RESEARCH PAPER

Seed Source Variation in Seed Morphology, Viability and Temperature-Dependent Germination of *Azadirachta indica* A. Juss. Across Agro-Climatic Zones of Uttar Pradesh, India.

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Abstract

The choice of seed lot impacts the performance of nurseries, but their apparent differences may also be due to the environment and handling, maternal history and sampling error. The morphology, viability, germination under 3 recorded conditions and early seedling growth were evaluated for sixteen coded neem (*Azadirachta indica* A. Juss.) seed sources. First, auditing raw replicates took place. The mean length of S2 seeds was corrected from 1.26 to 1.47 cm, redundant weight columns were excluded from multivariate analyses, and germination was modelled from counts rather than rounded percentages. A grouped-binomial model revealed a strong condition effect (Wald $\chi^2 = 14.82$, df = 2, $p < 0.001$); source and source-by-condition terms were non-significant. The germination pooled was 66.67% on sand, 54.58% at 20 °C, and 70.00% at 35 °C. After the Holm adjustment, the odds at 35 °C were 1.97 times those at 20 °C. The growth created a signal which was sharper. Randomized-block tests followed by 10,000 permutations within blocks confirmed differences in seedling height, shoot length, root length, seed length, seed thickness and vigour index. The highest source repeatability was achieved for seed thickness (90.63%), followed by shoot length (85.52%) and total height (82.39%). S9 had the highest recorded values of sand germination (93.33%), 35 C germination (86.67%), vigour index (1678.67) and the first principal component score. Regardless, the length, thickness, 1000-seed weight and viability of the seeds explained only 5.1% ($p = 0.960$) of the variation in sand germination.

The evidence suggests that S9 is the first-choice candidate for confirmatory testing. However, it is not the candidate who should immediately be declared genetically superior. The confirmation panel of S16 should include S2, S8 and S10 in documented, larger, multi-environment trials.

Keywords: Seed source, agro-climatic zones, genetic divergence, seedling vigour, path analysis.

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1. Introduction

Neem (*Azadirachta indica* A. Juss.) is a multi-purpose tropical tree. It gives shade, helps in land rehabilitation, provides timber, has medicinal use and has limonoids that are biologically active. Thus, planting has occurred well beyond the natural stands. Establishment still begins with seed, however, and neem seed can lose viability rapidly under poor storage. A nursery cycle may be influenced by lot identity, handling history, and sowing environment (National Research Council, 1992). The situation is complicated by differences in seed form and chemical composition: materials that seem to be alike need not to perform alike (Garg et al., 2024).

Does a collection perform more consistently than another with respect to a trait? It is uncertain for a long-lived outcrossing tree. Contributing factors could be genetic composition and/or maternal provisioning, mother-tree age, fruit maturity, collection site, storage duration, and post-harvest treatment. Nursery tests are still useful because they identify material for advancement. It is not possible for them, by themselves, to ascribe every difference to inheritance, nor does an early advantage necessarily persist in progeny tests or in a different environment in the field. Provenance studies in neem did show variations in height, root growth, biomass and composite suitability establishing the importance of careful preliminary screening (Jain & Dhar, 2008).

Comprehensive assessments evaluate the seed's size, germination, and early growth together. A study of 30 sources of Tamil Nadu showed significant variation across all three sectors. Thus, the combined pattern was used to identify good zones for planting-stock (Mayavel et al., 2023). The similar study conducted on progeny work in Punjab found that seed weight, germination, height, root traits and biochemical quality varied widely, however, the leading source changed depending on the trait studied (Dhakad &

Kaur, 2024). A single ranking might mask key trade-offs. A lot may germinate easily and grow slowly, while another may produce tall seedlings after only moderate establishment.

Germination depends on many factors. Temperature is key, but substrate, moisture, maturity, light and storage history can affect the response. Experiments controlled and carried out on the neem have testified the importance of temperature as well as the medium, but no one setting is best in every combination (Kumar et al. 2007). Dimension confers scant certitude. According to Kumar (2007), the grades of seed-size affected final germination but smaller or medium seeds did not lose favourable quality indices after storage. Maturity and density grading can alter viability and vigour, and poor moisture control hastens deterioration (Ponnuswamy et al., 1991). It is therefore necessary to directly model the testing environment and assess the physical seed characters as a hypothesis rather than proof of physiological quality.

The workbook utilizes a combined evaluation. Sixteen coded sources, three germination conditions, seed dimensions, weight, viability, leaf number, shoot and root growth are represented by replicated observations. Germination counts allow for binomial tests of source, condition and interaction. When there are replicates of continuous traits present, ANOVA will be blocked and variance-component estimation will be allowed. The predictors are examined using correlation and path analysis while multivariate separation are defined by principal components and Mahalanobis distance. Every approach tackles distinct uncertainties. When taken in unison they provide a stronger basis for selection than raw means or one conventional significance test.

The study had five objectives. The first task required mapping the variation in morphology, viability, germination and early growth S1-S16. They then tested sources and conditions, along with their interactions, on scales suited to each response. Estimates of phenotypic spread, between-source variation, broad-sense source repeatability, and expected selection response were supplied by variance components. A different analysis indicated how seed length in addition, thickness, weight, and viability correlated with sand germination and the direct effects as well. The status of candidate was reserved for sources based on various methods. The working hypothesis predicted that there would be more-pronounced differences in morphology and post-germination growth among the genotypes in comparison to those for final germination, which was measured from five seeds per replication and which was expected to respond strongly to condition.

2. Materials and Methods

2.1 Study material, identification, and limits of inference

S1-S16 are codes for the sixteen seed sources listed in Table 1. The manuscript deals with the species neem, *Azadirachta indica* A. Juss., as the companion methodology pages referred to azadirachtin-bearing material. The author must confirm identification prior to submission. The collection localities, with representative latitude, longitude, and altitude data, are presented in Table 1. Mother-tree records, fruit-maturity details, collection dates, seed-moisture values, storage durations, trial dates, experiment location and author affiliation were not provided. The claim is limited by those gaps. The recordings are thus referred to as coded seed sources or seed lots and their interpretation is limited to the recorded experiment. The conventional broad-sense heritability is the one calculated since it is available in the supplied methodology. However, the term ‘source repeatability’ is employed in discussion to avoid treating every between-lot difference as genetic.

