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Evaluation of genetic variability among *Azadirachta indica* A. Juss genotypes for seed attributes

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Abstract

In the present study, 40 genotypes of neem were collected from different regions of Telangana, Tamil Nādu, Karnataka and Rajasthan to evaluate their genetic variability for seed traits. Significant variation was observed among all the genotypes for all the seed characters. The seed attributes *viz.*, hundred seed weight, seed length and seed width contribute either independently or in conjunction with one another, in promoting high azadirachtin content. Hundred seed weight, germination percentage and azadirachtin percentage recorded higher GCV, PCV and genetic advance as percent of mean, whereas all the seed traits exhibited high heritability, which is indicative that selection based on these seed traits would be effective for the exploitation of genotypes yielding high azadirachtin content.

Keywords: *Azadirachta indica*, azadirachtin, genotypic coefficient of variance, phenotypic coefficient of variance, heritability, genetic advance as (%) of mean

Introduction

Azadirachta indica A. Juss belongs to the family Meliaceae. The genus 'Azadirachtin' is derived from a persian word "Azad-darakhat-e-hind" which mean "The free tree of India", indicates that the tree is free from insect and pest problems (Tomar *et al.*, 2009) [1]. It is commonly known as Neem, 'The tree of 21st century', 'Pharmacy in the backyard', 'Kalpavriksha', 'The wonder tree', 'The miracle tree' *etc.*, all these words would describe the versatile nature of the tree along with its immense utility. Neem is widely distributed throughout the tropics of Asia and Africa (Sombatsiri *et al.*, 1995) [21] and is believed to be originally native to Indian sub-continent. It occurs naturally in the drier and subtropical regions of India covering the states of Tamil Nadu, Karnataka, Andhra Pradesh, Telangana, Maharastra, Gujarat, Haryana, Punjab, Uttar Pradesh, Bihar, Odisha, and in the Shivalik hill regions of Himalayas (Parotta, 2001) [14]. Generally, it gets adapted to dry and harsh climatic conditions except frost weather and water-logged conditions. It can grow well with an annual rainfall requirement of 400-1200 mm and is tolerant to variety of soil types *viz.*, dry, clayey, sandy, sodic and laterite crusts and calcareous soils having pH of 6.2 or above (Tomar *et al.*, 2009) [1]. In India, neem is a highly domesticated species, often seen in farmlands, homesteads, around temples, as avenue trees, in wastelands and degraded lands and is seldom seen inside forests.

Studies reported that more than 200 bioactive compounds have been identified from different parts of the tree till date. Among them, the most important components are the tetranortriterpenoids groups, the Azadirachtin. The effectiveness of bio-pesticide is based on the concentration of azadirachtin, which varies from 0.05% to 4.24% in the seed depending on the time of harvest, geographical regions and storage conditions (Roy Choudhury, 2016) [18] with an average yield of 3.5% azadirachtin from kernels. Large variation is observed among neem trees for their oil and limonoid content in the seeds as these traits are governed by genetic and environmental factors (Salil *et al.*, 2014) [19]. Genetic variability plays a major role in the effective development of any plant breeding programme. Selection, which is the most important activity in all the plant breeding programmes, majorly depends on the extent of variability present in the germplasm to improve the heritability of certain polygenic characters like yield and other economically important characters (Zobel and Talbert, 1984) [22]. Very few studies have been carried out so far to find the existing variability of oil content and azadirachtin content in neem trees. The amount of variation in the species and the extent of variability exhibited by different parameters should be determined first to carry out tree improvement programmes to apply appropriate selection pressure to develop desired lines/genotypes.

This study focusses on determining the variability present in various seed parameters of selected neem genotypes to help breeders select appropriate selection methods and to predict gains from the selection.

Materials and Methods

The experimental material used for this present study consisted of 40 healthy individuals of Neem, which were spread across different areas of Telangana, Tamil Nadu, Karnataka and Rajasthan. Fruits were collected during June-August 2021 and these genotypes were assigned with accession number. Nearly 5kgs of fruits were collected from the crown of healthy and vigorous trees. The fruits were collected when they were greenish yellow in colour (Puri, 1999) [17]. After depulping, different seed parameters like seed length, seed width, hundred seed weight, germination percentage and azadirachtin percentage were recorded under this study. Three replicated samples were taken from each genotype and 100 random undamaged seeds (total 300 seeds) were measured for their length, width (in millimetres) and hundred seed weight (in grams).

The azadirachtin content present in the kernels of seed was estimated based on the standard HPLC method (Prabakaran *et al.*, 2019) [16] using three replicates from each accession. The data was statistically analysed using R open-source software Version 4.2.1 and Microsoft Excel. Analysis of variance was performed using Panse and Sukhatme (1967) [13].

Variability studies such as genetic variability, phenotypic variance, genotypic variance, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in broad sense, genetic advance and genetic advance as percentage of mean for all the seed traits were determined by using different methods suggested by Johnson *et al.* (1955), Burton (1952), Lush (1940) and Johnson *et al.* (1955) respectively.

