© 2023, Centre for Advanced Research in Agricultural Sciences
Research Journal of Agricultural Sciences
Volume 14; Issue 02 (Mar-Apr 2023); pp 516–521

Open Access
ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Full Length Research Article

Intra- Specific Variation in Seed and Seedling Characteristics among Selected Superior Phenotypes of Neem (*Azadirachta indica* A. Juss)

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Abstract

Azadirachta indica A. Juss (Neem), belonging to family Meliaceae, is one of the multi- purpose tree species of the world. In this context in the present study, 25 high fruits yielding CPT's of neem were selected from the western zone of Tamil Nadu and fruits were collected from these selected trees. Intra- specific variation in seed weight and seed diameter was significant (P≤ 0.05) among the half-sib families studied. The seeds were sown in mother beds (with pure sand) and observed that there was variation in commencement of germination of seeds from 9 DAS to 29 DAS. The peaking of germination was also varied from 11 DAS up to 22 DAS. The highest germination, shorter peak value and higher germination value were recorded in the family N- 25. From the biomass study, it is observed that leaf biomass, shoot biomass and total biomass registered significant variation, while root biomass and root/ shoot ratio recorded non-significant variation among families. Huge variation was noted in the quality indices viz., Seed Vigor Index, Sturdiness Quotient, and Dickson Quality Index Assessment. The paper discusses on the huge variation and need for identifying seed sources for quality planting stock production for various agroforestry systems.

***Key words*:** Intra-specific variation, Neem, Seed- seedling parameters, Seedling, Dickson quality indices, Quality planting stocks

Neem (*Azadirachta indica* A. Juss) is a prominent tree species of the Indo-Pakistan subcontinent, with multipurpose uses [1]. Neem is traditionally used in agroforestry systems are found as scattered trees on croplands and suitability of neem for agroforestry has been well reported [2-3]. Presently, India is the largest producer ofneem seeds in the world, mostly from Tamil Nadu, Uttar Pradesh and Karnataka [4]. It belongs to the Mahogany family (Meliaceae) and also one of the two species in the genus *Azadirachta*. Neem is a large tree growing to about 25 m in height with semi-straight to straight trunk, 3 m in trunk and spreading branches forming a broad crown which starts fruiting after 3-5 years of planting. From the tenth year onwards, it can produce up to 50kg of fruits annually [5]. Neem tree requires little water and plenty of sunlight and rainfall in the range of 450 to 1200 mm with wide temperature range of 0 °C to 49 °C. However, it has been introduced successfully even in areas where the rainfall is as low as 150 to 250 mm. It grows on almost all types of soil including clayey, saline and alkaline soil. Adult *A. indica* A. Jusstolerates some frost, but seedlings are more sensitive. It quickly dies in water logged soils. *A. indica* A. Juss requires large amounts of light, but it tolerates fairly heavy shade during the first few years. Biological and pharmacological activities attributed to different parts and extracts of these plants include antiplasmodial, antitrypanosomal, antioxidant, anticancer, antibacterial, antiviral, larvicidal and fungicidal activities [6].

Neem is normally propagated from seeds, either sown directly or raised in a nursery and transplanted as seedlings. Several problems are associated with neem seeds such as poor germination, short viability after harvest and poor storability [7]. Neem populations are heterogenous in all respects, owing greatly to differences in soil and climate. The trees themselves are known to have genetic variation in height, branching type, leaf form, and color [8]. Neem shows significant variation in seed weight, seed diameter, germination rate and seed vigour rate. There is a need for identification of biotypes, documentation, and collection of superior phenotypes to have enhanced growth and fruit yields by producing superior planting stocks. Superior phenotypes of neem are needed for enhancing production and supply of quality seeds in turn improved planting stocks for agroforestry systems.

Received: 23 Dec 2022; Revised accepted: 14 Mar 2023; Published online: 10 Apr 2023

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Citation: Shaju CH, Devanand PS, Velumani R, Buvaneswaran C. 2023. Intra- specific variation in seed and seedling characteristics among selected superior phenotypes of neem (*Azadirachta indica* A. Juss). *Res. Jr. Agril. Sci*. 14(2): 516-521.

