Int.J.Curr.Microbiol.App.Sci (2025) *14*(11): 145-156

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 Volume 14 Number 11 (2025)
Journal homepage: http://www.ijcmas.com

Review Article https://doi.org/10.20546/ijcmas.2025.1411.015

Integrative Review on Early Growth Traits of Neem (*Azadirachta indica* A. Juss): Exploring Seed Morphology, Seedling Physiology and Candidate plus Trees Selection

Narendra Kumar Shukla, Narendra Babu Shakya, Bhavna Yadav, Garima Singh, Pooja Yadav and Vishwadeep Shukla*

Department of Botany, Dayanand Anglo-Vedic (P.G.) College, C.S.J.M. University, Kanpur, U.P. – 208001, India

**Corresponding author*

Keywords

Vigor indices, drought tolerance, CPT selection, agroforestry, genetic improvement.

Article Info

Received: 14 September 2025
Accepted: 22 October 2025
Available Online: 10 November 2025

A B S T R A C T

The neem (*Azadirachta indica* A. juss) is a multipurpose evergreen tree that has economic, ecological, and medical benefits in regions that are tropical or semi-tropical. In addition to providing bioactive substances for industry, biopesticides, and medicine, the "village pharmacy" aids in agroforestry, soil conservation, and rural livelihoods. Early growth characteristics like seed shape, seedling physiology, and Candidate Plus Tree (CPT) selection are the foundation of long-term plantation management and genetic improvement. CPT selection ensures optimal seed supplies for long-term production, seedling physiology determines stress resistance, and seed attributes influence germination and vigor. This review compiles studies on these related characteristics, backs them up with figures and evaluation models, and identifies areas in which additional research is required, such as standardized evaluation methods, a more precise understanding of how genes interact with their environments, and methods for incorporating molecular breeding into our practice. It suggests strategies for advancing neem improvement efforts that have the dual goals of restoring the environment and generating benefits for society and the economy.

Introduction

Azadirachta indica A. Juss., more commonly referred to as neem, is a tree with many uses that is very important in tropical and subtropical areas. Its many medicinal, ecological, and monetary advantages have made it highly prized. Neem is a great source of bioactive chemicals like gedunin, azadirachtin, and nimbin, which have the ability to kill germs, fungi, viruses, and insects for medicinal purposes. Because of these components, neem has found a home in both the contemporary biopesticide and pharmaceutical sectors and in ancient medical systems like Ayurveda [1]. In addition to its therapeutic potential, neem is a significant contributor to forestry and environmental management due to its widespread use in windbreaks, roadside plantings, soil conservation, and agroforestry systems. Because it can grow in poor soils, endure droughts, and produce shade, lumber, feed, and other non-timber forest products, this tree is very important to rural life and ecological sustainability. Although neem's numerous applications have been extensively studied, its early development characteristics have not been as effectively incorporated into research and practice [2]. Early development factors such as seed morphology, seedling physiology, and the identification of Candidate Plus Trees (CPTs) are crucial in determining a plantation's long-term success and yield. Germination rates, viability, and storage behavior, to name a few examples, are all directly influenced by seed morphology, which includes the dimensions, form, mass, and characteristics of the seed coat.

Equally important for the survival and growth of seedlings in field circumstances, especially in semi-arid and dry regions where neem is often planted, is seedling physiology, which includes vigor, root-shoot balance, and stress tolerance [3]. Additionally, CPT selection frameworks offer a methodical approach for selecting superior phenotypes based on traits like adaptability, fruit production, and resistance to disease. This guarantees a consistent supply of superior seeds for extensive breeding and reforestation projects.

Despite the widespread interest in neem, most studies have focused on a single aspect of the plant, such as its medical applications, agronomic benefits, or ecological significance, rather than attempting to cover the entire spectrum, from seed to elite tree selection. Neem's genetic diversity and adaptability cannot be fully utilized because of this void. By integrating research on CPT selection, seed morphology, and seedling physiology, the current review aims to fill in knowledge gaps. By doing so, we hope to shed light on the importance of early growth traits in neem's domestication, plantation establishment, and genetic improvement efforts over the long run [4].

