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Geographical Variation in Seed and Seedling Traits of Neem (*Azadirachta indica* A. JUSS.) Among Ten Populations Studied in Growth Chamber

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(Received 17th February 1997)

Abstract

Nineteen biometric characters of seed and seedling of 10 populations of *Azadirachta indica* from Myanmar, Bangladesh, India, Pakistan and the Sudan were studied under growth chamber conditions. The results obtained by the use of principal component analysis (PCA) and cluster analysis of data revealed three distinct groups of populations. Provenances from the high rainfall areas were separated from lower rainfall areas in PCA. Correlation analyses between biometric traits and geoclimatic factors indicated the existence of ecoclinal variation in seed weight, shoot:root ratio, leaf number, and leaflet ratio. Leaflets of the northern provenances were narrower than those of the southern provenances, with a tendency for a separation between the northern (Pakistan, Myanmar and Bangladesh) and southern occurrences (Sudan and India). Provenances having lower shoot:root ratio and lesser number of leaves were separated with their corresponding mean annual rainfall. The results suggest that *A. indica* employs an adaptive strategy in response to water deficit at the initial phase of seedling growth and establishment.

Key words: Adaptation, *Azadirachta indica*, drought, ecoclinal variation, geographical variation, neem.

FDC: 165.52; 232.12; 164; 181.31; 181.525; 232.312.31; 232.318; 176.1 *Azadirachta indica*.

Introduction

Neem (*Azadirachta indica* A. JUSS.) is a multipurpose tree species native to the Indian subcontinent and Myanmar. It has been transferred to many of the warmer parts of the world. Apart from its value as a timber tree, neem has antiseptic, medicinal, insecticidal properties and sociocultural values (RADWANSKI and WICKENS, 1981; KOUL *et al*., 1989; MARAMOROSCH, 1991; MORDUE and BLACKWELL, 1993; AHMED, 1995; BISWAS *et al*., 1995; KETKAR and KETKAR, 1995). The effective exploration, identification, documentation and use of genetic resources of the neem is a prerequisite for its efficient use.

The exact origin of *A. indica* is not known. Some authors suggest it may lie in Myanmar and/or in parts of southern India such as Karnataka (TROUPS, 1921; VARTAK and GHATE, 1990). Nowadays, *A. indica* is widely distributed by introduction, mainly in the drier tropical and subtropical zone of Asia, Africa, the Americas, Australia and the South Pacific islands. The neem tree is well known for its drought resistance (SCHMUTTERER, 1995). The wide ranges of habitats indicate good adaptation to drier conditions. Neem thrives well in low rainfall (130 mm/year) areas. In high rainfall (3,000 mm/year to 4,000 mm/year) areas, it has failed completely (SCHMUTTERER, 1995). Neem performs well on a wide range of soil types. In its native environment, neem is found growing in mixed forests in association with *Acacia* and *Dalbergia spp.* (BENGE, 1989).

Knowledge of the distribution of genetic variability, breeding system of trees and of the evolutionary forces that have shaped them, is a prerequisite for tree improvement (MUONA, 1990). Such information is also needed for planning gene conservation and establishing breeding populations. Provenance testing is done for very practical reasons, to screen the naturally available genetic variation and to allow selection of the best available types for reforestation or further breeding (WRIGHT, 1976). Provenance research also aims at defining the genetic and environmental components of phenotypic variability between trees from different geographic origins (CALLAHAM, 1964). Several studies (HAMRICK *et al*., 1979, 1981; and HAMRICK and GODT, 1989) suggest that the extent of the geographic range correlates closely with the within population genetic variation in tropical tree species.

Variation in seed and seedling traits and its significance for seed source studies have been studied in a number of tree species. Various ecotypes of *A. indica* exhibit variation in several characters (ARORA, 1993). Growth and architectural analysis of neem showed significant variation in shoot elongation, height growth, branching pattern and internode length (BISHT and TOKY, 1993). Significant provenance variation in height growth and survival rates of neem was reported among 39 seed sources from India by RAJAWAT *et al*. (1994). Geographical variation in seed size, oil content and tree growth related characters has also been reported (VEERENDRA, 1995; SURENDRAN *et al.,* 1993; DWIVEDI, 1993). ERMEL (1995) showed distinct ecotypic differences in azadirachtin and oil contents of seed kernels.

