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Genetic Improvement in Neem-A Potential Multipurpose Tree: A Review

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ABSTRACT

Neem (*Azadirachta indica* A. Juss.) is an important woody angiosperm belonging to the order Rutales, family Meliaceae. Neem is a botanical relative of mahogany and is native to India and Burma.The studies on genetic improvement of Neem are not well documented so for the effective use of Neem resources, effective exploration and improvement required. The current paper aimed to review all the studies related to genetic improvement of neem to understand genetic variability in Neem and its improvement can be done in this medicinally important tree species. In thispaper various studies has been reviewed that provided status of the improvement work on Neem all over the world. Tree breeders assessed the genetic resources available for improvement, selecting genes with high utility and economic value and packaging them into genotypes that may be utilized to start commercial plantations. Researchers used biotechnological, traditional tools to improve this species. Despite its widespread ranged studies and numerous researches for better understanding breeding efforts must be expanded.

Key words: Genetic improvement, Molecular study, Morphology, Neem, Provenance.

Because of its various medical and agrochemical uses, neem (*Azadirachta indica* A. Juss.) is an important woody angiosperm belonging to the order Rutales, family Meliaceae. Neem is a botanical relative of mahogany and is native to India and Burma. It grows to be as tall as an oak and as wide as a locust, with masses of honey-scented white blossoms. It has a complex foliage that looks like walnut or ash and its bloated fruits resemble olives (Fig 1). It is rarely leafless and the shade it provides all year is one among the reasons it is appreciated in India. The Subcontinent is home to an estimated 18 million neem trees, the most of which are planted along roadsides or in clusters around markets or backyards to offer shade. Neem can withstand a lot of abuse. It can endure pollarding (repeated lopping at heights above 1.5 m), for example and its topmost trunk vigorously resprouts. It also coppices freely (repeated lopping at near-ground level). Because it is fed by a root system large enough to nourish a full-grown tree, regrowth from pollarding and coppicing can be extremely quick.

Assam and Burma are regarded to be the origins of neem (where it is common throughout the central dry zone and the Siwalik hills). The actual origin of neem is unknown: some believe it is native to the whole Indian subcontinent, while others say it is found in dry forest areas throughout South and Southeast Asia, including Pakistan, Sri Lanka, Thailand, Malaysia and Indonesia. India is the country where the tree is most frequently used. It grows from the southern tip of Kerala to the Himalayan hills, in tropical and subtropical climates, semiarid to wet tropical climates and at elevations ranging from sea level to 700 metres.

The tree is easily propagated-both sexually and vegetatively. It can be planted using seeds, seedlings, saplings, root suckers, or tissue culture. The seeds are fairly easy to prepare. The fruit drops from the trees by itself; the pulp, when wet, can be removed by rubbing against a coarse surface; and (after washing with water) the clean, white seeds are obtained. In certain nations-Togo and Senegal, for example-people leave the cleaning to the fruit bats and birds, who feed on the sweet pulp and then spit out the seeds under the trees.

It is used as a woodsource, an animal and poultry feed and as a manure and fertilizer as well as in a range of traditional medicines and pesticides (Parmar and Ketkar 1993). *Azadirachta indica* possesses better medicinal and bioactive properties than its close relatives such as Thai neem *(A. siamensis),* sentang or marrango tree *(A. excelsa)* and the Persian lilac or Chinaberry tree *(Melia azedarach)* (Lauridsen *et al.* 1991). Its wide array of phytochemicals are responsible for its biological activity against a plethora of insects, micro-organisms, nematodes *etc*. although the tree itself is susceptible to a range of pests and pathogens (Boa 1995). Azadirachtin is a tetraterpenoid found in higher concentration in seeds than other limonoid compounds.

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These Neem oil chemicals cause cytotoxic effects in various organs and tissues, as well as repellency, sterility and death Ceraeochrysaclaveri's cocoons undergo morphological and ultrastructural alterations after ingesting oil (Scudeler *et al*., 2014).