Reproductive Biology

Neem bears axillary, many-flowered inflorescences (thyrses/panicles) with small, white, fragrant flowers. Flowers are bisexual and male on the same tree (andromonoecy). Each flower is typically 5-merous; 10 stamens are fused into a staminal tube (monadelphous) surrounding the style; an annular nectary disc occurs at the ovary base; the ovary is superior and the fruit is a yellow drupe (usually one seed) [5]. These characters are diagnostic for Meliaceae and well described for *Azadirachta indica*.

Floral and Fructal Phenology

- Across India, timing varies with climate but follows a clear pattern:
- Flowering (typical): initiates February–April in many zones; warmer sites can start December–January.
- Fruit set & development: begins March–April.
- Fruiting/ripening: commonly June–August (can extend to September in some regions). Large-scale and regional studies report this window and also note inter-annual shifts with weather extremes (e.g., post-cyclone phenological changes observed in Odisha) [6].

Sexual and Breeding Systems

- Sexual system. Neem is andromonoecious: bisexual and male flowers occur on the same individual. Bisexual flowers are often protandrous (male phase slightly precedes female), promoting cross-pollination. Floral structure (staminal tube + exserted style/stigma) and nectar disc favor insect visitation (bees, other insects) [7].
- Breeding system & pollination. Field studies consistently find xenogamy (outcrossing) dominates, and natural fruit set can be pollinator-limited. Some controlled-pollination studies report self-incompatibility and obligate outbreeding, whereas others indicate self-compatibility is possible but contributes little to fruit set under natural conditions—i.e., effective reproduction still relies on cross-pollen and pollinator activity. The consensus for management is to treat neem as functionally outcrossing in plantations [8].

Seed Morphology

When growing *Azadirachta indica*, seed shape is one of the most important factors in germination, seedling health, and final harvest yield. The size, weight, and shape of seeds are all influenced by environmental conditions, mother tree genetics, and provenance. Seed size has a positive correlation with germination rates, seedling emergence rates, and vigor indices. On the other hand, seedlings grown from small, underdeveloped seeds tend to be weaker and have a lower chance of survival [9]. Importantly, removing the endocarp—the hard seed coat—improves oxygen exchange and imbibition, thereby reducing the mean germination time and making it a more practical aspect. Seeds without pulp have been found to germinate more quickly and uniformly than seeds with intact pulp or bulky endocarps, according to research. When fruits turn completely yellow, which indicates physiological maturity, they should be picked; this is also an important time to collect seeds.

Viability is low in fruits that are either overripe or not fully matured [10]. Neem seeds are classified as intermediate to recalcitrant due to their sensitivity to drying and rapid viability loss when stored. Therefore, it is best to store it for a short time in a dry, cold location. Spread neem seeds immediately or within two to three weeks of collecting them for best germination [11].

- Long and broad seeds usually have greater stored reserves, which allows seedlings to establish more rapidly under stress circumstances, and this correlation is inversely proportional to seedling vigor.
- Because bigger seeds often indicate greater endosperm development and higher germination potential, seed weight (100-seed weight) is a commonly used quality control metric in seed testing.
- Unless scarified or eliminated, the thickness of the endocarp may physically prevent or delay germination [12].
- Fully grown seeds have a consistent coat color of yellow to brown, whereas immature seeds with a greenish hue have poorer viability. This color acts as a visual maturity indicator for the seeds

Seedling Physiology

The physiological development of neem (*Azadirachta indica*) seedlings is of the utmost importance when they are planted in nurseries or field plantations. In harsh environments with extremes of water, salt, and temperature, strong seedlings have a better chance of surviving, growing rapidly, and flourishing [13]. When evaluating seedlings, some of the physiological characteristics that are utilized the most frequently are germination parameters, vigor indices, and stress tolerance indicators [14].