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Table 1. – Seed sources of *Azadiracta indica* used in the study.

Provenance code	Country of origin	Provenance	Latitude (Lat)	Longitude (Long)	Altitude (Alt) m	Mean annual rainfall (MAR) mm	Dry season	Date of seed collection	Date of seed received	Number of mother trees
S1	Myanmar	**Yezin**	19°51'N	96°16'E	100	1269.00	Nov-May	12.06.95	10.07.95	25
S2	Myanmar	**Myene**	22°03'N	95°13'E	76	809.00	Nov-April	20.06.95	10.07.95	30
S3	Bangladesh	**Chapai-Nawabganj**	24°41'N	87°54'E	17	1540.00	Oct-Aug	10.07.95	15.07.95	25
S4	Bangladesh	**Nazirhat**	22°38'N	91°44'E	15-25	2750.00	Sept-April	05.07.95	15.07.95	15
S5	India	**Chitradurga** (Karnataka)	14°02'N	76°04'E	615	417.00	–	03.07.95	29.07.95	30
S6	India	**Annur** (Tamil Nadu)	11°17'N	77°07'E	360	875.00	March-June	01.7.95	29.07.95	40
S7	India	**Ghatti** Subramanya (Karnataka)	13°22'N	77°34'E	950	741.00	March-June	08.07.95	29.07.95	16
S8	Pakistan	**Tibbi Laran** (Rahimyar Khan)	28°24'N	70°18'E	115	140.01	April-June Oct-Feb	10.08.95	18.08.95	20
S9	Pakistan	**Multan** (Cantonment area)	31°11'N	71°29'E	<150	276.00	Oct-Feb	07.08.95	18.08.95	25
S10	Sudan	**Geteina**	14°5'N	32° 2'E	380	156.00	Oct-June	10.08.95	15.08.95	15

Provenances are emphasized by bold letters

In view of the widespread use of neem, programs on provenance trials for the evaluation and improvement of genetic resources of the species have been initiated by the International Neem Network, coordinated by the Food and Agricultural Organisation (THOMSEN and SOUVANNAVONG, 1994). Twenty seven provenances from 11 countries in Asia and Africa are included in this program (MISHRA, 1995). This study attempts to gain a better understanding on how geoclimatic factors prevailing at different sites affect morphological differences between the populations during the seed and seedling stages. Thus, the present study aims at providing basic knowledge regarding the intraspecific variation which may be useful for tree improvement.

Materials and Methods

Genetic material

We received 200 g to 300 g of open pollinated bulked seeds of each of 10 provenances from Myanmar (Yezin S1 and Myene S2), Bangladesh (Chapai-Nawabganj S3 and Nazirhat S4), India (Chitradurga S5, Annur S6 and Ghati S7), Pakistan (Tibbi Laran S8 and Multan S9) and Sudan (Geteina S10) (*Table 1, Figure 1*).

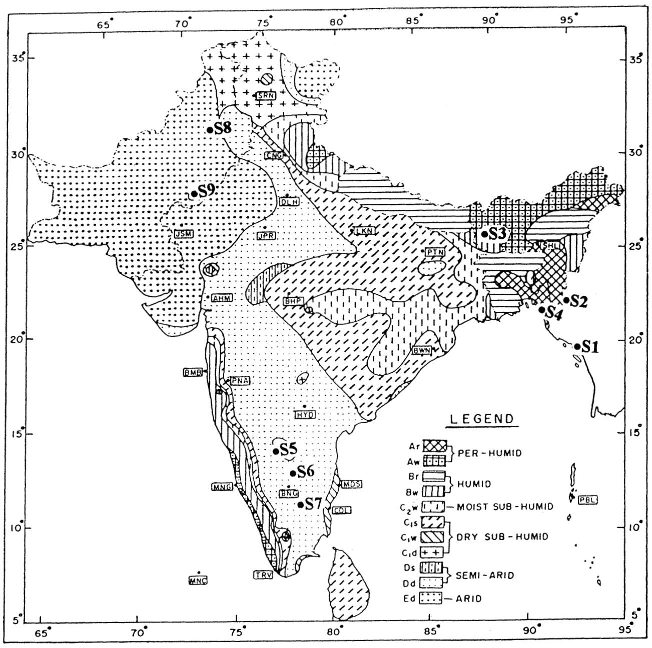

Figure 1. – Climatic classification (RAO, 1981) and geographical locations of 9 provenances (provenance S10 from the Sudan is not shown on the map). • represents the localities of provenances.