Neem uses a variety of pesticide chemicals to protect itself against a variety of pests. Its major chemical composition is a mixture of three or four related compounds, with another 20 or so lesser molecules that are still active in some form. These molecules are typically classified as "triterpenes," or "limonoids," a broad category of natural products. Azadirachtin, one of the first active compounds identified from neem, has proven to be the tree's primary insect repellent. Another feeding inhibitor, meliantriol, can induce insects to stop eating at extremely low quantities. Antiviral activity has been discovered in two new Neem components, nimbin and nimbidin.

Neem is andromonoecious, which means it produces both bisexual and staminate (functionally male) flowers on the same tree (Singh *et al*., 1996). The anthers in the unopened flower begin to dehisce around 08.00 hours and the pollen is ripe before the stigma becomes receptive (protandry) (Gupta *et al*., 1996). Bees and other small insects pollinate the flowers and the ovary is trilocularmeaning it has three chambers, each with two ovules. Polycarpy is a phenomenon in which the endocarp encloses more than one seed, frequently two and rarely three (Singh *et al*., 1995). Birds and mammals are the primary dispersers of the seeds. Kundu (in press) demonstrated that Neem outcrosses by utilizing three isozyme loci (tm = 0.90 and ts = 0.92) to estimate the outcrossing rate in a wild population from Bangladesh. Selfing has been seen in the species (Gupta *et al*., 1996) and self-pollination produces viable seed (Solanki, 1998). In the early stages of a breeding programme, one of the most significant characteristics considered by plant breeders is genetic diversity. There are a variety of approaches for evaluating plant populations, each with varying degrees of capacity to identify genetic changes (Morales *et al*., 2011). Agronomic, morphological and molecular variables, among other things, can be used to quantify genetic diversity. In response to worldwide, national and local research demands, many groups throughout the world have been focusing on improving the genetics of neem.

History of genetic improvement

Neem and its products have been the subject of increased investigation since the early 1980s. Many Indian research institutes, such as the Arid Forest Research Institute (AFRI) in Jodhpur, the Forest Research Institute (FRI) in Dehra Dun and the Institute of Forest Genetics and Tree Breeding (IFGTB) in Coimbatore, have extensive Neem research programmes, as have many research institutes in developed countries, such as the University of Keele in the United Kingdom, with their EU-funded AZTEC project. The International Neem Network (INN) was founded in 1994 in response to a growing global interest in the neem's therapeutic and insecticidal properties, as well as the issue that neem planted in many countries was regarded to be of poor genetic quality (Read, 1993).

The FAO is in charge of coordinating the Network, which aims to improve neem and its use by rural people (Thomsen and Souvannavong, 1994). The International Neem Network was founded in 1994 with the long-term goal of improving the genetic quality and adaptability of neem, as well as increasing its use as a development tool around the world, with a special focus on satisfying the needs of rural people (Thomsen and Souvannavong, 1994). The Network's partners agreed to engage in activities such as provenance research, seed collection and trade in order to develop internationally coordinated trials. They also opted to conduct study on seed physiology and technology, as well as genetic variety and reproductive biology, as well as chemical component variation.

The Network brings together national institutions from 21 nations across Asia, Africa, Latin America and Europe. The Indian Council for Forestry Research and Education (ICFRE, India), the Royal Forest Department (Thailand),

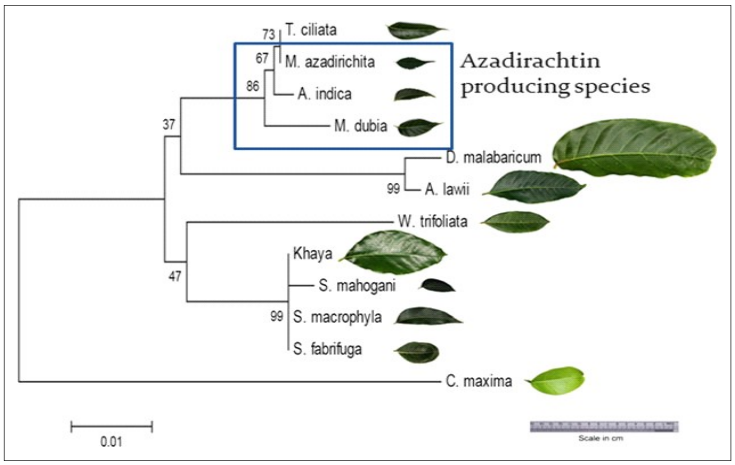

Fig 1: Dendrogram along with leaf image of Meliaceae plants based on rbcLgenesequence (Kuravadi and Gowada 2019).

the InstitutSénegalais de RechercheAgronomique (ISRA, Senegal), the Département Forestier du Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD-Forêt, France), the DANIDA Forest Seed Centre (DFSC, Denmark), FORTIP and the FAO are all members of the Network FAO is in charge of global coordination and it encourages inter-regional cooperation and information and genetic material exchanges.