Germination Parameters

- ➢ Germination Percentage (GP): GP is one of the simplest and most reliable metrics for assessing the potential and health of seeds. It is calculated by dividing the total number of seeds sown by the number of seeds that germinate [15]. A lot of seeds with a high GP have a good chance of growing into a healthy nursery stock.
- ➢ Mean Germination Time (MGT): MGT is used to determine the rate of seed germination. The production of synchronized seedlings of consistent size is enhanced by shorter MGT values, which signify quick germination and consistent emergence. A decrease in nursery efficiency and planting success is common when delayed germination is indicated by increased MGT levels [16].

Seedling Vigor Indices (SVI)

- Germination rate is just one measure of seedling vigor. Data on germination and growth characteristics, such as seedling length and biomass, are combined [17].
- To get the SVI-I, multiply the GP by the mean seedling length, which is the sum of the shoot length and root length. This gives a thorough evaluation of the strength of the seedlings that have germinated [18].
- For the purpose of selecting seedlings with improved nutrient consumption and stress tolerance, SVI-II uses GP multiplied by seedling dry weight, which represents the real buildup of biomass [19].

Physiological Stress Markers

- ➢ Relative Water Content (RWC): This shows how well the tissues of the leaf are hydrated. A higher RWC score indicates a higher capacity for water retention and drought resistance [20].
- ➢ Chlorophyll Content: It can be measured using spectrophotometry or portable SPAD meters to determine photosynthetic efficiency and stress tolerance. The breakdown of chlorophyll, which frequently occurs in response to stress, has an effect on seedling vigor [21].
- ➢ Membrane Stability Index (MSI): It determines how well cell membranes withstand stress by monitoring the loss of electrolytes. A high MSI indicates that the cells are functioning normally and are resistant to stress [22].
- Using GP and MGT, learn more about neem seed viability and emergence dynamics. By connecting germination success to real growth potential,
- SVI-I and SVI-II function as composite measures of vigor.
- The physiological indicators RWC, chlorophyll

content, and MSI aid in predicting how seedlings would respond to the unavoidable stress of outdoor environments [23].

Genetic Variability and Provenance Influence

Role of Provenance in Early Growth

A crucial component in the enhancement and long-term use of *Azadirachta indica* is its genetic diversity. The geographical origin of the seeds, or provenance, has a significant impact on the shape of the seeds, their germination behavior, and the strength of the seedlings. Seeds collected from semi-arid plains, dry zones, and alluvial regions in northern India may differ significantly in size, weight, oil content, and dormant behavior [24].

Neem populations adapt to various soil types, temperatures, and photoperiodic conditions in their respective regions, resulting in this diversity. In a controlled environment, seeds from wetter origins may be larger and have a higher germination rate, but they struggle under stress [25]. In contrast, seeds from drier provenances are often smaller but exhibit superior drought tolerance in the seedling stage. This demonstrates the significance of selecting plants from the appropriate origin for each planting location [26].

Heritability and Genetic Gain

Quantitative genetics has shown that many early development traits have moderate to high broad-sense heritability (H2). Because a significant portion of the observed variance is likely hereditary rather than environmental, this indicates that these qualities are suitable for systematic improvement selection. Heritability of seed characteristics, such as length, breadth, and 100-seed weight, typically falls within the range of 0.40 to 0.70, indicating that repeated selection may be able to improve seed quality [27]. Germination percentage (GP) and seedling vigor index (SVI), which both demonstrate strong heritability, are reliable selection criteria in genetic improvement initiatives. Heritable traits are frequently associated with significant phenotypic variation in Indian provenances, which amplifies the predicted genetic gain (G) from selection [28].

