

## Genetic Divergence in Pod and Seed Traits of *Robinia pseudoacacia* Linn. in North Kashmir Himalayas, India

### ABSTRACT

The present investigation was conducted to record variation of pod and seed traits among nine seed sources of *Robinia pseudoacacia* Linn. Study of variation is the first step for any breeding programme. Significant variations ( $p < 0.05$ ) were recorded for different morphological parameters of seed and seedling among the different provenances. Pods were collected from nine seed sources of Kashmir valley. Analysis of variance revealed highly significant differences among the pod and seed traits, thus exhibiting more variability. Seed source ( $S_2$ ) exhibited outstanding performance for the pod and seed traits (pod length- 72.66 mm; pod width- 13.57mm; seeds per pod- 7.92), whereas  $S_9$  showed the lowest values for pod length as 60.98mm) and  $S_8$  recorded minimum values for pod width (11.13mm) and number of seeds per pod (4.55). Maximum seed weight among sites (24.05 g) was observed in  $S_2$  and it was found statistically at par with  $S_4$  (24.02 g) and followed by  $S_6$  (24.00 g) with minimum observed in  $S_7$  (22.66 g). Furthermore, this variability appeared to be geographically structured and would be mainly genetically controlled.

**Key words:** *Robinia pseudoacacia*, variation, seed, source, pod, heritability

### INTRODUCTION

Genetic progress in desired characteristics for domestication is partly determined by the existing phenotypic diversity in the genetic base of plant populations. Traditional breeding programs have always assessed intra-specific genetic and genomic variation within and among populations and families involving thousands of individuals in attempts to increase selection differential and hence maximize genetic and genomic gains [1]. Variation within populations of tree species has been exploited in the selection of superior seed source for a given site and for evolving conservation strategies of genetic diversity within populations of tree species [2]. The seed sources represent the best available genetic material for planting as exhibited by the parental material [3].

In forest trees, a number of categories of variation exist that can be broadly grouped into Species, Geographic Sources (Provenances), Stands, Sites, Individual trees, and the variability within individual trees [4]. Genetic variation is the basis for tree improvement. It cannot always be seen directly, it must be studied through well-designed tests. The study of variation is the first step for forest tree improvement breeding programmes. Variability studies under various climatic conditions for a particular species is prerequisite and desirable for the genetic improvement and screening of the available variation for higher productivity and future breeding work. Any attempt to examine the extent and pattern of genetic variation may help in early evaluation of criteria for selection of some prominent traits both in laboratory and nursery condition, which may be related to subsequent performance in the field [5].

The genus *Robinia* is characterized by a variety of woody legume species, both shrubs and trees, all with pinnately compound leaves and diadelphous flower morphologies [6]. *Robinia* includes four species *Robinia hispida* (Bristly locust), *Robinia viscosa* (Clammy locust) and *Robinia neomaxicana* (New Mexican locust) and *Robinia pseudoacacia* (Black locust), sometimes called yellow locust, is a deciduous tree that belongs to the Fabaceae (legume) family and the Caesalpinioideae subfamily [7]. It has been introduced worldwide as a commercially important multipurpose tree because of its adaptability to environmental stresses, its rapid growth and the hardness of its wood [8]. Despite being the tree of tremendous potential and multifarious uses, it has only got limited research attention especially in Kashmir valley and no such records of provenance trails of *Robinia pseudoacacia* exist. Therefore, this study was

undertaken to investigate and evaluate the criteria for selection of some prominent traits, which may be related to subsequent performance in the field.

## MATERIALS AND METHODS

The study was carried out within the jurisdiction of three districts of North Kashmir viz., Baramulla, Kupwara and Bandipora (Table-1). From each district, three sites were selected based on distribution and abundance of the species. From each site (source), pods were collected during the month of September, 2020 from middle aged trees, located about 100 m apart from each other in order to avoid narrowing down of the genetic base due to inbreeding [9]. The seeds were extracted from pods and the samples so collected were kept in such a way that the identity of site and the individual tree could be maintained while putting them to further use in the laboratory.