Phylogeny of neem

Neem morphological reviews

In India and adjacent countries, neem trees are found in a wide range of agro-ecological conditions, with considerable variations in morphological (*e.g.*, seed weight, leaf form and phenology) and biochemical properties (*e.g.*, azadirachtin and oil content of kernels) Tiwari (1992). Seed shape (seed length and 20 seed weight) and oil content in *Azadirachta indica* A. Juss. (Neem) from five northern and western Indian provenances were investigated. For this investigation, trees with a wide range of girths were investigated. The highest average oil content was found in trees from the Hisarregion. Seed oil content was not consistently and significantly linked with morphological features of seeds in most provenances. The age of the tree had no bearing on the oil output (Kaura, 1998).

Kundu 1999, S. conducted research to investigate patterns of variability and evaluate genetic distance between neem seed morphometric and allozyme data (*Azadirachta indica*). Using starch gel electrophoresis, phenotypic diversity from open pollinated seed materials at 12 isozyme loci was investigated in four populations from Thailand, Bangladesh and Kenya. All four populations exhibited high levels of variation for seed parameters and isozymes. The Kenyan population have a close genetic link with Bangladeshi tree. Thailand's population had a long-distance relationship with the other three populations. This indicates that the Kenyan population originated in the Indian subcontinent. Cluster analysis of seed morphometric data, on the other hand, revealed three distinct populations. All other populations had a remote link with the Kenyan population. When calculating genetic distance, the study shows that allozyme data may not correspond to morphometric features.

Seed and biochemical indicators, according to Prabakaran *et al*. (2019), could be used as selection criteria for early and beneficial exploitation of high oil and azadirachtin producing genotypes. Their research verified the existence of significant genetic variation that can be used to save genetic resources in gene banks and future tree improvement programmes. Kundu conducted research in a wild population of Neem in Bangladesh in 1999 with the goal of estimating outcrossing rates and explaining genetic repercussions of seed development in the endocarp. To assess allozymes, cotyledons of sprouted open-pollinated seeds of individual trees were studied using starch-gel electrophoresis. To calculate the out-crossing rate, three loci with unambiguous Mendelian segregation were employed. The mating system was evaluated using a multilocus mixed mating model. Both multilocus (tm=0.90 0.024) and mean single-locus (ts=0.920.020) estimates revealed high outcrossing rates in the population. The difference between these two measures (tm–ts=0SE 0.038) was small, showing that the population did not suffer from 'biparental inbreeding.' When two or more loci were included, the degree of variance of the estimations of multilocus outcrossing rates fell. A total of 471 seeds were counted out of 440 endocarps in order to determine the relevance of polycarpy. This technique appears to be one approach to prevent inbreeding. The findings revealed that the neem population under study was mostly allogamous.

