Indian J. of Agroforestry Vol. 9 No. 2 : 106 - 110 (2007)

Divergence Studies in Plus Trees of Neem (*Azadirachta indica*)

N. Kaushik, S. Nautiyal*, J.C. Kaushik# and R.S. Beniwal#
CCS HAU, Regional Research Station, Bawal-123501

ABSTRACT

The performance of ninty plus trees of *Azadirachta indica* collected from different agroclimatic zones of Haryana was studied at HAU Regional Research Station, Bawal for genetic divergence studies. Data on seed morphology, nursery characters, oil content and fatty acid composition of oils was recorded. Six clusters were obtained based on D^2 statistic. Maximum plus trees (19) were under cluster 1. Maximum intra-cluster distance (125.928) shown by cluster III is an indicator of selection of parents for hybridization within the cluster. The maximum inter cluster distance (244.342) between cluster III and IV followed by cluster I and III (232.247) indicating wider genetic diversity between the trees in these groups and selection of parents from such cluster for hybridization programmes would help to achieve novel hybrids. These studies revealed that the geographical diversity is not the only factor in determining divergence, therefore, selection of genotypes should be based on genetic diversity. The hybridization between the more diverse genotypes of neem can produce genotypes with high heterotic vigor.

Key words: *Azadirachta indica, D^2 statistic, genetic divergence, plus tree*

1. INTRODUCTION

Neem (*Azadirachta indica* A. Juss.), belonging to the family 'Meliaceae' is a well known multipurpose tree of tropics, and one of the most valuable trees of semi-arid and arid region. The exact region of origin of *A. indica* is not known. Some authors suggest that it may lie in parts of southern India, such as Karnataka (Troup, 1921, Vartak and Ghate, 1990). It is cultivated in most parts of India except at high altitudes, in cold regions and damp sites (Sidhu, 1995). In India, neem occurs in the tropical dry deciduous and thorn forests (Anon.,1985). It thrives well in hot weather, where maximum temperature reaches as high as 50°C and tolerates cold up to 0°C, on altitude up to 1500 m (Hegde, 1993). The tree is well known for its drought resistance (Schmutterer, 1995). Neem performs well on a wide range of soil types including poor, shallow, stony or sandy, saline, alkaline and low acidic soils (Rawat, 1995). In its native environment, it is found growing in mixed forests in association with *Acacia* and *Dalbergia* spp. (Benge, 1989). The products of the tree are used to make the best biopesticide, and the timber is useful for various purposes. The ever-increasing emphasis on developing environmentally benign pest-control agents has brought neem, to the fore. Several reports confirming the effectiveness of neem-based products against various insect-pests have appeared in the literature and can be referred to the reviews by Saxena (1989), Schmutterer (1990), Singh (1993), Singh and Kataria (1991) and others.

Knowledge of the distribution of genetic variability, breeding system of trees and of the evolutionary forces that have shaped them, is a pre-requisite for tree improvement (Muona, 1990). Knowledge about the variability is not only important, but it is also essential to know genetic diversity among the available plant material, because the nature and degree of divergence in plus trees (genotypes) is useful for classifying them into groups on the basis of their diversity, particularly when overlapping for one or more characters is frequent. Besides its use in taxonomic problems, it also helps in choosing parents for specific breeding objectives to get desirable transgrassive segregates.

Hence, the current study was carried out to illicit information on the diversity of various plus trees of *Azadirachta* indica collected from different parts of Haryana.

2. MATERIALS AND METHODS

An extensive survey of three agro-climatic zones of Haryana was conducted to select the plus trees having 160-180 cm girth and height ranging from 10-15 m of neem during July and August for two consecutive years 2002 and 2003. The selection was made on phenotypic assessment of characters of economic importance, such as straightness, clear bole height, less crown spread, self pruning ability and free from pest and diseases. A total of 90 (nine provenances from three zones and 10 trees from each provenance) morphologically superior trees were selected and