**Table-1: Description of seed sources of *Robinia pseudoacacia* Linn. in North Kashmir Himalayas under study**

S. no.	District	Source Name	Source No.	Altitude (m)	Latitude (N)	Longitude (E)
1	Baramulla	Sherabad Khore	S1	1585	34°08'	74°34'
		Palhallan Pattan	S2	1692	34°07'	74°54'
		Kunzer Tangmarg	S3	1814	34°04'	74°30'
2	Bandipora	Sumbal tangpora	S4	1589	34°23'	74°65'
		Chewa Bandipora	S5	1601	34°28'	74°65'
		Kanibathy Bediyaar	S6	1965	34°40'	74°53'
3	Kupwara	Nutnusa Kupwara	S7	1081	34°51'	74°37'
		Potushai Lolab	S8	1706	34°53'	74°35'
		Watyam Chandigam	S9	1596	34°40'	74°34'

The whole data obtained was subjected to statistical analysis using RBD (factorial) design employing R-software. Genetic parameters were calculated in terms of mean, range, genotypic, phenotypic and environmental coefficient of variation, heritability (H), genetic advance and genetic gain as per cent of mean [10]. Genetic divergence was calculated by using non-hierarchical Euclidean cluster Analysis [11].

## RESULTS AND DISCUSSION

### VARIABILITY ESTIMATES AND GENETIC PARAMETERS

Perusal of the data in Table- 2 revealed significant variation among pod and seed traits. Among the studied seed sources, maximum pod length among of 72.66 mm was observed in S<sub>2</sub> followed by S<sub>5</sub> (67.15 mm). The minimum value was observed in S<sub>9</sub> (60.98 mm). Similarly, pod width was recorded maximum in S<sub>2</sub> (13.57 mm) followed by S<sub>5</sub> (12.78 mm), however minimum value was observed in S<sub>8</sub> (11.13 mm).

**Analysis** of variance indicated that maximum number of seeds per pod among sites was observed in S<sub>2</sub> (7.92) with minimum values obtained for S<sub>8</sub> (4.55). Likewise, maximum seed weight among sources was observed in S<sub>2</sub> (24.05 g) which was found to be statistically at par with S<sub>4</sub> (24.02 g) followed by S<sub>6</sub> (24.00 g) with minimum recorded in S<sub>7</sub> (22.66 g).

**Table -3** shows that among all pod and seed traits, pod length showed wide range of values (46.91 to 88.95 mm), followed by seed weight (21.92 to 24.96 g). Regarding to the genotypic coefficient of variation (GCV) was found maximum for number of seeds per pod (25.42 %) while minimum for seed weight (3.73 %). Phenotypic coefficient of variation (PCV) was found maximum for number of seeds per pod (27.07 %), the phenotypic coefficient of variation values were found slightly higher for all the parameters than genotypic coefficient of variation.

Heritability was found to be maximum (0.95) for seed weight. It was followed by pod length (0.91) and number of seeds per pod (0.88) whereas; pod width recorded the minimum heritability (0.87). Genetic advance was recorded maximum (21.77) for seed weight. Genetic gain was recorded maximum for number of seeds per pod (49.18 %) followed by pod length (20.41%).

**Similar** variations have been reported by different workers in different species, Kraszkiewicz [12] in *Robinia pseudoacacia*, Kumar *et al.* [13] in *Celtis australis*, Thakur *et al.* [14] in *Embllica officinalis*. High heritability with moderate genetic gain was observed for seed weight followed by pod length and number of seeds per pod. Highly significant and positive genotypic and significant positive phenotypic correlation was observed for pod width and pod length. All the pod and seed traits characters showed wide range of values (Table-2) indicating the extent of variation existing in the genotypes. The phenotypic coefficient of variation was higher than the genotypic coefficient of variation in all the characters which indicates that the traits were greatly influenced by the environment.

High heritability with moderate genetic gain was recorded for seed weight followed by pod length which revealed that traits were under strong genetic control. **Whereas, seed length and seed germination were found to have moderate heritability.** Wide range (17.74-49.18 %) was observed for genetic gain for evaluated characters. Therefore, selection for any of these traits would be a reliable measure for other traits.

**Table-2: Source variability patterns in pod length (mm), pod width (mm), number of seeds and 1000 seed weight of *Robinia pseudoacacia* Linn.**

District	Seed source	Pod Length	Pod Width	Mean Values	
				No. of Seeds per pod	1000 (g) Seed Weight
<b>Baramulla</b>	Sherabad Khore (S <sub>1</sub> )	65.10	12.49	4.67	22.91
	Palhallan Pattan (S <sub>2</sub> )	72.66	13.57	7.92	24.05
	Kunzer Tangmarg (S <sub>3</sub> )	63.12	12.43	4.90	23.67
<b>Bandipora</b>	Sumbal Tangpora (S <sub>4</sub> )	63.90	11.45	7.28	24.02
	Chewa Bandipora (S <sub>5</sub> )	67.15	12.78	7.55	23.93
	Kanibathy Bediyaar (S <sub>6</sub> )	65.22	12.39	7.07	24.00
<b>Kupwara</b>	Nutnusa Kupwara (S <sub>7</sub> )	61.20	11.28	7.12	22.66
	Potushai lolab (S <sub>8</sub> )	65.51	11.13	4.55	23.98
	Watyan Chandigam (S <sub>9</sub> )	60.98	12.30	6.02	23.33
<b>CD (At 0.05 level of significance)</b>		<b>1.69</b>	<b>0.31</b>	<b>0.52</b>	<b>0.10</b>