Table 1. Geographical distribution of the sixteen coded neem seed sources across the agro-climatic regions of Uttar Pradesh.

S. No.	Agro-climatic zone	Coded seed source	Latitude (°N)	Longitude (°E)	Altitude (m a.s.l.)
1	Tarai region	Lakhimpur (S1)	27.9500	80.7667	147
		Shahjahanpur (S2)	27.8793	79.9199	144
		Bareilly (S3)	28.3700	79.4000	173
		Pilibhit (S4)	28.6497	79.8724	172
2	Western Gangetic Plain region	Agra (S5)	27.1700	78.0300	169
		Mathura (S6)	27.5000	77.6700	174
		Kannauj (S7)	27.0700	79.9200	139
		Mainpuri (S8)	27.2300	79.0500	157
3	Eastern Gangetic Plain region	Sitapur (S9)	27.5700	80.6600	138
		Bahraich (S10)	27.5700	81.6000	124
		Kanpur Nagar (S11)	26.4300	80.3700	126

S. No.	Agro-climatic zone	Coded seed source	Latitude (°N)	Longitude (°E)	Altitude (m a.s.l.)
		Lucknow (S12)	26.8500	80.9500	123
4	Vindhya and Bundelkhand region	Mirzapur (S13)	25.1500	82.5800	80
		Prayagraj (S14)	25.4500	81.8400	98
		Kanpur Dehat (S15)	26.3800	79.9500	133
		Jhansi (S16)	25.4333	78.5833	284

Note. Agro-climatic zones and coded source localities are transcribed from Document 202. Latitude, longitude and altitude are representative locality or district-headquarters values compiled from ICAR-CRIDA contingency plans, India Meteorological Department station records and official district portals (accessed 21 August 2026); they are not coordinates of individual mother trees. For Kanpur Dehat, the reference point is Mati-Akbarpur district headquarters.

2.2 Traits Measured and Structure of the Experiment

The effect of sand, 20 °C, and 35 °C on germination was tested in three replications. Each replicate was made up of five seeds, for which there were 15 outcomes per source-condition cell resulting in 720 seed results overall. The sand-germination sheet recorded seedling measurements. In each replication, leaf number was determined on three seedlings and then their average taken for blocked analysis. Height, shoot length and root length were noted in three replications. Every observation's total height was equal to shoot plus root length. The identity was kept in descriptive reporting but never were deterministic forms entered together in a multivariate model.

For each source, three measurements represented seed length, and three more represented thickness. The workbook also provided the weights for 10, 100 and 1000 seeds. Only the 1000-seed weight entered into correlation, regression, principal-component, and distance analyses because those were exact scalar rather than the independent variables. Viability refers to the viable seeds out of ten tested. The Seedling vigour index I was calculated for each replication by multiplication of the sand germination percentage and total seedling length according to the multiple-criterion approach (Abdul-Baki and Anderson 1973).

2.3 Examination of Data and Preparation

No statistical model was fitted until the derived values were checked against the raw observations. The audit revealed a definitive mistake. For S2, 1.6, 1.6 and 1.2 cm lengths yield a mean of 1.4667 cm which is different from the worksheet's 1.26 cm. The modified value rounded to 1.47 cm for presentation was used. No changes were made to raw entries. Data for tables and figures were generated from the counts to obtain germination percentages, but the modelling was on the back end successes and failures. The source file did not give any temperature to sand treatment. For this reason, the terms '3 germination conditions' rather than '3 temperatures' are used for sand, 20 °C and 35 °C.

2.4 Statistical Model for Germination

Germination counts were fitted with a grouped-binomial generalized linear model with a logit link. The source, condition, replication and the source-by-condition interaction were fixed terms. The use of sum-to-zero coding allowed Wald tests to assess entire factors rather than coefficients associated with an arbitrary reference category. A Wilson 95% confidence interval was associated with pooled proportions. Due to the lack of statistical significance of the interaction, condition contrasts were estimated from an additive model. Odds ratios and 95% confidence intervals were calculated for these estimates. Holm's procedure was then applied to adjust for all three comparisons.

2.5 Blocked ANOVA and the parameters of variation

The continuous as well as replicate level of responses was analyzed as a randomized block design with source as treatment and replication as block. Each source F test used benefited from 15 and 30 degree of freedom. As the experiment was small, we note that normal-theory p values should be treated with caution, so we perform a robustness check. We independently permuted the source labels within each replication 10,000 times, then compared the observed F statistic to the resulting randomization distribution. Tukey's honest significant difference at $\alpha = 0.05$ produced compact-letter groupings where sources differed.

Variance components adhered to agricultural practices in the supplied methodology. The difference between source and error mean squares divided by the number of replications was used to find the between-source variance. Residual variance was indicated by the error mean square; phenotypic variance equaled the sum of the two components. Negative estimates attributed to source modeled at zero Calculating GCV, PCV and broad-sense source repeatability (H^2). The expected advance was assumed to have a 5% selection intensity with k = 2.06, and was expressed as a percentage of the mean (Burton and DeVane, 1953; Johnson et al., 1955). Partial eta-squared represented the size of the source effect on a common scale.

$$\sigma^2 s = (MSsource - MSerror)/r; \quad \sigma^2 e = MSerror; \quad \sigma^2 p = \sigma^2 s + \sigma^2 e$$

$$GCV = 100(\sqrt{\sigma^2 s}/\bar{x}); \quad PCV = 100(\sqrt{\sigma^2 p}/\bar{x}); \quad H^2 = 100(\sigma^2 s/\sigma^2 p)$$

2.6 Trait associations, path analysis, and multivariate structure

The Pearson coefficients were computed using non-redundant source means of seed length, seed thickness, 1000-seed weight, viability, germination under three conditions, number of leaves, and seedling height. There were thirty-six pairwise tests. In this regard, Benjamini-Hochberg false-discovery-rate adjustment was made to the nominal p values. We then subsequently asked whether seed length, thickness, 1000-seed weight, and viability had direct effects on germination in sand through a standardized multiple-regression/path model. Variance inflation factors checked collinearity. According to Wright (1921) and Dewey and Lu (1959) path-analytic logic, total correlations were partitioned into direct standardized coefficients and combined indirect effects.