MATERIALS AND METHODS

In the present study, 25 high fruit yielding candidate plus trees were selected from western zone of Tamil Nadu. Only fruits at the yellow green colour stage are picked from the branches. After depulping i.e., removal of fruit skin (exocarp) and pulp (mesocarp) from the neem fruit, seeds were extracted. Then, the seeds were kept in a cool and dry place and stored in polythene bag for further studies. Seeds and seedling traits were studied at Research Nursery of Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, India. For seed diameter, samples of 10 seeds were randomly selected from 25 selected Half-sib families. Each seed was measured for its diameter using vernier calipers (Tricle Brand reading to 0.01mm). For seed weight, samples of 100 seeds were randomly selected in triplicate from 25 Half-sib families and recorded weight (g) using electronic weighing balance. The seeds were sown in mother beds (with pure sand) 100 seeds as four replications; 25 seeds per replication were sown and watered immediately, then number of seeds germinated was counted and computed the percentage of germination.

1). Coefficient of variation (CV%) = Ratio of standard deviation to the mean and multiply by 100 proposed by [9].

$$CV\% = (\text{Standard Deviation} / \text{Mean}) \times 100$$

2). Germination percentage = Calculating results to calculate germination percentage, divide the number of healthy seedlings by the total number of seeds in the test and multiply by 100.

3). Peak Value (PV) = Maximum mean daily germination reached at any stage of germination period.

4). *Germination value (GV)*: It is the index of combining speed and completeness of seed germination was calculated to according to Czabator [10].

$$GV = \text{Final daily speed of germination} \times \text{Peak value}$$

5). *Germination energy or energy period*: Period from sowing to the till the day of peak germination [11].

Then after 30 days of growth of seedlings, height of the seedlings was recorded.

6). *Biomass study*: Triplicate (R_1, R_2 and R_3) seedlings from each half-sib family were sampled for biomass estimation. At the time of sampling, the age of the seedling was one year old. Fresh weight of sample seedlings was recorded for leaf, stem and root. The components were dried in hot air oven at 70 °C for 72 hrs and weighed separately after cooling and dry weight of shoot, root and leaf biomass content were determined and were expressed in g (Gram) per plant [12]. Shoot length, root length, leaf area and collar diameter were recorded at the time of harvest. The following derived parameters were also worked out as detailed below:

1). Root-shoot ratio is usually given as the ratio of the weight of the roots to the weight of the top of a plant.

$$\text{Root/Shoot ratio} = \text{Dry weight for roots} / \text{Dry weight for top of plant}$$

2). Seed vigour index is usually given as the ratio of the weight of the roots to the weight of the top of a plant [13].

$$\text{Seed vigour index} = \text{Germination (\%)} \times \text{Seedling height (cm)}$$

3). Sturdiness quotient of the seedling was derived by the ratio between seedling height and collar diameter [14].

$$\text{Sturdiness quotient} = \text{the Seedling height (cm)} / \text{the Stem diameter (mm)}$$

4). Dickson quality index assessment was made using the formula of Dickson *et al*. [15].

$$\text{Dickson quality index} = \frac{\text{Total biomass}}{\text{Height / Stem diameter} + \text{Shoot / Root ratio}}$$

The data from the seeds and seedling parameters were subjected to statistical analysis using SPSS 20- ANOVA Single factor for variability assessment.

RESULTS AND DISCUSSION

Variations in seed parameters

Observation on 100 seed weight and diameter was presented in (Table 1). The results revealed that 100 seed weight was maximum in family N- 24 (28.89±0.45 g) and the minimum was in family N- 25 (13.94±0.82 g). In Seed diameter, the maximum value was recorded in family N- 5 (6.9±0.24 mm) and the minimum value in N- 18 (5.48±0.19 mm). The CV value for 100 seed weight was very narrow ranging from 1.93% (in family N- 17) to 11.78% (in family N- 2), indicating uniformity of seed production in a single mother tree of neem. Similarly, the CV value for seed diameter was also narrow and which ranged from 1.59% (in family N- 25) to 18.47 (in family N- 12). The statistical analysis of data on 100 seed weight and also on seed diameter showed that there exists a significant variation among families of Neem with respect to seed weight and diameter (Table 1).