Evidence from Provenance Trials in Northern India

Numerous provenance tests carried out in the Indian states of Uttar Pradesh, Rajasthan, Haryana, and Madhya Pradesh revealed that the properties of seed and seedling neem differ significantly [29]. The germination rate, for instance, can be as low as 40% in dry-zone provenances and as high as 85% in wet alluvial provenances. Similarly, provenance has a significant impact on the growth of collar diameter and early seedling height. We can find the best elite provenances for various ecosystems thanks to this diversity. The following are particularly evident in Northern Indian provenances:

- Higher rates of germination in controlled environments.
- When watered, seedlings grow more vigorously.
- Impressive drought tolerance in experimental locations that are both dry and semi-dry.
- As a result, they are well-suited for upcoming breeding endeavors and Candidate Plus Tree (CPT) selection.

Candidate Plus Tree (CPT) Selection

Concept and Importance

When individuals in a population exhibit exceptional growth, shape, health, and reproductive success, they are referred to as Candidate Plus Trees (CPTs). Tree improvement initiatives rely on CPTs to plant seed orchards that consistently produce high-quality, uniform seed. CPT selection is even more important because of the neem (*Azadirachta indica*)'s genetic diversity and wide geographic distribution because only a small number of trees in wild populations consistently exhibit favorable characteristics [30-35].

- Through CPT selection, which is what forestry programs do, improve seed quality and vigor for plantation success.
- Make sure you can pass on favorable qualities by catching them.
- Remain resistant to pests, salinity, and drought.
- To expedite the improvement process, set up a system for clonal propagation or neem seed orchards.

Flowchart of CPT Selection Process (Figure 4)

The process of CPT identification and utilization can be visualized in the following stages:

- Step 1: Find areas with healthy neem populations, such as natural stands, plantations, or farmer's fields. This is the first step in the process, the reconnaissance survey.

- Step 2: Initial Evaluation: Leave out trees that are sick, misshapen, or have a poor yield; narrow down the list of possible CPTs.
- Step 3: Exact Measurements: Keep track of the plant's stress tolerance, yield, crown shape, and stem and seed quality.
- Step 4: Rating and Scoring: Prioritize potential trees by assigning numerical values using a weighted scoring matrix.
- Step 5: Documentation and Selection Pick out the best 5–10% of trees from every given population to guarantee variety in DNA.
- Step 6: Gather seeds from CPTs and evaluate their germination, vigor, and field performance as part of.
- Step 7: Planting Seed Orchards Transform CPTs into Seed Sources by Grafting, Clonal Multiplication, or Offspring Trials.

Explanation of the Matrix

- Stem form (20%): For optimal tree quality and lumber utilization, it is essential to have a straight bole free of defects.
- Crown design (15%): For optimal light interception and fruit production, symmetrical crowns with optimum branch angles are ideal.
- To guarantee genetic robustness, CPTs must demonstrate resistance to common pests and diseases, such as shoot borers and dieback, which accounts for 15% of the total.
- Fruit yield (20%): The availability of seeds is directly affected by the continually greater fruit and seed yields produced by superior plants [36].
- To guarantee excellent germination and vigor, the seeds from CPTs must be big, hefty, and well loaded (15%).
- Phenological stability accounts for 10% of the total. CPTs need to have predictable and consistent blooming and fruiting cycles so that seed is available every year.
- For climate-resilient plantations, it is important to choose trees with a 5% stress tolerance. These trees should be able to withstand conditions such as salty soils, drought, or high temperatures [37].

To avoid picking from a single site or family of trees and to ensure genetic diversity, foresters may objectively assess, rank, and choose the best candidates for seed programs by giving weights to each criteria [38].

Advanced Insights into Early Growth Traits

Seed Morphology: Beyond Basic Traits

Seed morphology plays a decisive role in the success of *Azadirachta indica* plantations. Beyond seed length, width, and 100-seed weight, morphological diversity across provenances provides insights into local adaptation. For example, seeds from semi-arid regions tend to be smaller yet more resilient, while those from humid provenances are larger but often less tolerant to stress [39]. Seed coat thickness and color are not only indicators of maturity but also influence oxygen diffusion and water uptake during germination. Research shows that pre-sowing treatments such as scarification, soaking in water, or mild acid treatments enhance germination uniformity by softening the hard endocarp. Comparative morphology studies highlight that seed traits directly correlate with early vigor indices, making them valuable markers for tree improvement programs [40].