We standardized the mean of the nine traits retained. Mahalanobis D^2 measured pair-wise multivariate separation from the inverse covariance matrix (Mahalanobis, 1936). The supplied legacy method mentioned Tocher grouping but not stopping value. In order to make the procedure reproducible, a source only entered a cluster if its mean D^2 from existing members was no greater than the largest nearest-neighbour distance in the dataset. The analyst defined this threshold, so cluster membership is exploratory; individual distances and component scores are given greater interpretive weight. All calculations were executed with Python numerical, statistical, and machine-learning routines with two-sided $\alpha = 0.05$.

Table 2. Seed dimensions, 1000-seed weight, and viability across the sixteen coded sources; means were recalculated from the raw observations.

Source	Seed length (cm)	Thickness (mm)	1000-seed weight (g)	Viability (%)
S1	1.47	0.613	200	100
S2	1.47	0.813	240	100
S3	1.57	0.717	260	90
S4	1.57	0.613	264	100
S5	1.23	0.517	150	100
S6	1.17	0.817	170	90
S7	1.47	0.510	220	90
S8	1.43	0.747	200	100
S9	1.67	0.510	230	90
S10	1.47	0.617	230	90
S11	1.40	0.610	200	90
S12	1.70	0.617	200	90
S13	1.37	0.623	248	100
S14	1.53	0.617	233	80
S15	1.27	0.627	150	100
S16	1.43	0.727	220	90

Note. Recalculation changed the S2 seed-length mean from 1.26 to 1.47 cm on the basis of 1.6, 1.6, and 1.2 cm. Since 10-, 100-, and 1000-seed weights were exact scalar multiples, only 1000-seed weight was retained in multivariate analysis.

Table 3. Source means for germination, early seedling growth, and vigour. Within height and vigour, unlike letters denote Tukey HSD differences at $\alpha = 0.05$.

Source	Sand germ. (%)	20 °C germ. (%)	35 °C germ. (%)	Leaves	Height (cm)	Shoot (cm)	Root (cm)	Vigour index
S1	60.00	53.33	66.67	1.89	12.10 de	8.83	3.27	726.00 b
S2	60.00	60.00	73.33	2.11	18.07 a	12.40	5.67	1089.33 ab
S3	60.00	66.67	66.67	2.00	10.57 e	7.77	2.80	628.00 b

Source	Sand germ. (%)	20 °C germ. (%)	35 °C germ. (%)	Leaves	Height (cm)	Shoot (cm)	Root (cm)	Vigour index
S4	73.33	53.33	66.67	2.00	12.10 de	7.70	4.40	890.00 b
S5	66.67	53.33	66.67	2.00	12.50 cde	7.50	5.00	832.00 b
S6	73.33	53.33	66.67	2.00	13.77 bcde	9.43	4.33	1026.00 ab
S7	60.00	53.33	66.67	2.00	10.23 e	7.50	2.73	614.00 b
S8	86.67	60.00	80.00	2.00	12.17 de	7.40	4.77	1039.33 ab
S9	93.33	60.00	86.67	2.22	18.07 a	11.57	6.50	1678.67 a
S10	60.00	46.67	66.67	2.00	18.10 a	12.00	6.10	1086.00 ab
S11	60.00	53.33	66.67	2.00	16.40 ab	11.70	4.70	984.00 b
S12	60.00	46.67	66.67	2.00	12.53 cde	7.83	4.70	752.00 b
S13	60.00	60.00	66.67	2.00	14.77 abcd	9.73	5.03	886.00 b
S14	66.67	46.67	66.67	2.00	16.03 abc	10.20	5.83	1070.00 ab
S15	53.33	53.33	66.67	2.00	16.60 ab	10.43	6.17	887.33 b
S16	73.33	53.33	80.00	2.11	17.57 ab	12.33	5.23	1287.33 ab

3. Results

3.1 Seed dimensions, weight, and viability

Seed formulation varied significantly by source. The mean length range was S6 (1.17 cm), and S12 was 1.70 cm. The thickness range was S7 and S9 (0.510 mm) and S6 (0.817 mm). The weight of a thousand seeds ranged from just 150 g in S5 and S15 to 264 g in S4. While the viability percentage dropped to 80% in S14, it attained 100% in six sources. The characteristics did not shift together. S6 seed and seedling (~3 weeks after sowing) had the shortest seeds and the greatest thickness. S12 seed had the longest seeds, but S12 did not have the greatest thickness or weight. Having just one 'large seed' class would conceal important contrasts among length, thickness and mass.

3.2 Germination response across conditions

By the raw percentages the leaders are suggested, but sampling uncertainty has weakened the apparent order. The percentage of sand germination ranged from 53.33% in S15 and 93.33% in S9, while 20 °C exhibited a narrower range of 46.67 -66.67%. At 35 °C, the values shifted from 66.67 to 86.67%. S9 took the lead on sand and was not inferior in the warmer treatment while S8 and S16 fared well at 35 °C and above. The focus shifted with modeling. The grouped-binomial source test yielded no significance (Wald $\chi^2 = 12.15$, df = 15, p = 0.667), and sources responses were not detectably condition-specific ($\chi^2 = 8.28$, df = 30, p > 0.999) Although the ranking can guide retesting, it does not establish unequal underlying germination probabilities.

Based on the statistical test, the main seed germination signal was supplied by the condition. Out of 240 seeds used in each setting, sand 160, 20 °C 131, and 35 °C 168 were germinated. The pooled estimates were 66.67%, 54.58%, and 70.00% with confidence intervals. Relative to sand at 20 °C the odds were lowered by 40.5% (OR = 0.60, 95% CI 0.41-0.86; Holm p = 0.013). There was no significant difference between sand and 35 °C (OR = 1.17, 0.79-1.72; p = 0.429). At 35 °C, the odds were almost double those observed at 20 °C (OR = 1.97, 1.35-2.87; Holm p = 0.001). The replication did not have a measurable effect. The outcome of this experiment gives a clear message; it is the environment and not the material that is superior for thermal insulation.

Table 4. Factor tests from the grouped-binomial model and adjusted pairwise contrasts among germination conditions.