Seed measurements

For determining the seed weight of 100 seeds, 300 seeds were taken at random and divided into 3 replications (100 + 100 + 100 = 300) (SNIEZKO and STEWART, 1989). Seed weight was recorded as soon as the seeds were received. To measure the seed size, a random sample of 100 undamaged seeds from each source was divided into three replications (30 + 30 + 40 = 100) and length (mm) and diameter (mm) of the seeds were measured with a slide caliper.

Seed germination

The seed coat or endocarp (hard shell) of each seed was removed by hand and the shelled seeds were placed in sterilized moistened sand and germinated in a germination chamber at 30 °C to 32 °C. After 7 days from sowing, the germinated seeds were transferred into perforated plastic pots (10 x 10 x 7 cm^3) containing a mixture of peat, sand and vermiculite (4 : 2 : 1) and placed in the greenhouse. The day/night temperatures and relative humidity were maintained at 22 ± 3 °C/18 ± 3 °C and 80% to 75%, respectively. The photoperiod was maintained at 12/12 hours daily dark/light period and photosynthetic light intensity was maintained at 500 to 700 µmol m^{-2} s^{-1} on the seedling canopy until October 31, 1995 (5 to 7 weeks). The seedlings were watered twice a week with 200 ml water per seedling.

Cotyledon measurements

The cotyledons were collected 3 weeks after transplanting and preserved at -20 °C. After cotyledons had been collected from all 10 provenances, samples of 100 cotyledons (in 3 replicates) were drawn at random from each provenance and measured for their maximum length, thickness and width in millimeters.

Seedling growth experiment

On November 1, 1995, 10 healthy seedlings of uniform height were chosen from each provenance and transferred to larger plastic containers (diameter 19 cm and volume 4250.78 cm^3) with the above mentioned rooting mixture. Seedlings were then placed in a growth chamber. A total of 100 seedlings were divided into 3 blocks in a randomized complete block design (30 + 30 + 40 = 100). The seedlings were watered twice a week with 300 ml of water. A balanced Kemira fertilizer (N 28%, P 5%, K 21%, S 1.8%, Mg 1.4% and trace elements) was applied twice a month. In the first month 20 mg, in the second and third months 30 mg and for the rest of the growing period 60 mg of fertilizer was applied in solution per seedling. The day/night temperatures and relative humidity were maintained at 30 ± 3 °C/20 ± 3 °C, and 80% to 75%, respectively. The photoperiod was maintained at 12/12 hours daily dark/light period and photosynthetic light intensity was maintained at 800 to 1000 µmol m^{-2} s^{-1} on the seedling canopy. The seedlings were harvested on May 25, 1996 after growing for 27 weeks.

Measurement of growth traits of seedling

After removal of the rooting media (by washing roots with water) seedlings were cut at root collar level beneath the cotyledon scars and their entire length was measured up to shoot tip as seedling height in centimeters. Collar diameter was measured at the point of cotyledon scars in millimeters. The distance between the cotyledon scars and lowest true leaf was measured in centimeters. Free water adhering to the root systems was blotted with blotting paper and total fresh weight recorded. The volumes of shoots and of roots were measured separately by the water displacement method as described by BURDETT (1978). The volume ratio of shoot:root was calculated. Oven dry weight was determined by drying seedlings in the oven at 80 ± 1 °C for 48 hours. The total number of leaves per seedling was recorded. Total leaf area was measured with the LI-COR Model LI-3000 portable area meter. Leaf length was measured on 10 consecutive fully mature leaves (*Figure 2*) and mean values were used for analyses of data. Internode length over 10 consecutive leaves was measured in centimeters. To determine the leaflet ratio, width and length of 5 leaflets selected from a fully expanded mature leaf (excluding terminal leaflet) were measured as shown in *figure 2*. Leaflet areas of the same 5 leaflets were measured with the above mentioned area meter. The means were used for statistical analysis. The method of PINYOPUSARERK *et al.* (1993) for assessment of leaf traits was followed.