Results and Discussion

Analysis of variance of seed traits *viz.*, seed length, seed width, hundred seed weight, germination percentage and azadirachtin percentage revealed the presence of significant variations among different genotypes (Table 1).

Table 1: Analysis of variance for seed traits of *Azadirachta indica*

Source	d.f	Mean sum of squares				
		Seed Length	Seed width	Hundred seed weight	Germination percentage	Azadirachtin percentage
Treatments	39	5.76**	0.87**	41.84**	1349.61**	0.12**
Replication	2	0.13	0.04	0.06	0.5	0.000091
Error	88	0.07	0.03	0.08	1.45	0.000057
CD (5%)		0.426	0.261	0.468	1.960	0.012
CD (1%)		0.565	0.346	0.621	2.599	0.016

Genetic variability estimates like phenotypic variance (PV), genotypic variance (GV), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability, genetic advance as (%) of mean were presented for every seed trait (Table 2).

The proportion and extent of genetic variation present among the accessions for all the seed traits can be judged by estimating PCV and GCV. Germination percentage registered the highest for both PCV and GCV (53.21% and 53.12% respectively), followed by azadirachtin percentage (24.93% and 24.89%) and hundred seed weight (22.57% and 22.51). Low PCV and GCV values were exhibited by Seed length (9.98% and 9.82%) and seed width (8.27% and 7.92%) (Table 2).

The magnitude of both PCV and GCV for all the traits in the study were found to be almost equal, which indicates the insignificant effect of environment on the expression of these traits (Abdul Manan *et al.*, 2018). This presents remarkable scope for effective selection in these traits in neem. Similar variation was reported in *Pongamia Pinnata* (L.) Pierre (Jaisankar *et al.*, 2014 [8]; Deebe *et al.*, 2011 [4]; Naresh *et al.*, 2007 [12]), *Acacia catechu* (Gupta *et al.*, 2012) [7] and *Casuarina equisetifolia* (Mahadevan *et al.*, 1999) [11].

The degree of reliability of a genotype to be exploited for future improvement programs depends on the magnitude of heritability present for a trait. It is evident from the current study that the heritability in broad sense was recorded highest by all the seed traits *viz.*, germination percentage (0.99),

azadirachtin percentage (0.99), hundred seed weight (0.99), seed length (0.97) and seed width (0.97) (Table 2). The findings of the current study are similar with those of Bindyalaxmi *et al.* (2022) [3] and Kala *et al.* (2017) [9] in *Bixa orellana*, Prabakaran *et al.* (2019) [16] in *Azadirachta indica*, Divakara, 2014 [6] in *Madhuca latifolia* Macb., Gupta *et al.* (2012) [7] in *Acacia catechu*, Singh *et al.* (2012) [20] in *Pinus wallichiana*, Divakara *et al.* (2010) [5] in *Pongamia Pinnata* (L.) Pierre, Kaushik *et al.* (2007) [10] in *Jatropha curcas* L.

Genetic advance as (%) of mean registered high for germination percentage (109.24) followed by azadirachtin percentage (51.23) and hundred seed weight (46.22) (Table 2). Similarly genetic advance as % of mean was found higher in hundred seed weight of *Azadirachta indica* (Prabakaran *et al.*, 2019) [16], *Pongamia Pinnata* (L.) Pierre (Pavithra *et al.*, 2013) [15], *Jatropha curcas* L. (Atul *et al.*, 2013) [2], *Casuarina equisetifolia* (Mahadevan *et al.*, 1999) [11]. Germination percentage was found to be the highest in genetic advance as % of mean in *Pongamia Pinnata* (L.) Pierre (Pavithra *et al.*, 2013) [15], *Acacia catechu* (Gupta *et al.*, 2012) [7].

Genetic gain from any tree improvement programmes depends on the type and extent of genetic variability present in the population. The best gains are obtained from those characteristics that are strongly under genetic control and have a wide range of variability (Zobel, 1971) [23]. Heritability provides an index of the relative role of heredity and environment in the phenotypic expression of various traits (Kaushik *et al.*, 2007) [10].

Table 2: Genetic estimates of seed traits of *Azadirachta indica*

Character	Phenotypic variance	Genotypic variance	GCV	PCV	Heritability	Genetic advance (%) of mean
Seed length	1.97	1.89	9.82	9.98	0.97	19.86
Seed width	0.31	0.28	7.92	8.27	0.92	15.61
100 seed weight	14.00	13.91	22.51	22.57	0.99	46.22
Germination %	450.84	449.39	53.12	53.21	0.99	109.24
Azadirachtin %	0.04	0.04	24.89	24.93	0.99	51.23

Equivalent coefficient of variance for both phenotypic and genotypic coupled with high estimates of heritability (broad sense) for all the seed traits revealed the transmissible nature of the variability present. Very high heritability estimates registered by all traits also envisaged the minimal effect of environment on these traits. High heritability coupled with high GCV, PCV and GA as percent of mean were noticed for the traits viz., hundred seed weight, germination percentage and azadirachtin percentage which further suggested the role of additive gene action and the likelihood of the experimental material for future improvement programmes through selection.

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