Effect/contrast	Statistic or OR	df or 95% CI	p	Interpretation
Seed source	12.15	15	0.667	Factor test
Germination condition	14.82	2	<0.001	Factor test
Replication	0.72	2	0.699	Factor test

Effect/contrast	Statistic or OR	df or 95% CI	p	Interpretation
Source x condition	8.28	30	1.000	Factor test
20 °C vs sand	0.60	0.41-0.86	0.013	Odds ratio (Holm p)
35 °C vs sand	1.17	0.79-1.72	0.429	Odds ratio (Holm p)
35 °C vs 20 °C	1.97	1.35-2.87	0.001	Odds ratio (Holm p)

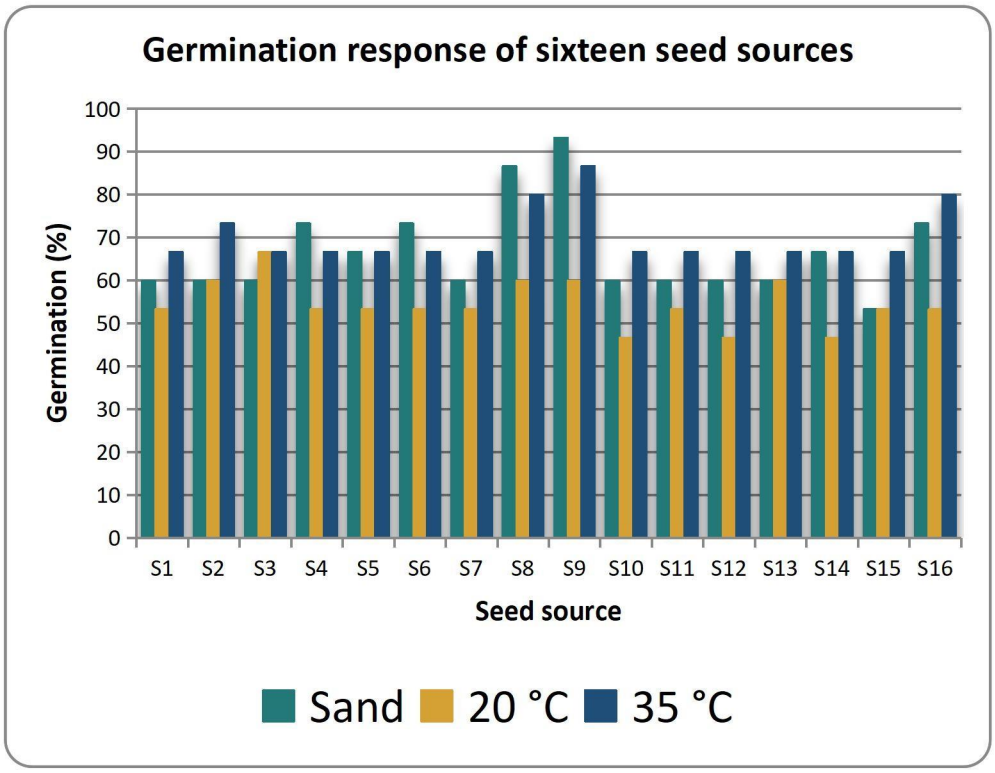


Figure 1. Germination percentages observed for each coded source under sand, 20 °C, and 35 °C. The figure shows raw means; formal modelling supported a condition effect, but not source or source-by-condition effects.

3.3 Early seedling growth and vigour

The sources were differentiated much more at the time of growth than at the final germination. The full height the plant attained came from S7 at 10.23 cm to S10 at 18.10 cm. Close behind was S2 and S9 at 18.07 cm and S16 at 17.57 cm. The shoot length varied in S2 and S8. It was 12.40 cm in S2 and 7.40 cm in S8. The roots showed different patterns, from 2.73 cm in S7 to 6.50 cm in S9. Heights ($F = 15.03$, $p < 0.001$), shoot length ($F = 18.72$, $p < 0.001$), root length ($F = 3.35$, $p = 0.002$), seed length ($F = 10.26$, $p < 0.001$), and thickness ($F = 30.02$, $p < 0.001$) were found to have strong source effects. All outcomes passed the randomization check, with permutation $p \leq 0.0011$. In contrast, leaf number showed little dispersion, with no supported source effect ($F = 1.69$, $p = 0.108$).

The vigour index combined two phases that matter in practice: establishment and later elongation. According to the means our source calculated from S7 - 614.0 to S9 - 1,678.67, and clear effect ($F = 4.02$, $p < 0.001$; permutation $p < 0.001$) was. S9, placed by Tukey lettering, was alone in the highest displayed group. Be cautious when interpreting this visual separation: three replications offered insufficient precision to distinguish S9 from every other strong source. S2, S6, S8, S10, S14 and S16 remained statistically comparable to S9. S9 scored higher than S1, S3, S4, S5, S7, S11, S12, S13 and S15. The claim that is defensible is thus particular: S9 displayed the strongest integrated performance, though no doubt several candidates could appear equally vigorous.

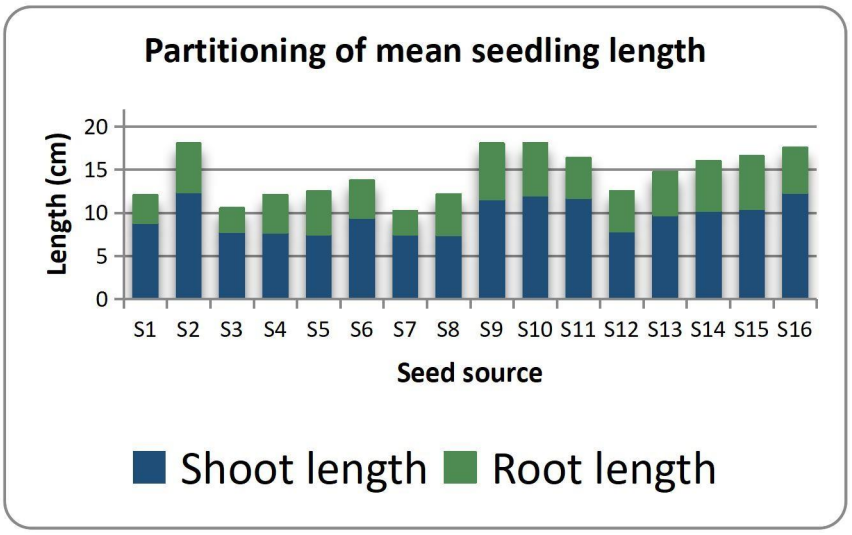


Figure 2. Mean shoot and root lengths by source. The stacked total equals seedling height because height was recorded as the arithmetic sum of shoot and root length.