Provenance trails

Several research institutes, mostly in South and Southeast Asia, have created neem provenance trials or micro-propagated clonal material from plus tree selections (Chamberlain, 1999). The FCRI (TNAU) has the oldest provenance experiment, which was started in 1991. AFRI also has a provenance trail, which was started in 1992 and now contains around 40 provenances from India's ten states. CAFRI, Jhashi, established a neem provenance trial with 26 provenances from central India (Solanki, 1998). After a seven-month growing time in the field Seed, twenty neem (*Azadirachta indica* A.Juss.) provenances were evaluated at three sites of the international provenance trials in Bangladesh and India. The findings of this study revealed that ecoclimatic factors influence growth, with correlations between origin latitude and CD and latitude and SV percent percent at sites I and II suggesting clinal variation in neem (Kundu 1998). During the years 1995 to 1997, 36 provenance experiments were set up in Bangladesh (2), Burkina Faso (1), Chad (1), India (6), Lao (1), Mali (2), Myanmar (3), Nepal (2), Nicaragua (1), Pakistan (1), Philippines (2), Senegal (3), Sri Lanka (1), Sudan (1), Tanzania (4), Thailand (2) and Vietnam (2) (Child *et al*. 2001). In July 1996, an international neem provenance trial was launched at Butwal Research Station in Nepal's western Terai region. It included seed supplies from 23 provenances from ten nations, including Nepal (Lamichhane and Thapa, 2010). Using Mahalanobis D^2 analysis, the genetic diversity of ninety-nine trees of neem (*Azadirachta indica* A. Juss) selected from various locations of Haryana and Delhi was investigated for morphological and biochemical features of seed. Nine clusters were formed from these trees. Geographic diversity in neem did not have a one-to-one relationship with genetic diversity, according to the clustering pattern. The current study's findings demonstrated that geographic diversity is not the main criterion for determining divergence and that genotype selection should be based on genetic diversity. Hybridization between neem trees/genotypes with higher divergence can result in genotypes with greater hetroticvigour (Dhillon *et al*., 2009).

A number of studies have found that the concentration and quality of chemical elements in plants are changed significantly by the agro-climatic and phyto-geographic circumstances to which they are exposed (Gupta *et al*. 1998). Kaushik *et al*. (2007) investigated the content of azadirachtin in neem (*Azadirachta indica* A. Jusieu) seeds collected from various parts of India. Azadirachtin concentrations ranged from 200 to 16,000 ppm (mg/g of seed kernel). Climate and habitat were discovered to have an impact on azadirachtin content. The highest levels of azadirachtin were found in Neem tree populations in India's southern states. Intraprovenance heterogeneity in azadirachtin A and B content and oil % was investigated in 43 Indian provenances. The interprovenance variability of twenty-eight individual neem trees from five provenances in different agroclimatic zones was also investigated. Using reversed phase analytical HPLC, the azadirachtins were measured. The amount of oil and azadirachtin in different provenances varied greatly. Azadirachtin A levels in kernels ranged from 556.9 to 3030.8 mg kg^{-1}, whereas azadirachtin B levels varied between 43.1 and 590.6 mg kg^{-1} across the provenances studied. An analysis of variance revealed substantial differences in oil content, azadirachtin A, total azadirachtin (A + B) and the A:B ratio among distinct Neem provenances. Within a single provenance, there were individuals with high and low azadirachtin levels and this pattern was observed in all five agroclimatic areas of the country. Variations in azadirachtin content in neem trees from different provenances revealed that environmental conditions such as rainfall, humidity, or temperature had no effect on the neem trees' azadirachtin content (Sindhu *et al*., 2003). Sindhu *et al*. (2003b) investigated quantitative variances in thirty-three provenances. RP-analytical HPLC was used to measure nimbin and salanin levels. Individuals with a high salanin and nimbin levels were recognised as elite. Nimbin concentrations ranged from 18.2 to 636.8 mg kg^{-1} kernel weight, while salanin concentrations ranged from 45.4 to 1830.3 mg kg^{-1} kernel weight. Individual trees from the same agro-climatic zone showed varied trajectories, indicating that nimbin or salanin synthesis was not controlled by environmental factors. It can be determined that neem trees have individual genetic peculiarities. This leaves plenty of room for tree enhancement through half-sib progeny trials and clonal multiplication. 60 Neem seed samples were gathered from various regions of Rajasthan, India and analysed by GLC to determine the fatty acid composition variability. Individual fatty acid variability was found to be quite high. The percentages of palmitic acid, stearic acid, oleic acid and linoleic acid varied from 16 to 34 per cent, stearic acid from 6 to 24 per cent, oleic acid from 25 to 58 percent and linoleic acid from 6 to 17 per cent. This variability can be used to help with tree selection and research into neem genetic variability. These selections can also be used to improve the tree's genetics (Kaushik and Virt, 2002).