Seedling Physiology: Stress and Performance Indicators

Seedling physiology offers a functional perspective on how neem responds to environmental pressures. In addition to germination percentage (GP) and mean germination time (MGT), photosynthetic efficiency (chlorophyll fluorescence, stomatal conductance) has emerged as a critical parameter for assessing stress resilience [41]. Seedlings exposed to salinity stress, for instance, exhibit reduced relative water content (RWC) and altered chlorophyll stability, but genotypes from drier provenances maintain higher membrane stability index (MSI) under the same conditions [42]. Nutrient uptake efficiency—particularly nitrogen and phosphorus assimilation—also determines seedling vigor in degraded soils.

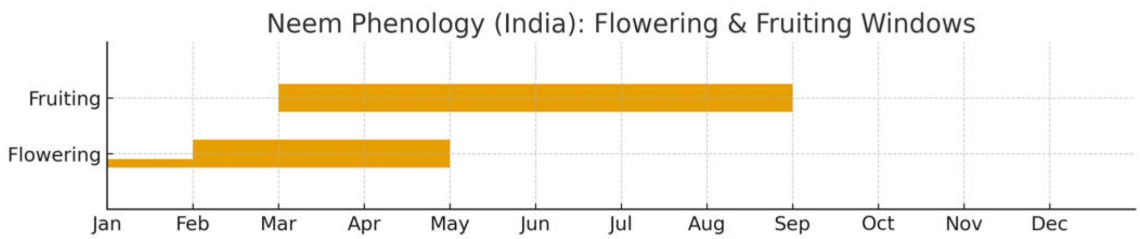

Figure.1 Variation in Neem Seed Morphology

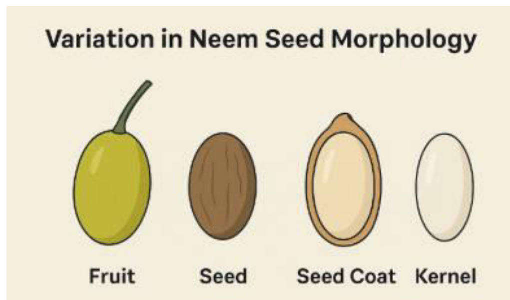

Figure.2 Flowchart of Seedling Growth Assessment Parameters

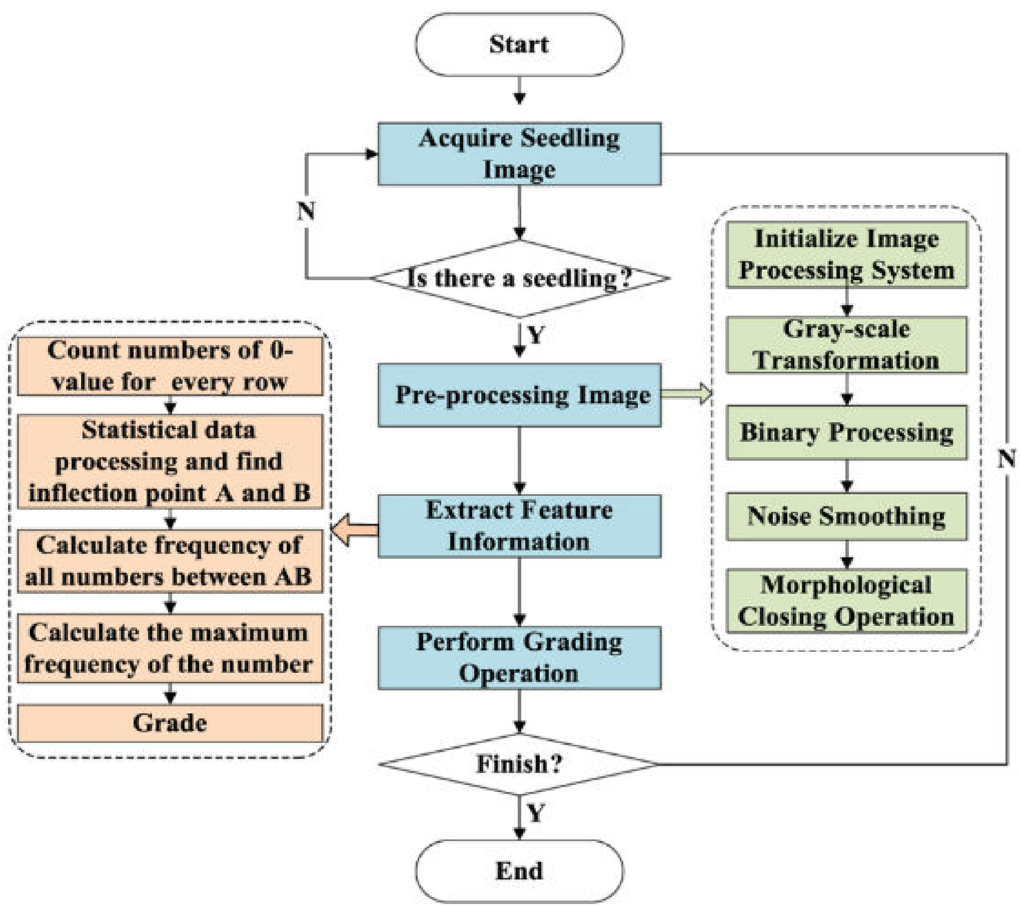

Figure.3 Key Physiological Markers for Seedling Stress Tolerance

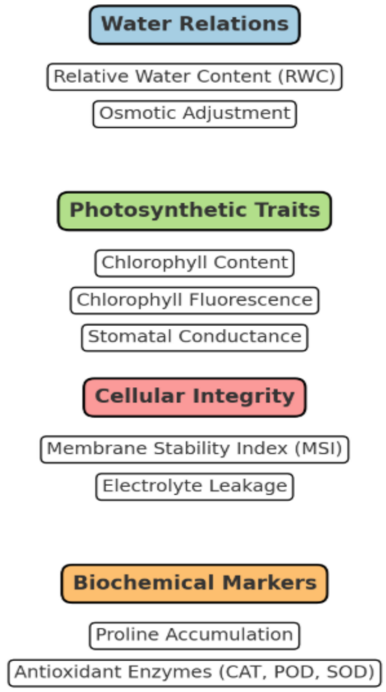

Figure.4 Circular Flow Representation of CPT Selection

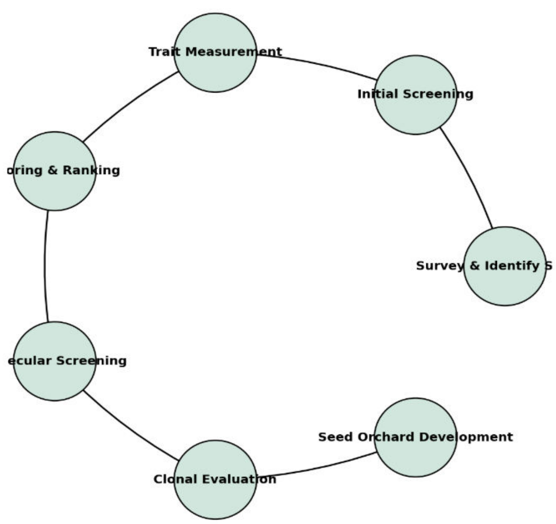

Figure.5 Conceptual Linkage between Seed Traits, Seedling Physiology, and Field Survival