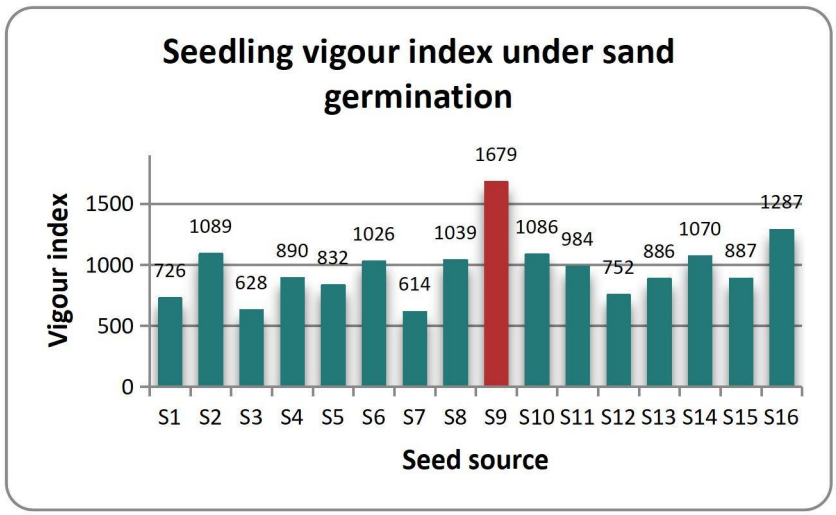


Figure 3. Mean seedling vigour index under the sand treatment. S9 produced the largest value; inferential source comparisons appear in Table 3.

3.4 Variation, source repeatability, and expected advance

Residual variation occurred in every character so PCV was greater than GCV in all cases. The empty space itself conveyed meaning. There was the greatest increase in final germination and root length, indicating a large within-source or condition-specific effect. For instance, on sand, GCV was 9.94%, PCV was 24.55% while source repeatability dropped to 16.39%. At 20 °C estimates were even lower (8.39%) and at 35 °C (8.33%). This pattern of variance agrees with the binomial model. This small trial was not under sufficiently stable source control to support strong source-level claims for final germination.

Morphology and shoot growth displayed opposite trends. Source repeatability for seed thickness was 90.63%, shoot length 85.52%, total height 82.39% and seed length 75.53%. The expected advance, represented in terms of mean, for thickness 28.88%, shoot length 36.71%, height 34.90% and seed length 17.01%. Root length had a moderate repeatability estimate of 43.98%. Its projected advance, nevertheless, 27.07% shows a source signal inside a comparatively large residual spread.

Vigour has a $H^2 = 50.19\%$ for expected advance that equals 34.42% of the mean. This research trial indicates that selection on germination growth and physical seed characteristics is stronger than selection on germination percentage.

Table 5. Randomized-block source tests, permutation checks, effect sizes, and variance-based selection parameters.

Trait	Mean	F(15,30)	p	Permutation p	Partial η^2	Error CV	GCV	PCV	H^2 (%)	GA (% mean)

Trait	Mean	F(15,30)	p	Permutation p	Partial η²	Error CV	GCV	PCV	H² (%)	GA (% mean)
Germination - sand (%)	66.67	1.59	0.137	0.150	0.443	22.44	9.94	24.55	16.39	8.29
Germination - 20 °C (%)	54.58	1.27	0.276	0.398	0.389	15.63	4.73	16.33	8.39	2.82
Germination - 35 °C (%)	70.00	1.27	0.278	0.375	0.389	14.13	4.26	14.75	8.33	2.53
Leaves (no.)	2.02	1.69	0.108	0.191	0.458	4.80	2.30	5.32	18.67	2.05
Seedling height (cm)	14.47	15.03	<0.001	<0.001	0.883	8.63	18.67	20.57	82.39	34.90
Shoot length (cm)	9.65	18.72	<0.001	<0.001	0.903	7.93	19.27	20.84	85.52	36.71
Root length (cm)	4.83	3.35	0.002	0.001	0.627	22.37	19.82	29.89	43.98	27.07
Seed length (cm)	1.45	10.26	<0.001	<0.001	0.837	5.41	9.50	10.93	75.52	17.01
Seed thickness (mm)	0.64	30.02	<0.001	<0.001	0.938	4.73	14.73	15.47	90.63	28.88
Seedling vigour index	967.25	4.02	<0.001	<0.001	0.668	23.49	23.58	33.29	50.19	34.42

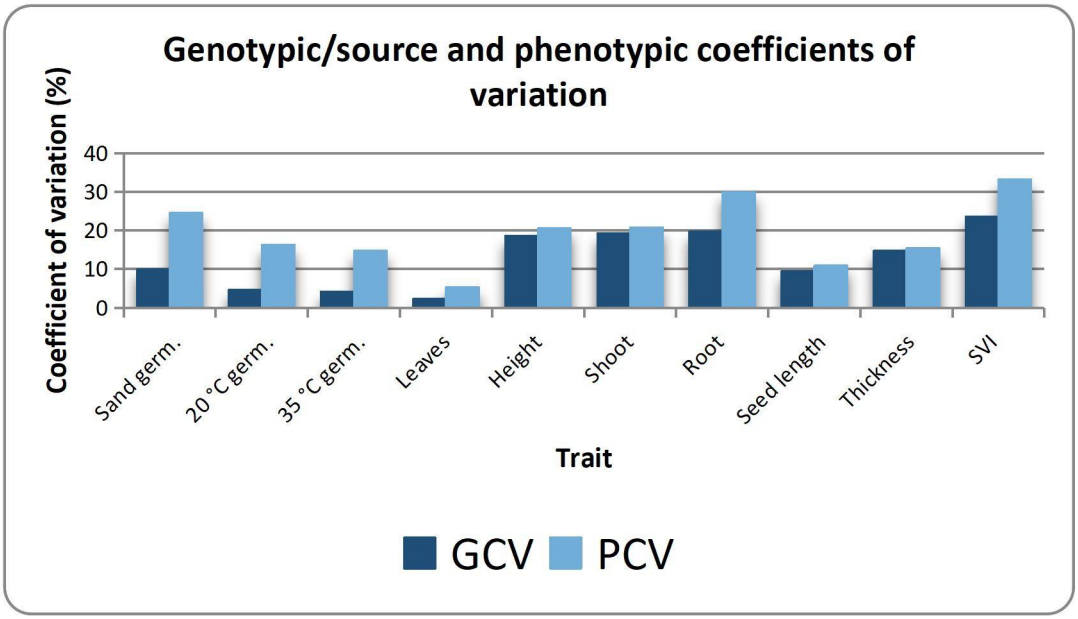


Figure 4. Source/genotypic and phenotypic coefficients of variation for the recorded traits. Larger PCV-GCV separation indicates a stronger residual or within-source contribution.