Table 1 Analysis of variance (ANOVA) and co-efficient of variation (CV) for seed weight and seed diameter among different half-sib families of *Azadirachta indica* A. Juss in Tamil Nadu

Half sib families	100 Seed weight (g)	Seed diameter (mm)	CV (%) (100 Seed weight)	CV (%) (Seed diameter)
N_1	23.56±0.59	6.78±0.10	4.361	2.673
N_2	20.97±1.42	6.07±0.23	11.777	6.742
N_3	19.34±0.43	6.20±0.12	3.93	3.549
N_4	16.43±0.71	5.88±0.31	7.52	9.306
N_5	24.14±0.80	6.90±0.24	5.757	6.264
N_6	27.62±0.99	6.59±0.44	6.227	11.778
N_7	17.64±0.50	6.05±0.06	4.932	1.749
N_8	19.97±0.30	6.18±0.28	2.639	7.984
N_9	20.35±0.53	6.25±0.12	4.561	3.371
N_{10}	17.72±0.66	6.39±0.10	6.454	2.837
N_{11}	19.01±0.36	6.66±0.16	3.325	4.162
N_{12}	19.72±0.53	6.85±0.73	4.711	18.471
N_{13}	25.21±0.74	6.25±0.08	5.118	2.405
N_{14}	17.57±0.68	6.25±0.16	6.771	4.484

N_{15}	20.99±0.72	6.83±0.61	6.018	15.623
N_{16}	20.13±0.25	5.73±0.18	2.211	5.671
N_{17}	20.16±0.22	5.62±0.18	1.935	5.782
N_{18}	17.34±0.80	5.48±0.19	8.068	6.032
N_{19}	24.73±0.69	6.45±0.20	4.833	5.549
N_{20}	26.21±0.93	6.85±0.12	6.186	3.142
N_{21}	18.28±0.88	5.56±0.10	8.359	3.195
N_{22}	23.96±0.53	6.87±0.37	3.88	9.408
N_{23}	18.88±0.37	5.51±0.17	3.456	5.442
N_{24}	28.89±0.45	6.40±0.19	2.733	5.233
N_{25}	13.94±0.82	5.64±0.05	10.205	1.597
Grand mean ± S.E	20.91±0.63	6.24 ±0.21	5.438±0.48	6.097±0.83
F- Value	28.79395	2.923		
P- Value	1.54E-21	0.000685		
Statistical significance	Significant	Significant		

As observed in the present study, Prabakaran *et al*. [16] also recorded the average value of 100 seed weight as 20.23g in neem. Similarly, Khatri *et al*. [17] have also reported variation in seed thickness values varying from 7.20 mm to 19.15 mm in *Simarouba glauca* DC and such variation in parameters of different seed lots could be ascribed to both internal and external conditions operating at the time of seed development. Age and reproductive character of the mother tree can also have influence on seed parameters [18].

Table 2 Seed germination parameters for *Azadirachta indica* A. Juss among different half-sib families in Tamil Nadu

Half sib families	Germination (%)	Peak value	Energy period	Germination value
N_1	25	0.81	16	0.65
N_2	72	2.32	11	5.39
N_3	78	2.52	12	6.33
N_4	40	1.29	11	1.66
N_5	43	1.39	14	1.92
N_6	65	2.1	15	4.39
N_7	81	2.61	12	6.82
N_8	34	1.1	16	1.21
N_9	61	1.97	14	3.87
N_{10}	36	1.16	22	1.34
N_{11}	62	2	16	4
N_{12}	59	1.9	12	3.62
N_{13}	59	1.9	12	3.62
N_{14}	80	2.58	16	6.65
N_{15}	88	2.84	16	8.05
N_{16}	72	2.32	15	5.39
N_{17}	69	2.23	14	4.95
N_{18}	10	0.32	16	0.11
N_{19}	90	2.9	12	8.42
N_{20}	56	1.81	16	3.26
N_{21}	98	3.16	16	9.99
N_{22}	94	3.03	12	9.19
N_{23}	95	3.06	12	9.39
N_{24}	84	2.71	15	7.34
N_{25}	99	3.19	15	10.19
Mean ±S. E	66.000±4.82	2.129±0.15	14.320±0.15	5.110±0.61