Vegetative propagations in Neem

Gehlot *et al*. (2015) found that cutting diameter, auxin content and rooting substrate had significant effects on adventitious rooting from neem hardwood cuttings. The proportion of roots, the number of roots, the length of the roots and the number of leaves were all measured. The diameter of the hardwood cuttings used ranged from 0.5 to 1.5 cm, with the 0.5-1.5 cm diameter showing the most roots. When grown in a sand rooting medium, IBA (500 mg L^{-1}) resulted in a greater rooting percentage (80%) with 6.82 sprouts, 53.06 roots, 7.13 cm root length and 7.0 leaves per rooted hardwood cuttings. The use of hardwood cuttings and the establishment of optimal rooting methods proven to be crucial in the multiplication of *A. indica*. Palaniswamy and Pramod Kumar established the vegetative propagation strategy (adventitious roots in branch cuttings) of neem in 2001. Only in the leaf fall season did 1000 ppm IBA produce 80 percent rooting and a luxuriant root system in mature tree branch cuttings (February). Juvenile cuttings taken from young seedlings, on the other hand, exhibited rooted in most of the months. The method is advised for bulk multiplication of superior trees in order to build clonal seed orchards and afforestation projects.

Polyploidal studies in neem

In neem, the utility of polyploid and mutant breeding is still debatable. For the production of transgenic neem, gene transfer techniques could be effective (Naina *et al*., 1989). Genes that control drought tolerance and genes that produce a lot of azadirachtin-A could be valuable. The most successful and time-efficient technique for short-term Neem improvement is the establishment of seed production areas (SPO) from provenance experiments. Seed orchards (SO) are recommended for long-term tree production as well as fruit yield.

Understanding the genetic nature of natural populations, their evolution and the utility of genetic variety is required for neem improvement. The efficient use of neem requires effective exploration, identification, documenting and exploitation of its genetic resources. The systematic breeding of neem for bioactive chemicals or other numerous uses has yet to begin. In 1996, the FAO coordinated a programme on provenance trials for the International Neem Network, which began evaluating and improving the species' genetic resources (Anonymous, 1998).

Biotechnological innervations

Barka *et al*. (2021) used RAPD to examine the genetic diversity of 27 randomly selected neem trees from different agro-ecological zones in Northern Nigeria. A total of 9 primers were used, however only 5 of them were effective (OPA-02, OPA-03, OPA-15 and OPA-19). The visible DNA bands in the various tree samples differed according to these primers. There were genetic differences among the trees that were sampled. There were differences in

percentage polymorphism, with Borno State tree samples having the greatest percentage (97.44 per cent) compared to those in Yobe State with no polymorphism. Bhandare *et al*. (2021) employed RAPD molecular markers to assess genetic diversity in populations of *Azadirachta indica* from various parts of north Karnataka. Only three of the five random decamer primers employed resulted in polymorphic banding patterns. There were a total of 23 distinct DNA bands that could be reliably obtained, with 18 (78.26%) of them being polymorphic. Using PyElph 1.4 and PAST software, the polymorphisms were scored and the data was presented as a binary matrix for phylogenetic analysis. The 20 populations were divided into two groups using cluster analysis based on Jaccard's similarity coefficient and UPGMA. On average, the principal component analysis detected 37% of the overall variation. Ana *et al*. (2013) The genetic diversity of 54 accessions from the Embrapa Coastal Tablelands Germplasm Bank (GBN) using random amplified polymorphic DNA (RAPD) markers (Sergipe, Brazil). A model-based Bayesian technique (Structure), molecular variance analysis (AMOVA) and the Jaccard coefficient were used to analyse the accessions. The marker data revealed that GBN is divided into three distinct groups, as evidenced by genetic structure and genetic variability, allowing for the development of appropriate GBN management and use techniques.