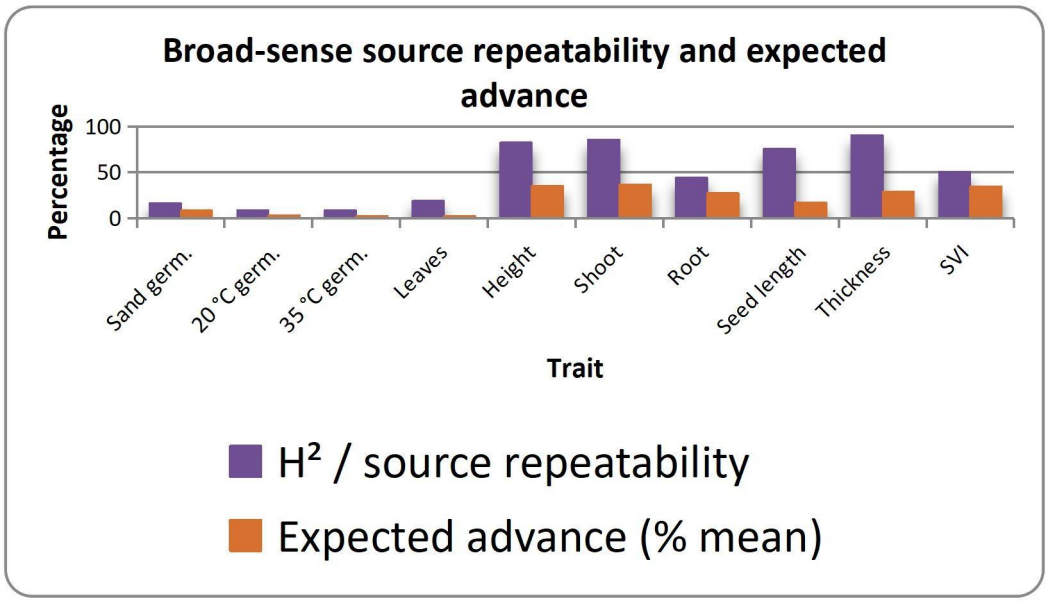


Figure 5. Broad-sense source repeatability and expected advance expressed as a percentage of the mean. These values describe the present experiment and should not be interpreted as narrow-sense heritability.

3.5 Correlation and path analysis

The correlation matrix has two reliable links after false-discovery-rate adjustment. Germination on sand tracked closely germination at 35 °C ($r = 0.803$, $q = 0.0049$) The figure 35 °C which gave the best germination was also correlated to number of leaves. There were three coefficients that were nominally significant but exceeded the adjusted threshold. Seed-length with 1000-seed weight ($r = 0.650$, $p = 0.006$, $q = 0.077$); sand germination with leaves ($r = 0.561$, $p = 0.024$); leaves with height ($r = 0.591$, $p = 0.016$). With 16 sources, unadjusted correlations do not automatically mean there is a link. Thus, the interpretation is based on the adjusted results.

The path model produced a helpful null result. Length, thickness, 1000-seed weight and viability are all accounted for 5.1% (adjusted $R^2 = -0.294$; $F = 0.15$; $p = 0.960$) of variation in sand germination. None of the standardized direct effects were significant: β of 0.295 for length, 0.107 for thickness, -0.111 for weight and -0.001 for viability. The explanation was not collinearity, because every variance-inflation factor was below 2.2. While physical grading may aid in handling as well as uniformity, these measurements did not serve as a replacement for direct germination tests.

Table 6. Pearson correlation matrix for nine non-redundant source means. Double asterisks mark FDR-adjusted q < 0.05; single asterisks mark nominal p < 0.05 only.

Trait	Seed length	Thickness	1000-wt	Viability	Sand germ.	20 °C germ.	35 °C germ.	Leaves	Height
Seed length	1.000	-0.245	0.650*	-0.322	0.197	0.009	0.285	0.292	-0.032
Thickness	-0.245	1.000	0.071	0.086	0.027	0.288	0.103	0.018	0.126
1000-wt	0.650*	0.071	1.000	-0.190	0.089	0.308	0.123	0.232	0.021
Viability	-0.322	0.086	-0.190	1.000	-0.066	0.371	-0.000	-0.185	-0.176
Sand germ.	0.197	0.027	0.089	-0.066	1.000	0.245	0.803**	0.561*	0.091
20 °C germ.	0.009	0.288	0.308	0.371	0.245	1.000	0.372	0.297	-0.168
35 °C germ.	0.285	0.103	0.123	-0.000	0.803**	0.372	1.000	0.790**	0.394

Trait	Seed length	Thickness	1000-wt	Viability	Sand germ.	20 °C germ.	35 °C germ.	Leaves	Height
Leaves	0.292	0.018	0.232	-0.185	0.561*	0.297	0.790**	1.000	0.591*
Height	-0.032	0.126	0.021	-0.176	0.091	-0.168	0.394	0.591*	1.000

Table 7. Standardized path/regression results for germination under the sand treatment.

Predictor	Total r	Direct beta	Summed indirect	p for direct effect	VIF
Seed length	0.197	0.295	-0.098	0.509	2.16
Seed thickness	0.027	0.107	-0.080	0.743	1.18
1000-seed weight	0.089	-0.111	0.200	0.791	1.92
Viability	-0.066	-0.001	-0.065	0.997	1.12

Model summary: $R^2 = 0.051$; adjusted $R^2 = -0.294$; $F(4,11) = 0.15$; $p = 0.960$; residual path coefficient = 0.974.

