										1	-0.274	-0.659**
Latitude											1	0.082
Altitude												1

* Correlation is significant at 0.05 level (2-tailed), ** Correlation is significant at 0.01 level (2-tailed)

germination traits were considered largely under maternal influences but were strongly controlled by micro and macro habitats, besides the age and general health of the parent trees (Isik 1986).

Significant positive correlation between seed weight and cone length ($r = 0.612$), seed weight and cone diameter ($r = 0.610$) showed that seed weight in the species depend more on the cone size. Seed germination was found positively correlated ($p < 0.01$) with seed weight, seed diameter, cone size and number of seeds per cone in the study. Though the seed characters are significantly different between PTs yet they do not show any trend with latitude and altitude. However latitude and altitude are found positively correlated ($p < 0.05$) with cone weight and seed diameter, respectively (Table 3). This observation could be explained on the basis of presence of rich soil at higher altitude forests of Himalayas (Dhir 1963). Khalil (1986) also reported variations in seed quality and some juvenile characters in 110 provenances of *Picea glauca* covering the whole of the natural ranges.

Genetic variability

The variability among the genotypes is commonly used as an estimate of total genetic variation and used to calculate the degree of genetic control for a particular trait (Foster and Shaw 1988). The estimates of phenotypic and genotypic variances as well as the coefficient of phenotypic and genotypic variation of different characters in *P. kesiya* are shown in Table 4. The relative amount of variation in different characters can be formed by comparing the coefficient of phenotypic and genotypic variation of each character. In general, both the coefficient of phenotypic and genotypic variation was of comparable magnitude for all the ten characters studied. PTs exhibited a wide range of variability in terms of variance, coefficient of variability, broad sense heritability, genetic advance & genetic gain and provide an opportunity to screen out the desired characters in the species.

The heritability expresses the degree to which a character is influenced by heredity as compared the environment. The heritability estimates (broad sense) were found highest in seeds/cone (0.93) followed by seedling diameter (0.89) and seed weight (0.80) of PTs of *P. kesiya* in this study.

These characters also showed high heritability values (>75) coupled with maximum genetic gain. Traits with such values indicate the presence of good amount of heritable additive components and are under strong genetic control. However, other characters studied showed low to moderate heritability and genetic gain. The findings of this study revealed that PTs of *P. kesiya* expressed both phenotypic and genotypic differences in the seed, cone and seedling characteristics which might be due to the

Table 4 Genotypic variance, phenotypic variance, coefficient of variation, heritability and genetic gain in cone, seed and seedling traits of *Pinus kesiya*

Characters	GV	PV	GCV	PCV	h^2	Gs	G. Gain
Cone fresh weight	187.26	244.48	30.00	34.00	0.77	24.80	54.00
Cone length	71.73	94.88	12.07	13.87	0.75	15.17	21.62
Cone diameter	8.88	13.18	7.68	9.36	0.67	5.04	12.99
Seed length	0.10	0.23	6.00	9.00	0.43	0.43	8.00
Seed diameter	0.04	0.07	7.43	9.84	0.57	0.31	12.00
Seed weight	0.08	0.10	17.00	19.00	0.80	0.52	31.00
Seeds/cone	299.47	322.57	22.00	23.00	0.93	34.40	44.00
Seed germination	13.59	40.14	4.95	8.50	0.34	4.42	5.93
Seedling length	29.80	38.97	18.38	21.02	0.76	9.83	33.11
Seedling diameter	2.11	2.35	34.69	36.64	0.89	2.83	67.66

GV Genotypic variance, PV phenotypic variance, GCV genotypic coefficient of variance, PCV phenotypic coefficient of variance, h^2 Heritability, Gs genetic advance, G gain is genetic gain

differences in genetic makeup of different PTs and environmental factors i.e. genotypic and environmental interaction. Significant genetic variability and association were also recoded among 40 CPTs for pod and seed traits in *Pongamia pinnata* (Kaushik et al. 2007). Johnsen et al. (1989), Volkar et al. (1990), Singh and Chaudhary (1993) and Singh and Thapliyal (2012) reported that the heritability estimates along with estimates of expected genetic gain is more useful than the heritability alone in predicting the resultant effect for selecting the best genotypes for a given trait.

Conclusions

Plantations raised through intensively selected and tested material have many advantages over plantations raised from unknown seed source. The genetically improved plantations could produce higher utilizable biomass and significantly reduce wastage during wood processing. Improved plantations of the species can also play a complementary role in conservation through compensatory afforestation and provide necessary timber and industrial raw material which otherwise would come from natural forests. The presence of significant variations in cone, seed and seedling traits as well as occurrence of genetic variability in terms of variance, coefficient of variability, broad sense heritability, genetic advance and genetic gain in different PTs of *P. kesiya* provide an opportunity to screen out the desired characters, so as to improve this important tree species and make use of it in further afforestation/ reforestation programmes in the region. In addition a high genetic variation as measured and shown in the species in this study is fundamental for the maintenance and long-term stability of any forest ecosystem.

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