---

* Division of Physiology, FRI, DehraDun

Department of Forestry, CCSHAU, Hisar-125004

Table 1. Seed collections sites, provenances and agro-climatic zones

Tree No.	Site	P	Z	Tree No.	Site	P	Z	Tree No.	Site	P	Z
T_1	Mayar	HISAR	ARID	T_1	Kauokar	KARNAL	SEMI-ARID	T_1	Yamunanagar	YAMUNANAGAR	SUB-ARID
T_2	Hisar			T_2	Pehowa			T_2	Jagadhri-I		
T_3	Central SF			T_3	Samora			T_3	Jagadhari-II		
T_4	Chikenwas			T_4	Indri			T_4	Radaur		
T_5	Agroha			T_5	Umari			T_5	Bilaspur		
T_6	Bropal			T_6	Kurukshetr			T_6	Sadora		
T_7	Fatehabad			T_7	Dadupur			T_7	Buria		
T_8	Ding			T_8	Karnal			T_8	Damla		
T_9	Jodhkan			T_9	Bustra			T_9	Chachrauli-I		
T_{10}	Bhuna			T_{10}	Girbari			T_{10}	Chachrauli-II		
T_1	Akoda	BHIWANI		T_1	Panipat-I	SONIPAT		T_1	Ismaylabad	AMBALA	
T_2	Ghasola			T_2	Panipat-II			T_2	Barara		
T_3	Ch. Dadri			T_3	Nai Boli			T_3	Ambala-I		
T_4	Bhiwani			T_4	Jhatipur			T_4	Ambala-II		
T_5	Prem Nagar-I			T_5	Teha			T_5	Ambala cantt-I		
T_6	Prem Nagar-II			T_6	Murthal			T_6	Ambala cantt-II		
T_7	Milakpur			T_7	Sonipat-I			T_7	Jatwara		
T_8	Jatu Luhari			T_8	Sonipat-II			T_8	Nariangarh-I		
T_9	Bawanikhera			T_9	Rai			T_9	Nariangarh-II		
T_{10}	Jeeta kheri			T_{10}	Kundali			T_{10}	Maulana		
T_1	Bawal	REWARI		T_1	Sisana	ROHTAK		T_1	Panchkula-I	PANCHKULA	
T_2	Saharanwas			T_2	Kansala			T_2	Panchkula-II		
T_3	Buroli			T_3	Rohtak-I			T_3	Rajpur		
T_4	Dahina			T_4	Rohtak-II			T_4	Barwala		
T_5	Jhajjar			T_5	Kheri			T_5	Pinjore-I		
T_6	Dujana			T_6	Meham			T_6	Pinjore-II		
T_7	Dighal			T_7	Madina			T_7	Morni		
T_8	Kathuwas			T_8	Mokhra			T_8	Chandigarh		
T_9	Bhora kalan			T_9	Kalanaur			T_9	Kalka-I		
T_{10}	Jorikalan			T_{10}	Lahali			T_{10}	Kalka-II		

P- Provenance, Z - Zone

sufficient amount (2.00 kg) of good quality mature fruits were collected from these trees (Table1, Fig.1). The minimum distance between the trees was 2 km. Within 24 hours of collection, fruits were de-pulped and seeds were washed thoroughly and air-dried under shade for 2 - 3 days. Thereafter, seeds were kept in muslin cloth bags and stored at room temperature. The observations on Seed length (mm), Seed breadth (mm), 100 seed weight (g), Shoot length (cm), Root length (cm), Collar diameter (cm), Oil content (%), Oleic acid (%), Palmitic acid (%), Stearic acid (%), Myristic acid (%), Specific gravity (g cm^{-3}), Saponification value [mg of KOH (g of $oil)^{-1}$], Acid value (%) were recorded. The data were statistically analysed and genetic divergence was estimated by Mahalanobis D^2 statistics, the genotypes were grouped on the basis of minimum generalized distances using Tocher's method as described by Rao (1952).

3. RESULTS AND DISCUSSION

Based on the relative magnitude of D^2 values, 90 plus trees were grouped in to six clusters, maximum numbers of 25 trees were accommodated in cluster V, followed by 19 in cluster I, 18 in cluster II, 17 in cluster IV, 5 in cluster III and 6 in cluster VI (Table 2). A critical examination of the clustering pattern indicated that geographical diversity need not necessarily be related to genetic diversity.

The intra and inter cluster distance among the genotypes was of varying magnitude (Table 3). Cluster III exhibited maximum intra cluster distance (125.928) indicated that trees in this cluster were more diverse than the other clusters. Minimum intra cluster distance (69.893) was shown by cluster IV. The maximum inter cluster D^2 value was obtained between cluster III and IV (244.342) followed by clusters III and I. The minimum distance (86.599) between clusters IV and V revealed that the trees belonging to these clusters were relatively closer.

The cluster means for different characters under study revealed considerable genetic differences between the groups (Table 4). Clusters III and VI recorded highest mean value for shoot length and root length, respectively. Whereas cluster V registered the highest mean value for collar diameter, seed breadth and 100-seed weight. Cluster III and I exhibited highest mean value for oil content and seed length, respectively. This indicated that none of the clusters contained trees with all the desirable characters, which could directly selected and utilized in breeding programme.