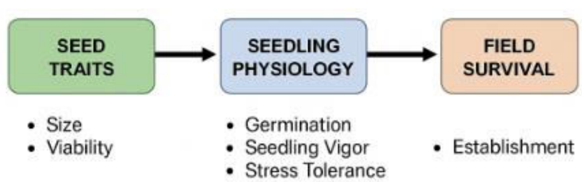

Table.1 Important Seed Morphological Traits and their Implications

Trait	Range	Importance
Seed length (mm)	9–16	Larger seeds show higher and faster germination
Seed width (mm)	6–10	Wider seeds associated with better seedling vigor
100-seed weight (g)	20–45	Strong indicator of seed quality and viability
Endocarp thickness	0.5–1.5	Thicker endocarps delay germination
Seed coat color	Yellow–brown	Indicator of maturity and viability

Table.2 Key Physiological Parameters in Neem Seedlings

Parameter	Formula / Method	Application
Germination Percentage (GP, %)	(Germinated seeds ÷ Total seeds) × 100	Basic measure of seed viability
Mean Germination Time (MGT, days)	$\Sigma(n_i \cdot t_i) \div \Sigma n_i$	Indicates speed and uniformity of germination
Seedling Vigor Index I (SVI-I)	GP × Mean seedling length	Combines germination with elongation growth
Seedling Vigor Index II (SVI-II)	GP × Seedling dry weight	Integrates germination with biomass accumulation
Relative Water Content (RWC, %)	(FW–DW) ÷ (TW–DW) × 100	Drought tolerance and water status indicator
Chlorophyll Content	Measured by SPAD meter or spectrophotometer	Proxy for photosynthetic capacity and stress adaptation
Membrane Stability Index (MSI, %)	(1 – Conductivity at high temp ÷ Conductivity at low temp) × 100	Measures cellular resilience under stress

Table.3 Scoring Matrix for CPT Selection

Criterion	Weight (%)	Indicators
Stem form	20	Straight bole, absence of forking, minimal defects
Crown architecture	15	Symmetry, optimal branch angle (45–60°)
Health & vigor	15	Resistance to pests, diseases, and dieback
Fruit yield	20	Mean 3-year average fruit production
Seed quality	15	High 100-seed weight, % well-filled seeds
Phenological stability	10	Consistent flowering and fruiting each year
Stress tolerance	5	Drought and salinity resilience, observed survival
Total	100	Weighted performance score

Physiological stress markers like proline accumulation, antioxidant enzyme activity (CAT, POD, SOD), and osmotic adjustment indicators provide a deeper understanding of drought and temperature tolerance [43]. By integrating these physiological responses into nursery evaluation, researchers can predict field survival and long-term growth performance with greater precision [44-45].

Genetic Variability and Provenance Influence

Neem's genetic variability is central to its adaptability. Provenance trials across northern and central India reveal significant differences in seed size, germination behavior, and seedling height. For example, provenances from Uttar Pradesh often show higher germination rates under irrigated conditions, whereas Rajasthani provenances exhibit superior drought resistance. Quantitative genetic studies confirm moderate to high heritability (H^2 = 0.40–0.70) for seed weight, germination percentage, and vigor indices, indicating strong potential for genetic gain through selection [46].

The growing use of molecular tools such as RAPD, ISSR, and SNP markers has further refined our understanding of neem's genetic diversity [47]. These markers help identify elite genotypes with superior stress tolerance, disease resistance, and reproductive stability. Coupling field-based provenance trials with molecular insights ensures more reliable candidate plus tree (CPT) selection [48]

Candidate Plus Tree (CPT) Selection: Modern Approaches

CPT selection forms the cornerstone of neem improvement programs. While traditional selection relies on visual scoring (stem form, crown symmetry, fruit yield), modern strategies integrate quantitative trait scoring, molecular fingerprinting, and remote sensing. For example:

- Scoring Matrix: Combines morphological, physiological, and reproductive traits with weighted indices.
- Clonal Evaluation: Grafting or tissue-culturing CPTs enables replicated trials under uniform conditions.
- Molecular Screening: Identifies CPTs with high genetic diversity and resistance alleles.
- Remote Sensing & GIS: Drone-based multispectral imaging can assess canopy vigor, crown architecture, and stress signatures at landscape scale.