Table-3: Mean, range, GCV, PCV, heritability, genetic advance and genetic gain of pod & seed characters of *Robinia pseudoacacia*

Characters	Mean	Range	Coefficient of Variance (%)		Heritability	Genetic Advance	Genetic gain (%)
			Genotypic	Phenotypic			
Pod Length	64.98	46.91-88.95	10.37	10.85	0.91	13.26	20.41
Pod Width	12.20	8.18-15.38	9.18	9.79	0.87	2.16	17.74
No. of seeds/pod	6.34	3.2-10.80	25.42	27.07	0.88	3.11	49.18
Seed weight	23.61	21.92-24.96	3.73	3.82	0.95	21.77	27.50

## CORRELATION STUDIES

**Appraisal** of Table-4 revealed that pod length had positive and highly significant genotypic correlation with pod width (0.79), seed weight (0.76), number of seeds per pod (0.41). Highly significant and positive correlation was projected by pod width with seed length (0.98), significant and positive correlation with seed width (0.33) and number of seeds per pod (0.33). Likewise, phenotypic correlation estimates (Table-4) indicated that pod length had positive and highly significant phenotypic correlation (0.600) with seed weight, number of seeds per pod (0.293), seed width (0.285) and pod width (0.218). Highly significant and positive correlation was projected by pod width with number of seeds per pod (0.891), significant and positive correlation with seed weight (0.790) and seed width (0.272). **However**, number of seeds per pod showed significant and positive correlation with seed weight (0.262). Rest of the combinations showed non-significant association.

**Strong** correlation at genotypic level suggests either the existence of linkage or pleiotropy or both between the correlated characters. This can be exploited for indirect selection as a mechanism for the genetic improvement of the species [15].

**Table-4: Genotypic and phenotypic correlation among pod & seed characters of *Robinia pseudoacacia***

Characters		Pod Length	Pod Width	No. of seeds/pod	Seed weight
Pod Length	G	1	0.79 **	0.41 **	0.76 **
	P		0.21 *	0.39 **	0.60 **
Pod Width	G		1	0.33 **	0.18
	P			0.89**	0.79 **
No. of seeds/pod	G			1	0.69 **
	P				0.26*
Seed weight	G				1
	P				

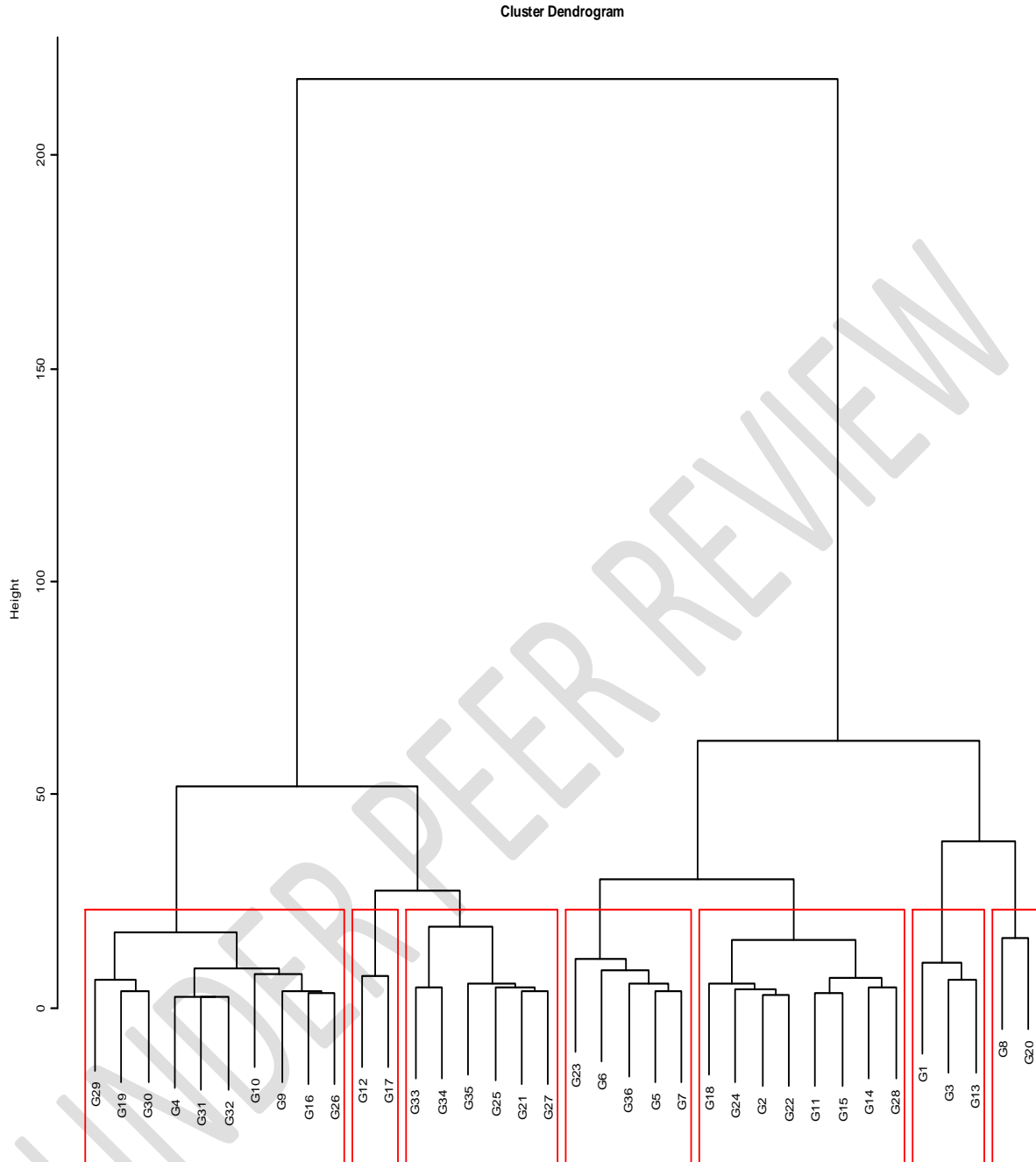
### **Dendrogram based cluster analysis of pod & seed traits of *Robinia pseudoacacia***