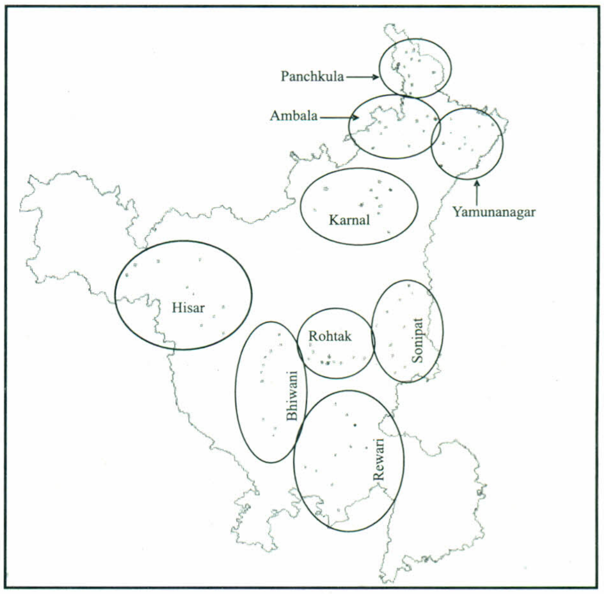

Fig. 1. Seed collection sites

Table 2. Distribution of different trees in clusters based on D^2 statistics

Cluster	Number of trees in the cluster	Tree's site
I	19	Mayar, Saharanwas, Rohtak II, Milkpur, Mokhra, Prem Nagar II, Lahali, Jhatipur, Jagadhari II, Umari, Meham, Girbari, Central SF, Ambala Cantt I, Jorikalan, Kurukshetra, Chachrauli I, Kalka II, Damla
II	18	Bropal, Dighal, Panipat II, Fatehabad, Chandigarh, Jagadhari I, Samora, Kalka I, Kalanaur, Pehowa, Sonipat II, Bilaspur, Bawal, Bhuna, Maulana, Ambala Cantt II, Rajpur, Pinjore I
III	5	Prem Nagar I, Dujana, Sadhora, Pinjore II, Narainagarh I
IV	17	Hisar, Radaur, Ch. Dadri, Jeetakheri, Jodhkan, Jatulohari, Dahina, Karnal, Nai boli, Barara, Kathuwas, Ismaylabad, Sisana, Kauokar, Panchkula I, Madina, Yamunanagar
V	25	Chikenwas, Ding, Jhajjar, Barwala, Chachrauli II, Ambala I, Ghasola, Indri, Bhiwani, Buria, Murthal, Bawani khera, Teha, Panchkula II, Agroha, Buroli, Bustra, Kheri, Bhora kalan, Dadupur, Kundali, Rai, Panipat I, Naraingarh II, Morni
VI	6	Akoda, Kansala, Sonipat I, Ambala II, Rohtak I, Jatwara

Table 3. Average inter and intra cluster distance (D^2 values) among six clusters in neem

Clusters	I	II	III	IV	V	VI
I	74.925	91.354	232.247	136.910	95.081	145.818
II		78.783	215.730	165.456	119.438	158.303
III			125.928	244.342	232.195	168.601
IV				69.893	86.599	126.010
V					66.366	124.080
VI						89.972

The D^2 statistic, which is based on several

Table 4. Cluster mean values for different characters in neem

Clusters Characters	I	II	III	IV	V	VI
Shoot length (cm)	69.254	73.513	73.913	71.271	72.607	70.333
Root length (cm)	32.926	33.459	30.547	29.033	35.571	38.917
Collar diameter (cm)	0.886	0.770	0.799	0.785	0.905	0.893
Seed length (mm)	12.920	12.632	11.807	11.868	12.720	11.148
Seed breadth (mm)	6.913	6.787	6.781	6.528	6.945	6.847
100 seed weight (g)	19.719	20.706	20.047	17.908	20.939	19.750
Oil content (%)	40.170	38.213	45.130	39.935	38.497	43.680
Oleic acid (%)	60.218	65.098	55.893	93.751	51.697	48.156
Palmitic acid (%)	19.250	20.818	20.420	19.337	19.621	21.298
Stearic acid (%)	14.365	16.809	16.127	15.976	15.958	15.772
Myristic acid (%)	0.631	1.926	1.553	0.409	0.364	5.247
Specific gravity (g cm^{-3})	0.879	0.892	0.912	0.905	0.904	0.884
Saponification value [mg of KOH (g of oil)$^{-1}$]	164.298	196.741	222.867	197.275	203.133	209.667
Acid value (%)	15.507	20.172	17.260	24.541	17.499	21.100

characters, is one of the powerful tools to assess the relative contribution of different component traits to the total diversity to quantify the degree of divergence between population to understand the trend of evolution and choose genetically diverse parents for obtaining desirable recombination. The clustering pattern in this study revealed that trees from different provenances (geographic region) were grouped together in a cluster and vice-versa suggested that geographical diversity did not necessarily represent the genetic diversity. It is reported that in *Grewia optiva,* 24 genotypes were grouped into 4 clusters but the grouping of genotypes into clusters was not related to their geographical origin (Sehgal *et al.*, 2003). The inclusion of geographically different plus tree families in the same cluster was attributed by Anand *et al.* (2005) to the fact that the factors other than geographical diversity might be responsible for their genetic uniformity. The trees/provenances that originated in one region had been distributed into different clusters indicated the trees with same geographic origin could have undergone change for different characters under selection.