3.6 Principal components and multivariate divergence

Three main components summarized 69.79% of standardized variation. PC1 explained 33.58% of the data goodness; PC2 another 19.47%; PC3 16.75%. The first axis encompassed a broad establishment-growth profile. The positive coefficients with a greater magnitude corresponded to germination of temperatures at 35 °C (0.523), leaf number (0.514), sand germination (0.432), seed length (0.277), total height (0.263), and 1000-seed weight (0.249). S9 had a score of PC1 5.08 which was above all others. The more scores that followed were substantially lower: S16 scored 2.15, S2 1.47 and S8 1.33. PC2 presented an opposing contrast, where seed viability and germination at 20 °C were inversely related to seed length and weight. The axes confirm that the sources cannot be ordered adequately by one physical character.

Mahalanobis distance showed prominent comparative analyses between pairs without a simple cluster. The farthest distance was between 6-9 (D^2=29.46); 12-13 was next farthest (27.82); 10-14 was closest (4.62). Utilization of the modified Tocher rule assigned sixteen sources to six exploratory clusters. The mean inter-cluster distance of maximum was 25.04 between the clusters V and VI. Such organizations can be used to represent a broader confirmation panel or future crossing design but do not indicate final genetic classification. The analysis of nine traits with just sixteen sources and the threshold stopping was defined for this dataset.

Table 8. Trait coefficients for principal components 1-3.

Trait	PC1	PC2	PC3
Seed length	0.277	-0.505	-0.335
Seed thickness	0.056	0.381	-0.063
1000-seed weight	0.249	-0.313	-0.507
Viability	-0.107	0.529	-0.227
Sand germination	0.432	0.135	0.040
20 °C germination	0.230	0.415	-0.488
35 °C germination	0.523	0.175	0.099
Leaves	0.514	0.023	0.194
Seedling height	0.263	-0.015	0.536

Explained variance: PC1 = 33.58%, PC2 = 19.47%, PC3 = 16.75%; cumulative variance through PC3 = 69.79%.

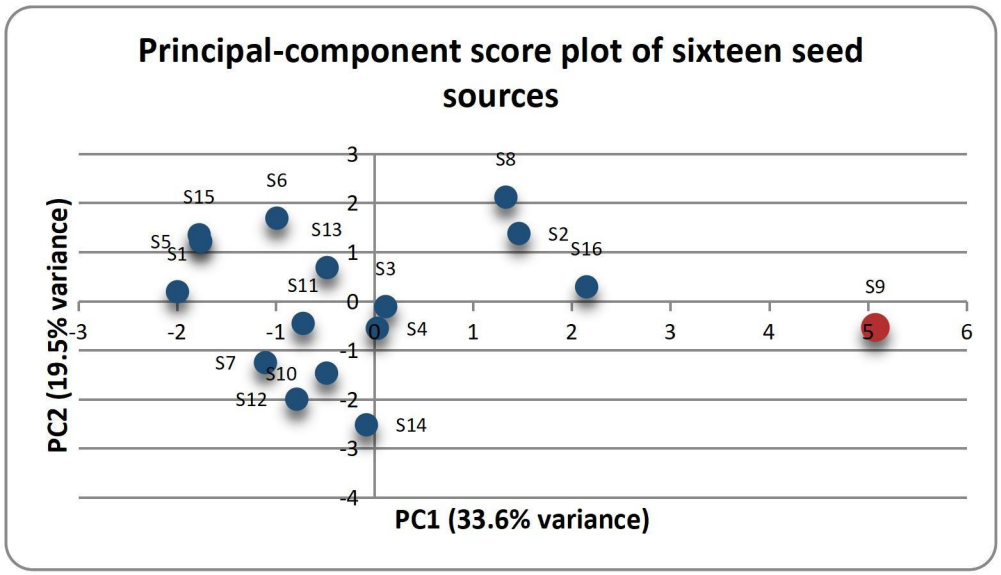


Figure 6. Principal-component score plot for the sixteen coded sources. S9 occupies a distinct position on the favourable side of PC1.

Table 9. Exploratory modified Tocher clusters with the principal Mahalanobis-distance summaries.

Cluster	Sources	Mean intra-cluster D^2
I	S10, S14, S11, S13, S1, S15, S5	11.22
II	S7, S16	13.00
III	S6, S8	18.21
IV	S2, S12	18.43
V	S4, S9	23.81
VI	S3	0.00

Distance summary: maximum individual D^2, S6-S9 = 29.46; minimum individual D^2, S10-S14 = 4.62; modified stopping threshold = 16.29.

3.7 Candidate sources supported across analyses

No source corresponded with S9 on as many independent criteria. The best germination (highest) was observed on sand and at 35 °C; longest mean root, largest vigour index, most favourable score for PC1. A strong second candidate emerged in S16 owing to its high warm-condition germination, good height and second-largest PC1 score. The value of S2 is associated with shoot growth, total height and thickness whereas S8 is stronger for germination than height. Although S10 had the highest seedlings, it had one of the lowest germination percentages at 20 °C. Hence, a portfolio interpretation relatively defensible than winner-takes-all ranking. S9 must be confirmed first, while S16, S2, S8 and S10 maintain complementary performance profiles potentially matching different nursery or breeding objectives.

4. Discussion

The key finding of germination trials was that treatment matters more than code source. Performance at 20 °C was less than under sand or 35 °C, and sources showed no systematic condition-specific rank change. Neem biology makes this pattern so. It is known that the temperature and the medium influence germination. The best response is also known to depend on the precise combination tested (Kumar et al. 2007). Earlier work with 13 neem seed sources likewise found significant effects of temperature and substrate, with the highest germination and mean daily germination at 30 °C on top of paper, whereas 20 °C was unfavourable (Shringirishi et al., 2001). Due to the absence of temperature recording on the sand setting, it cannot be translated into thermal level Nevertheless, the combined result was 35 °C and exceeded 20 °C. Consequently, nursery recommendations indicate full germination environment. Source mean without test conditions is an incomplete measure.

A non-significant source term calls for careful evaluation, not dismissal. The outcome does not imply that all sixteen lots possess the same biological potential. The differences based on 15 seeds per source-condition cell could not be separated from binomial uncertainty and residual variation. S9's raw lead is helpful in determining what to retest. However, on its own, it cannot sustain a higher underlying germination probability. All three conditions indicate low repeatability. A stronger follow-up requires many

more seeds and time to event measures-germination speed, mean daily germination and germination value-which have discriminated neem provenances in bigger studies (Mayavel et al., 2023).