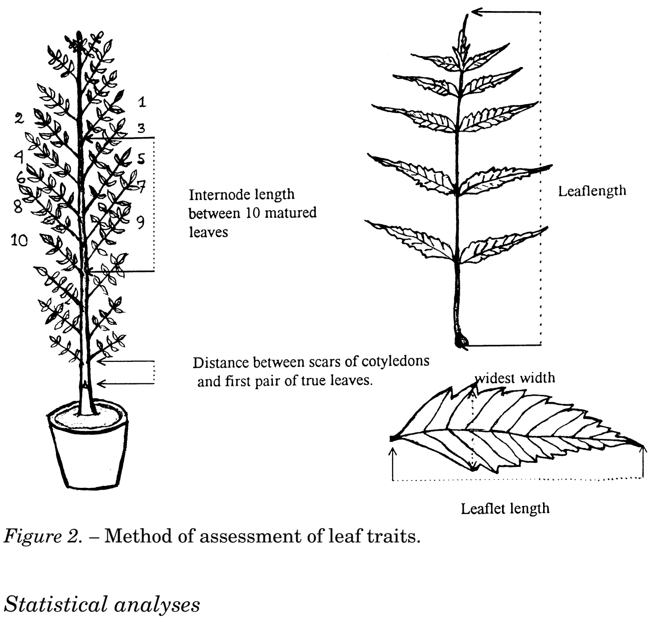

Figure 2. – Method of assessment of leaf traits.

Statistical analyses

Data were analyzed on estimates of simple statistical parameters, analysis of variance (ANOVA), variance components, correlation and principal component analysis (PCA). The multiple comparison procedure involving TUKEY's Honestly Significant Difference (HSD) test (P = 0.05) was used to compare the provenance means. Regression analyses were conducted to investigate relationships between biometric traits on the one hand, and geoclimatic data on the other. These analyses were carried out by applying the Statistical Analysis Systems (SAS) (1989) software package version 6.08.

To investigate distance ('similarity/dissimilarity') relationships among populations, a cluster analysis was carried out by applying SYSTAT Inc. (1990) software package, which applies the 'PEARSON distance' and 'Single linkage' methods. Two-way ANOVA was used for individual measurements, while provenance means were used for correlation, regression, PCA and cluster analyses.

Results

The statistical parameters (mean, standard error of the mean (SE) and coefficient of variation (CV)) for 19 measured traits along with the statistical significance at the 5% level of probability are presented in *table 3*. Seed length varied from 15.27 mm to 11.71 mm (S3 and S9). Seed diameter varied from 7.47 mm to 6.55 mm (S3 and S6). Seed weight varied from 28.35 g to 11.41 g (S3 and S2). For most of the growth related traits, S1, S3, S5 and S7 showed superior, S9, S4 and S2 medium and S8, S10 and S6 poor performance. Provenances S1, S3, S5 were significantly (P = 0.05) different from S6, S10 in height growth (*Table 3*). Cumulative height growth curves of 10 provenances are illustrated in *figure 4*. The variation in leaf size of Indian and Bangladeshi provenances is illustrated in *figure 3a*. Higher shoot: root ratios were observed in S4 and S3 (8.42 – 8.16) and lower ratios in S6 and S10 (5.41 – 4.13). Shoot: root ratios of S4 and S3 were significantly different from that of the relatively low shoot:root ratio of S10. The variation in root system among 10 provenances is illustrated in *figure 3b*. Leaflet ratio varied from 0.41 (S10) to 0.32 (S9) which values are significantly different. There were very highly significant (P < 0.0001) differences among the provenances for all measured traits. In contrast, differences between individuals within the provenances were generally very small. For seedling traits, the leaf length showed the highest (41.95%) relative

Table 2. – Measured and calculated morphometric characters of seeds, cotyledons and seedlings of *A. indica*.

A. Seed characters:
1. Seed length (SL)
2. Seed diameter (SD)
3. Seed weight of 100 seeds (SW)

B. Cotyledon characters:
4. Cotyledon length (CL)
5. Cotyledon width (CW)
6. Cotyledon thickness (CT)

C. Growth related characters of seedlings:
7. Seedling height (HT)
8. Collar diameter (CD)
9. Distance between cotyledons and first true leaf (DCL)
10. Fresh weight of seedling (FW)
11. Oven dry weight of seedling (DW)
12. Volume of seedling (Vol.)
13. Shoot:root ratio (St:Rt)

D. Leaf related characters:
14: Total number of leaves per seedling (TL)
15. Total leaf area per seedling (TLA)
16. Leaf length (LL) *(Figure 2)*
17. Internode length between ten mature leaves (INL) *(Figure 2)*
18. Leaflet ratio = width of leaflet / length of leaflet (LtR) *(Figure 2)*
19. Leaflet area (LtA)

variance component and shoot:root ratio the lowest (16.58%). All relative variance components of the seedling traits may be attributed to genetic variation.