These methods not only accelerate seed orchard development but also ensure that plantations are climate-resilient, disease-tolerant, and genetically robust [49].

Nursery–Field Linkages and Survival Strategies

The integration of nursery practices with field establishment is pivotal for plantation success. Seedlings with optimal collar diameter, balanced root-to-shoot ratio, and lignified stems are more likely to survive in semi-arid soils. Hardening techniques—such as gradual exposure to sunlight, controlled irrigation, and nutrient regulation—prepare seedlings for field conditions [50]. Field trials confirm that nursery-measured traits like SVI-I, SVI-II, and chlorophyll stability strongly correlate with survival percentages after transplantation. Site preparation, mulching, and protection from grazing further enhance establishment success [51]. Long-term monitoring reveals that genotype × environment interactions play a defining role in growth trajectories, emphasizing the need for feedback loops between nursery trials and field plantations [52].

Nursery and Field Linkages

The transfer of the plants from the nursery to the field is essential to the success of the planting projects. Because field outcomes are directly influenced by the quality of nursery techniques, the connection between the nursery and the field is essential for efficient afforestation [53]. Proper nursery management is necessary to ensure that seedlings have the desired characteristics, such as the right height, collar diameter, balanced root-to-shoot ratio, and well-lignified stems. This includes the target plant concept, substrates with good drainage, and the use of deep containers to prevent root coiling. To help seedlings adapt to tough field circumstances, hardening procedures include exposing them to sunlight gradually, watering them less, and controlling their nutrients [54]. Plant performance in the field can be accurately predicted by physiological indicators like chlorophyll stability and relative water content, as well as nursery-evaluated characteristics like root-to-shoot ratio, seedling vigor indices, and collar diameter. In addition to careful site preparation, mulching, and grazing protection, planting at the beginning of the monsoon rains further enhances field performance [55]. Managers may improve processes and zero in on characteristics most predictive of success with the use of data provided by field monitoring of survival and growth. Therefore, when the nursery and field stages are well integrated, neem plantings are productive and long-lasting, particularly in stressed-out and degraded areas [56].

In conclusion, the neem tree (*Azadirachta indica* A. Juss) grows in areas that are tropical or semi-tropical. Due to its adaptability and numerous applications, including agroforestry and medicine, it contributes significantly to sustainable development. Combining seedling physiology and seed morphology with Candidate Plus Tree (CPT) selection is the only way to realize the plant's full potential. Seedling physiology predicts adaptation and survival, ceremonial propagation via translocation (CPT) selection ensures genetically superior planting stock, and seed features influence germination and vigor. Combining these elements improves the efficiency of afforestation, plantation production, and genetic improvement over time. Harmonizing traditional applications with current scientific methods is essential to ensure that neem can fulfill its dual roles as a "village pharmacy" and a foundational species for ecological restoration, climatic resilience, and sustainable bioresource exploitation.

Author Contributions

Narendra Kumar Shukla: Investigation, formal analysis, writing—original draft. Narendra Babu Shakya: Validation, methodology, writing—reviewing. Bhavna Yadav:—Formal analysis, writing—review and editing. Garima Singh: Investigation, writing—reviewing. Pooja Yadav: Resources, investigation writing—reviewing. Vishwadeep Shukla: Validation, formal analysis, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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How to cite this article:

Narendra Kumar Shukla, Narendra Babu Shakya, Bhavna Yadav, Garima Singh, Pooja Yadav and Vishwadeep Shukla. 2025. Integrative Review on Early Growth Traits of Neem (*Azadirachta indica* A. Juss): Exploring Seed Morphology, Seedling Physiology and Candidate plus Trees Selection. *Int.J.Curr.Microbiol.App.Sci.* 14(11): 145-156**doi:** https://doi.org/10.20546/ijcmas.2025.1411.015