Seed germination parameters

Data on germination parameters are presented in (Table 2) and the results revealed a significant difference among progenies. The minimum germination was in family N- 18 (10%) and maximum germination was in family N- 25 (99%). Similarly, minimum peak value was recorded in family N- 18 (0.32) and maximum peak value was recorded in family N- 25 (3.19). Energy period varied from 11 to 22 days. Minimum energy period was recorded in families N- 2 and N- 4 and maximum energy period was recorded in family N- 10. Germination value was varying from 0.11(in family N- 18) to 10.19 (in family N- 25) (Table 2). Jain [19] also revealed that germination of *Azadirachta indica* A. Juss seeds in sand, paper, beds and polybags was highly significant ($P \leq 0.01$) among all provenances. He also reported that seed thickness and seed weight are significantly correlated with germination. Khatri *et al*. [20] also recorded variation in seed germination, peak value germination, and energy period and germination value in *Simarouba glauca* DC. Existence of variation in the germination of seed lots from different provenances, seed lots / families is reported by several workers [21-22]. Existence of poor germination in seed lots could be ascribable to release of inbreeding effects known to occur in seeds from natural stands [23] and seeds collected from isolated trees where selfing predominates [24]. As observed in the present study, the existence of huge variation in germination (from 10% to 99%) in neem obviously demands for selection of superior seed source or mother trees for quality planting stock production.

Table 3 Analysis of variance (ANOVA) for seedling parameters among different half-sib families of *Azadirachta indica* A. Juss in Tamil Nadu

Half sib families	Seedling height (cm)	Collar diameter (mm)	Leaf area (cm^2)
N_6	44.66±2.09	8.91±0.23	3.48±0.30
N_7	59.95±0.76	9.99±0.74	2.93±0.25
N_{15}	35.03±3.03	9.97±0.29	3.85±0.14
N_{16}	57.35±3.43	11.30±0.76	3.12±0.29
N_{19}	73.33±1.28	9.91±0.56	3.18±0.28
N_{21}	59.5±4.69	10.77±0.10	3.36±0.23
N_{22}	61.4±3.89	11.46±0.49	3.67±0.53
N_{23}	53.3±2.25	10.58±0.24	3.34±0.28
N_{24}	70.8±1.73	10.81±0.48	4.57±0.45
N_{25}	65.68±5.58	10.21±0.29	3.16±0.24
Grand mean ± S.E	58.1± 4.43	10.39±0.41	3.46±0.29
F- value	12.67683	2.548267	2.139
P- Value	1.79E-06	0.039089	0.075
Statistical significance	Significant	Significant	Non- significant

Variations in seedling parameters

Data on seedling height, collar diameter and leaf area are presented in (Table 3). The observation revealed that the seedling height was maximum in family N- 19 (73.33±1.28 cm) and minimum in family N-15(35.03±3.03cm). In collar diameter, the maximum value was recorded in family N- 22 (11.46±0.49 mm) and minimum value was recorded in family N- 6 (8.91±0.23 mm). In leaf area, the maximum value was observed in family N- 24 (4.57±0.45 cm^2) and the minimum value was observed in family N- 7 (2.93±0.25 cm^2). The statistical analysis of data on seedling height and collar diameter showed a significant variation, however, leaf area showed non- significant variation among different families of neem (Table 3). Prabakaran *et al*. [25] also reported significant variation in shoot length, collar diameter and leaf area among the progenies of *Azadirachta indica* A. Juss. A plethora of researchers [26-28] have also reported the existence of variability in growth parameters of *A. indica* A. Juss due to different genotypes at nursery stage and these differences vary with soil and climatic conditions. Eco-climatic attributes play an important role in the differentiation of neem populations and thereby affect their growth during the early growth stages [29].