Multivariate analysis

Correlation coefficients (*r*) among all possible combinations of variables both the biometric traits and geoclimatic data are summarized in the *tables 4a* and *4b*, respectively. Correlations between the measured traits and geoclimatic data are of special interest (*Table 4b*). Shoot:root ratio (St:Rt) and total number of leaves (TL) were highly significantly ($P < 0.01$) correlated with the mean annual rainfall. Positive significant ($P < 0.05$) correlations were found between seed length (SL), shoot:root ratio and total number of leaves on the one hand,

Table 3. – Statistical parameters (Stat. param.), mean, standard error of the mean (SE) and coefficient of variation (CV), of the seed and seedling traits (variables) studied for 10 provenances (S1 to S10) of *Azadirachta indica*.

Traits	Stat. param.	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Seed											
SL (mm)	MEAN	13.63^{bcd}	13.90^{bc}	15.27^{a}	13.96^{bc}	14.53^{ab}	12.08^{e}	12.85^{ecd}	12.72^{cd}	12.61^{cd}	11.71^{e}
	SE	0.213	0.147	0.167	0.191	0.310	0.369	0.306	0.260	0.279	0.260
	CV	15.595	10.548	10.936	13.584	21.348	30.507	23.812	20.460	22.150	22.174
SD (mm)	MEAN	7.01^{abc}	7.088^{ab}	7.471^{a}	7.176^{ab}	7.235^{ab}	6.553^{c}	6.996^{abc}	6.968^{bc}	7.005^{abc}	7.394^{ab}
	SE	0.105	0.072	0.095	0.130	0.097	0.133	0.131	0.124	0.090	0.101
	CV	14.913	10.548	12.704	18.165	13.455	20.255	18.569	17.781	12.910	13.647
SW (g)	MEAN	16.457^{cd}	11.407^{d}	28.350^{a}	27.953^{ab}	22.663^{abc}	21.770^{c}	17.167^{cd}	17.293^{cd}	17.293^{cd}	17.837^{c}
	SE	0.726	0.429	1.392	1.443	1.476	1.965	1.059	0.776	0.776	1.453
	CV	7.643	6.511	8.504	8.939	11.281	15.631	10.683	7.768	7.768	14.111
Cotyledon											
CL (mm)	MEAN	12.723^{c}	13.203^{bc}	14.115^{a}	13.509^{ab}	13.566^{ab}	13.139^{bc}	13.047^{bc}	12.568^{c}	13.196^{bc}	12.721^{c}
	SE	0.201	0.137	0.146	0.193	0.181	0.119	0.124	0.168	0.130	0.212
	CV	15.759	10.409	10.319	14.311	13.372	9.065	9.477	13.348	9.859	16.691
CW (mm)	MEAN	5.888^{c}	5.919^{c}	6.941^{ab}	6.691^{ab}	6.396^{bc}	7.057^{a}	7.120^{a}	6.680^{ab}	6.931^{ab}	6.386^{bc}
	SE	0.119	0.111	0.165	0.126	0.138	0.107	0.111	0.133	0.158	0.117
	CV	20.185	10.409	10.319	14.311	13.372	9.065	9.477	13.348	9.859	18.252
CT (mm)	MEAN	3.438^{bc}	3.654^{b}	3.400^{bc}	4.358^{a}	4.124^{a}	3.304^{c}	3.262^{c}	3.410^{bc}	3.654^{bc}	3.554^{bc}
	SE	0.054	0.068	0.071	0.094	0.078	0.058	0.059	0.070	0.050	0.082
	CV	15.822	18.636	20.804	21.609	18.980	17.662	18.213	20.504	14.953	22.998
Seedling											