Using eight isozyme systems, researchers looked at the genetic variance among 22 neem provenances from the International Provenance Trial in KamphengPhet province. These enzyme systems' 12 gene loci were discovered in 22 provenances. Thai and Indian Neems differed in terms of DIA-A, DIA-B, GOT, MDH-C and SKDH. The levels of provenance variation in GOT, G-6PDH, IDH, MDH-A, MDH-B, PGM-A and PGM-B were moderate to high. The average predicted heterozygozygosity (He=0.226) and Wright's FST (0.58) were also high, indicating that provenances had a lot of genetic variety. Between Thai and Indian neems, the genetic distances (D) were large (D>1.00). D levels among Thai and Indian neem provenances, on the other hand, were low (Wathinee *et al*., 2017). RAPD markers were used to assess the genetic diversity of 12 Neem populations collected in Nigeria. There was no difference in genetic composition across the 12 populations using this marker (Awodele *et al*., 2011). Kurawadi *et al*. (2015) used next-generation sequencing technology to sequence the genomes and transcriptomes of Neem to uncover genes and pathways. 267 Mb of Illumina and 454 sequencing reads were assembled, accounting for 70% of the predicted size of the neem genome. They found 44,495 genes in the neem genome, with 32,278 of those genes being expressed in neem tissues. The genome of the neem has 32.5 per cent (87 Mb) repetitive DNA sequences. This research provides genetic, transcriptomic and amount data for the top three neem metabolites, which can speed up basic research on neem and help researchers, better understand biochemical processes. In 2017, Bhambhani *et al*. created and analyzed transcriptome datasets from leaf and fruit, identifying members of neem gene families. The expression of a number of genes differs between the leaf and the fruit, implying that they are involved in the manufacture of fruit-specific triterpenoids. AFLP markers were used to assess genetic diversity in 37 neem accessions from various eco-geographic regions of India and four exotic lines from Thailand. According to the cluster study, neem germplasm in India has a broad genetic foundation, with genetic similarity coefficients ranging from 0.74 to 0.93, In contrast; the four Thai lines formed a small genetic foundation with similarity values ranging from 0.88 to 0.92. The high level of genetic variation detected by AFLP analysis in Neem accessions suggests that it is an effective marker technology for identifying genetic relationships between genotypes and estimating genetic diversity, allowing for the development of appropriate conservation and tree improvement strategies (Singh *et al*., 1999). The applicability of amplified fragment length polymorphism (AFLP) markers in establishing clonal fidelity of tissue culture (TC)-raised Neem plants is investigated by Singh *et al*. (2002). Across the mother plant, TC progenies and other Neem accessions used as controls, seven AFLP primer combinations generated a total of 334 amplified fragments. A total of 239 amplified pieces were monomorphic between the mother tree and its TC progenies. Because of their extended generation period, AFLP markers have proven to be an ideal technique for routine examination and certification of genetic fidelity of micropropagated plants prior to commercialization, especially in tree species.

Tissue culture in neem

Seed is the traditional method of propagation for neem. Cutting-grown trees typically lack the deep tap root of seed-grown trees, making them more susceptible to dryness and severe winds. Suckers, which are commonly generated, especially in dry environments, can also be used to propagate neem. Neem can be pollarded and coppiced to increase timber production and it often regrows extremely quickly following such treatment. Aseptic cultures of Neem have been established from most tissue types, including anthers, bark, cotyledons, leaves and stems, with the surface sterilization process being depending on the parent plant (Fig 2). Narayan and Jaiswal (1985) looked at neem callus differentiation (NAA). The auxin NAA alone, at concentrations ranging from 0.05 to Img l^{-1}, resulted in root development that was best at 0.5 mg l^{-1}. Concentrations of the cytokinin BA (0.05-Img l^{-1}) on the other hand, promoted differentiation to create shoot buds, which thereafter developed into shoots. On Wood and Braun medium supplemented with Kn, BA and adenine sulphate, Ramesh and Padhya (1990) investigated the formation of adventitious shoot buds from leaf discs. They found that, despite minimal growth on the basal medium, adding Kn or BA alone resulted in callus formation and adding Kn and BA together (4 11M each) resulted in the creation of up to 10-12 shoots per explant in 4 weeks. The influence of tree