Dendrogram was formed in order to anticipate genetic differentiation among pod and seed traits to see their clustering pattern (Fig-1). A critical audit of dendrogram indicates that the distribution of various genotypes into clusters and within cluster was somewhat random.

The dendrogram exhibited seven clear clusters. According to dendrogram the genotypes (4, 9, 10, 16, 19, 26, 29, 30, 31 & 32) were separated from the rest of the genotypes giving information about their most diverse nature, which is categorized as cluster 1. The remaining genotypes exhibited in six clear clusters. Overall cluster 1 was found to be 50 per cent similar with cluster 4 and 5.

Cluster 2 comprises of two genotypes viz., 12 and 17 having 35 per cent similarity with cluster 1. Cluster 3 was categorized by six genotypes viz., 12, 25, 27, 33, 34 & 35 which exhibited 35 per cent similarity with cluster 1 and 2. Cluster 4 comprises of five genotypes viz., 5, 6, 7, 23 & 36. The cluster fifth is a major cluster having eight genotypes viz., 2, 11, 14, 15, 18, 22, 24 & 28. The genotypes 1, 3 & 13 are categorized by cluster sixth having 40 per cent similarity with cluster 1. The cluster seventh comprises of two genotypes viz., 8 & 20, having 40 percent similarity with cluster 1 and 7.

Dendrogram was created in order to visualize genetic differentiation among various progenies and to see their clustering pattern. A critical perusal of dendrogram reveals that the distribution of various genotypes into clusters and within cluster was somewhat random. The dendrogram exhibited seven clear clusters in pod and seed characters. According to dendrogram the genotypes (4, 9, 10, 16, 19, 26, 29, 30, 31 & 32) were separated from the rest of the genotypes giving information about their most diverse nature, which is categorized as cluster 1. The remaining genotypes exhibited in six clear clusters. Overall cluster 1 was found to be 50 per cent similar with cluster 4 and 5. The present results are in line with the findings of Lal *et al.* [16], Kumar *et al.* [17] in *Dalbergia sissoo*, Sherry *et al.* [18] in *Prosopis species* and Wang *et al.* [19] in *Dalbergia sissoo*.



**Figure- 1. Dendrogram based cluster analysis of pod traits of *Robinia pseudoacacia***

**Conclusion:** In forest trees, a number of categories of variation exist that can be broadly grouped into Species, Geographic Sources (Provenances), Stands, Sites, Individual trees, and the variability within individuals. Genetic variation is the basis for tree improvement. It cannot always be seen directly; it must be studied through well-designed tests. This kind of study on variation is the first step for forest tree improvement breeding programmes.