Post-germination characteristics provided a much firmer answer. According to the results, height, shoot length, root length, seed length, and thickness all differed by source. The pattern was reinforced by large partial η^2 values and relatively narrow gaps between PCV-GCV for several traits. Similar source-related variation in height and root attributes has been reported in nursery and provenance trials (Jain and Dhar 2008). Research indicates that rankings for growth, roots, oil, and azadirachtin may differ (Dhakad & Kaur, 2024). Selection should start with a stated goal. The height and the shoot length appear especially responsive but the H^2 refers to repeatability among these lots and not additive genetic inheritance.

Highly repeatable means more than simply a pretty table entry. For height and shoot length, the random measurement noise did not create most of the separation amongst the source means. Physical growth of a child can be relied upon. S10 was the tallest, but germination at 20 °C was only 46.67%, besides root performance was not consistent. S9 provided a bigger profile by combining establishment, root extension and total growth. Those choices took its vigor index far beyond the lowest sources. Composite measures are important because at first the planting stock must emerge, and then the planting stock must grow. Established vigour methods follow this same logic rather than allowing one trait to dictate the decision (Abdul-Baki & Anderson 1973).

The size of the seed did not provide the shortcut expected. There was some correlation between length and weight, but length, thickness, 1000-seed weight, and viability failed to predict germination on sand. By contrast, a multi-source study of *Acacia nilotica* reported positive associations of germination with 100-seed weight and viability, indicating that such relationships may be species- and population-specific (Shekhar et al., 2002). Neem literature has been put forward as a weak link. Kumar (2007) reported evidence that size grading does not determine final germination and that both smaller or medium seeds can retain favourable vigour after storage. While reserve quantity may matter, it works in tandem with age, moisture history and deterioration. The findings of Ponnuswamy et al. (1991) demonstrated that the viability and seedling vigour were altered substantially by density class and storage environment. In the present evidence, a simple nursery rule for example choosing the heaviest seeds for the best germination is ruled out.

The null path result becomes credible due to two data-audit decisions. As shown by the variance-inflation factors, collinearity was modest; severe predictor overlap did not erase a real signal. The columns for the 10 and 100 seed weights were duplicated due to their identical nature to the 1000 seed weight column. Keeping them would actually be the same evidence. The shoot and root length were also excluded from the matrix containing total height; their deterministic sum would deform the covariance estimates. Sixteen sources continue to offer limited power for subtle or nonlinear effects, meaning small relationships may have gone unnoticed. The model rules out a strong consistent linear relationship between the seed characters as determined from measurement and sand germination

Through multivariate analysis, various distinct outcomes transfigured into a selection strategy. Earlier multivariate work on 43 neem ecotypes likewise used correlation, path and cluster analyses to characterize seed-trait variability and genetic divergence (Shukla et al., 2002). PC1 was the joint tendency to germinate in warm conditions and in sand, produce leaves, increase in height and have larger seed measurements. S9 occupied a less prevailing place on that axis. The following were S16, S2 and S8, but each reached that position through a different combination of traits. This variety is important. The confirmation panel built only around S9 would test one promising profile. The addition of S16, S2 and S8 would test balanced growth and shoot and thickness advantages and germination-oriented performance. The Mahalanobis distance adds information about the contrast, and not the quality. The greatest distance between sources was found between S6 and S9; however, highly divergent sources are not beneficial. Its effectiveness is dependent on the breeding or nursery goal.

The evidence has well-defined limits. Exact collection-site coordinates, mother-tree identity, fruit maturity, and storage history were not available; therefore, genetic, maternal, and site effects cannot be separated. Germination was done five seeds per replication resulting in coarse 20 percentage point steps and weak level power at source. It wasn't supplied exact duration and detailed assessment protocols so that comparison with published absolute values was limited. Using nine traits drawn from just sixteen sources these multivariate distances are exploratory of cluster membership. The main analyses revealed the condition effect and source differences for continuous traits to be statistically robust despite these constraints. It would be unreasonable to extend them beyond the coded lots and registered settings.

Explain differences not simply rank them next experiment or test. Every locality requires many recorded open-pollinated families with large seed samples and standardized moisture and storage. Replicated nursery blocks would support hierarchical source-and-parent models. More than one nursery environments should be used before field trials for the material. An extensive trait set germination rate, collar diameter, biomass, survival, oil and azadirachtin content will take selection beyond early length. There could be considerable chemical variation in neem seed which may not correlate to morphology (Garg et al., 2024). It is recommended that you don’t bulk S9 deployment; rather, advance it along with like candidates into a verifiable confirmation stage.

5. Conclusion

Two findings are certainly within the scope of this experiment. It was the test environment that chiefly affected germination. Compared to the sand and 35 °C treatments, the 20 °C treatment performed poorly, and

there was no evidence for the overall source effect nor the source-by-condition interaction. The performance was different after germination. Coded sources differed strongly in seed thickness, shoot length, total height, seed length, root length and vigour. The statistical tests outcomes are not reliant on just that single statistic: conventional F tests, 10,000 within-block permutations, variance components, effect sizes and multivariate structure all point in the same direction.

S9 is the first source to definitely retest. This source produced 93.33 percent germination on sand while sustaining 86.67 percent germination at 35 °C with the greatest mean root length and vigor index and a positive score on PC1 well above all other sources. There's no sufficient evidence to show genetic superiority. The source main effect was not significant to its obvious germination advantage and the lack of provenance and maternal records prevents genetic attribution. S16 or S2, S8 and S10 should be retained in the confirmation panel because they contribute different strengths and protect the programme from selection on one early characteristic.

Similarly useful, a second conclusion is that seed length, thickness, weight, and laboratory viability did not reliably predict germination. Visual grading cannot substitute for testing within the confines of a defined environment. The next stage should be done in phases verify the identity and collection history of each source, repeat germination with increasing sample size, assess families in replicated nurseries, then test growth, survival and biochemical quality at various locations. In that form, the current research provides a defensible screening decision. It determines which evidence is strong or weak and what materials are worth investing in and which need more work.

Declarations

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