Table 4 Analysis of variance (ANOVA) for dry matter allocation in different biomass components among different half-sib families of *Azadirachta indica* A. Juss in Tamil Nadu

Half sib families	Leaf dry weight (g per seedling)	Shoot dry weight (g per seedling)	Root dry weight (g per seedling)	Total biomass (g per seedling)	Root / Shoot ratio
N_6	9.50±1.08	11.55±1.49	13.17±1.75	34.22±4.32	1.15±0.10
N_7	6.35±1.48	10.91±0.97	15.64±1.34	32.91 ±2.35	1.46±0.20
N_{15}	6.00±0.95	13.84±1.32	19.17±2.99	39.01±5.26	1.37±0.16
N_{16}	11.89±0.68	21.27±0.56	21.89±3.54	55.05±4.78	1.02±0.16
N_{19}	6.83±0.65	11.87±1.43	13.74±2.92	32.44±5.00	1.13±0.12
N_{21}	9.81±0.46	12.52±1.27	17.62±3.28	39.95±5.01	1.39±0.14
N_{22}	9.34±1.35	17.28±0.52	18.06±1.17	44.68±3.04	1.04±0.06
N_{23}	10.76±0.97	17.83±1.59	23.26±1.69	51.85±4.25	1.34±0.22
N_{24}	11.08±1.22	15.79±0.17	17.77±0.75	44.64±2.14	1.12±0.04
N_{25}	7.17±0.73	17.02±3.17	17.07±2.04	41.26±5.94	1.05±0.16
Grand mean ± S.E	8.94±0.89	14.98±1.24	17.73±2.14	41.61±4.21	1.207±0.13
F- value	4.426	5.239	1.857	4.182	1.198
P- Value	0.002	0.0009	0.119	0.003	0.348
Statistical significance	Significant	Significant	Non- Significant	Significant	Non- Significant

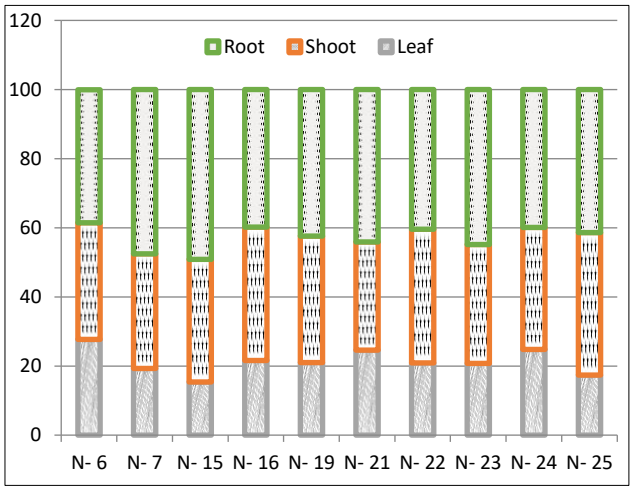

Fig 1 Bar chart (% of DMP in leaf, shoot and root)

Dry matter production

Data on dry matter allocation in leaf, shoot and root were presented in (Table 4) and the bar graph for % of DMP (Dry Matter Production) in leaf, shoot and root of different half-sib families of neem were presented in (Fig 1). The observation revealed that the leaf biomass was maximum in family N- 16 (11.89±0.68 g) and was minimum in family N- 15 (6.00±0.95 g). For shoot biomass, the maximum value was recorded in family N- 16 (21.27±0.56 g) and minimum value was recorded in family N- 7 (10.91±0.97 g). For Root biomass, the maximum value was recorded in family N- 23 (23.26±1.69 g) and minimum value was recorded in family N- 6 (13.17±1.75 g). Total biomass was maximum in family N- 16 (55.05±4.78) and minimum was in family N- 19 (32.44±5.00). Root / shoot ratio was maximum in family N- 7 (1.46±0.20) and minimum was in family N- 16 (1.02±0.16 g). The statistical analysis of data on leaf biomass, shoot biomass and total biomass showed a