## References

1. Alain T, Michael A, Marie-Louise A, Alice M, Ann D, Zacharie T, Ramni J. and Fabiola N. Tree-to-tree variation in fruits of three populations of *Trichoscypha acuminata* (Engl.) in Cameroon. *In Scientific African*, 2019;7(1): 235.
2. Rawat K, Kumar P, and Nautiyal S. Variability studies in different seed sources of *Pinus wallichiana* in India with special reference to seed and germination characteristics. **In:** FRI, Dehradun, 2006; **pp.** 64-79.
3. Mborora A, BarnekovLilles JP, Schmidt, L., Angaine, P., Meso, M., Omondi, W., Ahenda, J, Mutua, N.A., Orwa, C. and Jamnadass, R., Tree Seed Source Re-classification Manual. *World Agroforestry Centre*, Nairobi, Kenya, 2009; **pp.** 34.
4. Zobel B J, Thorbjornsen E. and Henson, F. Geographic, site, and individual tree variation in wood properties of loblolly pine. *Silvae Genetica*, 1960; 9(6): 146-158.
5. Ginwal H S, Rawat P S. and Srivastava RL. Seed source variation in growth performance and oil yield of *Jatropha curcas* L. in Central India. *Silvae genetic*, 2004; 53(4): 186-192.
6. Isely Ds. and Peabody FJ. *Robinia* (Leguminosae: Papilionideae). *Castanea*, 1984; 49(4): 187-102.
7. Smith JP. Black locust (*Robinia pseudoacacia*) **In:** *Vascular plant families*. Mad River press, Inc., Eureka, California, 1977; **pp.** 320.
8. Jung SC, Matsushita N, Wu B Y, Kondo N. Shiraishi, A. and Hogetsu, T., Reproduction of a *Robinia pseudoacacia* population in a coastal *Pinus thunbergii* windbreak along the Kujukurihama Coast, Japan. *Journal of Forest Research*, 2009; 14(1): 101-110.
9. Ginwal H S, Phartyal SS, Rawat PS. and Srivastava R L. Seed source variation in morphology, germination and seedling growth of *Jatropha curcas* L. in Central India. *Silvae genetica* 2005; 54(2): 76-80.
10. Johnson H W, Robinson HF and Comstock R E. Estimation of genetic and environmental variability in Soybean. *Agronomy Journal*, 1955; 47(7): 314-318.
11. Beale, E. M. L., Euclidean cluster analysis. A paper contributed to 37<sup>th</sup> session of the International Statistical Institute, 1969, **pp.** 341-347.
12. Kraszkievicz A. Productivity of black locust (*Robinia pseudoacacia* L.) grown on a varying habitats in Southeastern Poland. *Forests*, 2021; 12: 470.
13. Kumar R, Mehtab H, Kumara A, Kumar AB, Kaushalb R, Dobhal S, Guptab A K, Banyal R, Kumara M, Kumard S. and Verma K. Seed source variation in *Celtis australis* in Indian Himalayas. *Global Ecology and Conservation*, 2021; 26(1): 14-69.
14. Thakur S, Kamal S, Charan S. and Atul G. Variation in seed and fruit characters of some commercially important agroforestry species. *Indian Forester*, 2008; 134(11): 1505-1511.
15. Juma I, Geleta M, Hovmalm HP, Nyomora A, Saripella GV, Carlsson A S, Fatih M. and Ortiz R. Comparison of morphological and genetic characteristics of *Persea americana* Mill. grown in Tanzania. *Genes*, 2021; 12: 63.
16. Lal S, Sharma OC, Singh DB, Rather SA, Padder B A. and Qureshi I. Genetic Divergence of Horticultural Traits among Olive (*Olea europea* L.) Genotypes Grown in Temperate Climate. *Int.J.Curr.Microbiol. App.Sci*, 2017; 6(8): 2120-2130.
17. Kumar A, Dobhal S. and Sharma S. Assessment of genetic diversity in different clones of *Dalbergia sissoo* Roxb. by RAPD markers. *African Journal of Biotechnology*, 2011;10(35): 6686-6694.
18. Sherry M, Smith S, Patel A, Harris P, Hand P, Trenchard L. and Henderson J. RAPD and microsatellite transferability studies in selected species of *Prosopis* (section

Algarobia) with emphasis on *Prosopis juliflora* and *P. pallid.* *Journal of Genetics*, 2011; 90(2): 251-264.

19.Wang B Y, Shi L, Rua ZY. and Deng J. Genetic diversity and differentiation in *Dalbergia sissoo* (Fabaceae) as revealed by RAPD. *Genetic Molecular Research*, 2011; 10 (1): 114-120.

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