Maximum intra-cluster distance (125.928) shown by cluster III is an indicator of selection of parents for hybridization within the cluster. The maximum inter cluster distance (244.342) between cluster III and IV followed by cluster I and III (232.247) indicating wider genetic diversity between the trees in these groups and selection of parents from such cluster for hybridization programmes would help to achieve novel hybrids. The present study supports the results obtained by various workers in different species *viz.*, Manga and Sen (2000) in *Prosopis cineraria*, Anand *et al.* (2005) in *Bauhinia variegata*. The minimum inter-cluster distance indicated that trees in these clusters were of close relationship. Hence, selection of parents from these two clusters is to be avoided.

Theoretically speaking, the clusters which are having more inter cluster distance and high mean value would produce divergent trees. Therefore, those trees should be selected which are having high inter cluster distance. Cluster III and cluster IV are having high inter cluster distance. So, the trees belonging to these clusters could be selected as parents for breeding programme.

REFERENCES

Anonymous 1985. *The Wealth of India Raw Materials*, Vol. I (Revised edition). Publications and Information Directorate, CSIR, New Delhi. 504 p.

Anand, R.K., Chauhan, K.C. and Huse, S.K. 2005. Genetic divergence studies in plus tree families of *Bauhinia variegata* Linn. for its growth and biomass traits. *In: Multpurpose Trees in the Tropics: Management and Improvement Strategies* (Eds. Tiwari, V.P and Srivastava, R.L.), AFRI, Jodhpur (India) pp. 744-748.

Benge, M.D. 1989. Cultivation and propagation of neem tree. In: Focus of phytochemical pesticides Vol. I. *The Neem Tree* (Ed. M. Jacobson), CRC Press, Boca Raton, Florida. pp.1-8.

Hedge, N.G. 1993. Neem production and development - constraints at grass root level. *In: Souvenir of World Neem Conf.* held at Bangalore, India, from 24-28,1993. pp.I-7.

Manga, V.K. and Sen David, N. 2000. Genetic diversity among different genotypes of *Prosopis cineraria* Druce. *Indian J. Forestry* 33(3): 291-295.

Muona, O. 1990. Population genetics in forest tree improvement. *In: Plant population genetics, breeding and genetic resources* (Eds. Brown, H. D., Clegg, M.T., Kahler, A.L. and Weir, B.S.). Sinauer Associates Inc., Sunderland, Massachusetts. pp. 282-298.

Rao C.R.1952. *Advanced Statistical Methods in Biometric Research.* John Wiely and Sons Inc., New York.

Rawat, G.S. 1995. Neem (*Azadirachta indica*) - Nature's Drugstore. *Ind. For.* 121: 977-980.

Saxena. R.C. 1993. Scope of neem for developing countries. *In : Souvenir of World Neem Conf.* held at Bangalore, India, from Feb 24-28, 1993, pp. 30-36.

Schmutterer, H.1990. Properties and potential of natural pesticides from the neem tree (*Azadirachta indica*). *Ann. Rev. Entomol.*.35: 271-297.

Schmutterer, H. 1995. The tree and its characteristics. *In: The Neem tree Source of unique natural products for integrated pest management, medicine and other purposes* (Ed.Schmutterer,H.).Weinheim,NY.pp.1-34.

Sehgal, R.N., Rathore, A. and Chauhan, S.K. 2003. Divergence studies in selected genotypes of Grewia opitva. *Indian J. Agroforestry* 5 (1&2): 99-102

Sidhu, D.S. 1995. Neem in agroforestry as a source of plant derived chemicals for pest management. *Ind. For.* 121: 1012-1021.

Singh, R.P. and Kataria, P.K. 1991. Insects, nematodes and fungi evaluated with neem (*Azadirachta indica A. Juss*) in India. *Neem Newsletter* 8(1): 3-10.

Singh, R.P. 1993. Bioactivity against insect-pest. In : Neem research and development (Eds. Randhawa, N.S. and Parmar, B.S.). Publication 3. Society of Pesticide Science. New Delhi. pp. 109-122.

Troup, R.S. 1921. *The silviculture of Indian forest trees.* Claredon Press, Oxford, UK. Vol. 1.

Vartak, V. and Ghate, V.1990. Ethnobotany of neem. *Biol. Ind.* 1: 55-59.