significant variation. However, root biomass and root/shoot ratio showed non- significant variation among families of Neem (Table 4). Saka *et al*. [30] revealed that trunk component of *Azadirachta indica* A. Juss contains larger amount of above ground biomass content than the branch and leaf of the tree. There is a significant variation ($P<0.05$) among the three components (Bole, branch and leaf) in biomass and carbon stock. Kumar *et al*. [31] also recorded variation in growth, biomass, carbon stock, and climate resilience potential in *Celtis australis* L., in Indian Himalayas. Total biomass (TB) trends differed significantly in all the seed sources. Non-significant estimates for total biomass (TB) production in one-year old plant show the production of uniform biomass in the species. Growth, biomass production, and carbon stock of tree species are affected by the environmental factors, anthropogenic activities, and genotypic responses [32].

Table 5 Quality indices for *Azadirachta indica* A. Juss among different half-sib families in Tamil Nadu

Clonal ID	Seed vigour index	Sturdiness quotient (Height / stem diameter ratio)	Quality index
N_6	2902.9	5.012	5.553
N_7	4855.95	.6.001	4.411
N_{15}	3082.64	3.514	7.987
N_{16}	4129.2	5.075	9.032
N_{19}	6599.7	7.4	3.803
N_{21}	5831	5.525	5.777
N_{22}	5771.6	5.358	6.983
N_{23}	5063.5	5.038	8.13
N_{24}	5947.2	6.549	5.821
N_{25}	6502.32	6.433	5.514
Mean±S.E	5068±419.1	5.590±0.34	6.301±0.53

Quality indices

Seed vigor index, sturdiness quotient and quality index data are presented in (Table 5). Seed vigor index was maximum in family N- 19 (6599.7) and was minimum in family N- 6 (2902.9). Sturdiness quotient was maximum in family N- 19 (7.400) and was minimum in family N- 15 (3.514). Quality index was maximum in N- 16 (9.032) and minimum in family N- 7 (4.411) (Table 5). Binotto *et al*. [33] described those correlations between growth variables and the Dickson quality index in forest seedlings, ratio of shoot dry matter to root dry matter can be considered as an effective, safe index to evaluate seedling quality, stem base diameter is found to be strongly correlated with all parameters, along with root dry matter, both in *E. grandis* W. Hill ex Maiden and in *P. elliottii* Engelm. Significant correlations are found between the Dickson Quality Index (DQI) and variables height, stem base diameter and days after emergence, indicating a satisfactory outcome due to the fact that the assessment of these variables has a non-destructive nature and thus facilitates and enables experimentation in forest nurseries. Fonseca *et al*. [34] also reported that DQI is highly correlated with all morphological parameters in *Trema micrantha* (L.) Blume. Quality indices of the planting stocks are highly varying among different half-sib families of neem in the present study. This clearly implies the need to select superior mother trees for planting stock production.

CONCLUSION

In this study, there is a huge intra specific variation among seed morphology parameters, seed germination parameters, seedling morphology parameters, dry matter allocation in leaf, shoot and root biomass and quality indices as seed vigour index, sturdiness quotient and quality index of different half- sib families of neem. This clearly implies the need to select superior mother trees or superior seed sources for planting stock production for agroforestry purposes.

Acknowledgement

The authors gratefully acknowledge the funding support rendered by National Medicinal Plants Board, Government of India to conduct this study. The authors also thankful to the Director, Institute of Forest Genetics and Tree Breeding, Coimbatore and Director General, Indian Council of Forestry Research and Education, Dehra Dun for providing the opportunity to conduct the experiments in Research nursery.

Abbreviations

CPT- Candidate Plus Tree, DAS- Days After Sowing, DMP- Dry Matter Production, g- Gram, mm- Millimeter, cm- Centimeter, cm^2- Centimeter square, °C- Degree Celsius, %- Percentage.

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