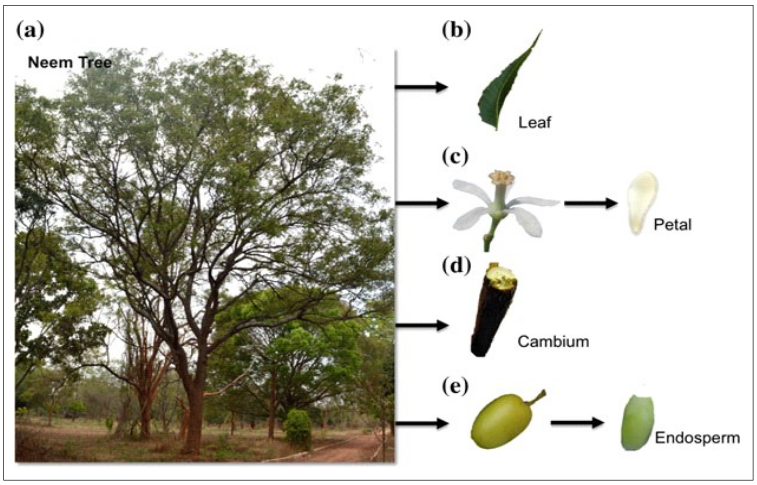

Fig 2: Explants for Neem tissue culture (Mohan *et al.,* 2019).

age and phytohormones (cytokinins and auxins) on micropropagation of neem was investigated by Shrinidhi *et al*. (2008) by using nodal segments from mature (15-year-old) trees and green-house-grown juvenile (1.5-year-old) seedlings. Modified MS medium (MS-RMN) supplemented with a mixture of 2.0 mg/l 6-Benzylaminopurine (BA) and 0.3 mg/l indole-3 butyric acid (IBA) proved efficient in shoot bud sprouting in both juvenile and adult trees. Maximum numerous shoots were induced on modified MS medium supplemented with 1 mg/l BA + 0.5 mg/l 1-naphthalene acetic acid (NAA) (NAA). In half-strength MS medium supplemented with 2.0 mg/l IBA, the highest frequency of rooting was observed. Elongated shoots were culturing in basal MS media for 2 weeks before being subculturing in rooting medium. The influence of age of explants source was not observed in rooting stage. Singh and Chaturvedi published a paper in 2009 that documented the culture conditions required to induce and maintain recurrent somatic embryogenesis in Neem for the first time. Out of various treatments tested, the somatic embryos were induced directly from immature zygotic embryos of neem on MS + TDZ (0.1 µM) + ABA (4 µM), in more than 76 percent cultures. Direct secondary somatic embryogenesis occurred from primary somatic embryos on MS + IAA (5 µM) + GA3 (5 µM) in 12.5 per cent cultures. Repeated subcultures at regular intervals were used to sustain the explant's and primary embryos' embryogenic competence for a long time. Only about 10% of these somatic embryos were transformed into plantlets. The most ideal explant for the study in neem is young zygotic embryos, which demonstrated the greatest embryogenic response in the shortest amount of time, followed by immature cotyledons. Early dicotyledonous stage immature zygotic embryos were efficient in directly producing somatic embryos on the explants (Chaturvedi *et al*., 2004). To find a successful regenerating method, researchers used Neem shoot tip explants. Shoot tips were obtained from Neem embryos cultivated in DKW medium enriched with BAP and medium without hormones, which were alternated. The existence of cotyledons altered the initial development of the shoot. Organogenic potential was also seen in a basal callus removed from an *in vitro* stem base. Plant lines derived from each seed have diverse features in some circumstances. There were no significant variations in *in vivo* development between Neem plants propagated from callus and those propagated from shoot tips (Laureen *et al*., 2015).

CONCLUSION

Tree breeders assessed the genetic resources available for improvement, selecting genes with high utility and economic value and packaging them into genotypes that may be utilized to start commercial plantations. Tree breeders also look for superior genotypes in existing provenances and reproduce them clonally to take advantage of both additive and non-additive genetic influences that regulate commercial attributes. Neem has traditionally been propagated by seed, however it is a stubborn plant that quickly loses seed viability. Neem's heterozygous nature makes uniform plant selection of azadirachtin high-yielding and fast-growing trees from seedlings difficult. As a result, the micropropagation approach can aid in the uniform synthesis of azadirachtin in cloned plants. Although somatic embryogenesis in Neem has been intensively explored, proper germination and plantlet development from somatic embryos, as well as scale-up investigations remain of interest. The usage of various markers is one of the molecular level research efforts. AFLP and RAPD have been frequently used. Despite its widespread range and numerous applications, *ex situ* conservation, characterization and breeding efforts must be expanded. The understanding of genetic variety is thought to aid in the genetic improvement of the species.

Conflict of interest

All authors declare that they have no conflict of interest.

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