HT (cm)	MEAN	104.30^{a}	85.70^{ab}	102.30^{a}	85.30^{ab}	100.95^{a}	64.10^{b}	96.10^{ab}	75.80^{ab}	89.40^{ab}	75.80^{b}
	SE	6.987	8.139	6.549	7.976	9.107	6.157	5.299	5.944	6.441	7.285
	CV	21.185	30.033	20.244	29.571	28.528	32.153	17.436	24.797	22.785	35.118
CD (mm)	MEAN	7.135^{a}	5.765^{bcd}	6.885^{ab}	5.94^{abcd}	6.375^{abc}	4.587^{d}	6.19^{abc}	5.70^{bcd}	6.33^{abc}	5.41^{cd}
	SE	0.225	0.414	0.403	0.392	0.308	0.185	0.228	0.227	0.339	0.235
	CV	9.988	22.716	18.509	20.883	15.299	12.728	11.667	12.570	16.950	13.738
DCL (cm)	MEAN	2.89^{a}	2.60^{ab}	1.96^{bc}	1.71^{c}	2.88^{a}	2.27^{abc}	2.48^{abc}	2.37^{abc}	2.66^{ab}	2.27^{abc}
	SE	0.264	0.148	0.119	0.224	0.124	0.178	0.185	0.213	0.163	0.139
	CV	28.925	18.040	19.271	41.484	13.577	24.847	23.573	28.413	19.431	19.376
FW (g)	MEAN	62.625^{a}	37.442^{abcd}	60.40^{ab}	39.76^{abcd}	52.139^{abc}	20.192^{d}	46.48^{abc}	36.26^{bcd}	44.59^{abcd}	28.08^{cd}
	SE	5.401	5.986	5.799	6.012	9.725	2.429	3.604	4.518	4.690	3.648
	CV	27.274	50.553	30.362	47.818	58.983	38.037	24.523	39.400	33.260	41.084
DW (g)	MEAN	16.527^{a}	9.723^{abc}	16.397^{a}	10.597^{abc}	13.26^{ab}	5.248^{c}	11.85^{abc}	8.96^{bc}	11.71^{abc}	6.97^{bc}
	SE	1.461	1.185	1.865	1.674	2.286	0.604	0.985	1.261	1.414	1.015
	CV	27.96	59.019	35.972	49.965	54.511	36.399	26.287	44.452	38.222	46.051
Vol (cc)	MEAN	63.52^{a}	41.20^{abc}	61.43^{a}	40.496^{abc}	46.769^{ab}	18.50^{c}	45.93^{ab}	33.11^{bc}	43.44^{abc}	25.77^{bc}
	SE	6.172	6.922	5.961	6.932	7.695	2.395	4.749	5.425	5.540	4.033
	CV	30.728	53.129	30.680	54.134	52.030	40.931	32.711	51.812	40.336	49.497
St/Rt	MEAN	7.125^{ab}	5.48^{ab}	8.163^{a}	8.42^{a}	7.02^{ab}	5.41^{ab}	7.08^{ab}	5.58^{ab}	5.54^{ab}	4.13^{b}
	SE	1.985	0.511	0.654	0.481	1.011	0.507	0.312	0.364	0.339	0.303
	CV	88.114	29.485	25.341	18.021	45.523	29.611	13.948	20.635	19.367	23.224
Leaf											
TL	MEAN	40.90^{a}	232.40^{abc}	41.60^{a}	40.60^{ab}	32.30^{abc}	25.70^{c}	33.70^{abc}	29.70^{bc}	27.20^{c}	25.80^{c}
	SE	2.972	2.296	2.857	2.495	3.664	1.521	1.585	2.305	0.998	1.965
	CV	22.978	22.408	21.715	19.436	35.871	18.710	14.873	24.540	11.600	24.088
TLA (sq cm)	MEAN	2421.90^{a}	1493.00^{bc}	2421.90^{a}	1745.90^{ab}	1816.90^{ab}	846.30^{c}	1682.30^{abc}	1525.30^{bc}	1707.30^{ab}	1086.80^{bc}
	SE	200.117	197.474	205.394	222.613	267.824	101.172	151.274	176.241	138.445	136.074
	CV	26.145	41.803	26.819	40.321	46.615	37.805	28.435	36.538	25.644	39.593
LL (cm)	MEAN	22.908^{a}	19.626^{abc}	23.505^{a}	19.85^{abc}	22.756^{a}	16.148^{c}	22.517^{a}	20.291^{ab}	23.123^{a}	18.471^{bc}
	SE	0.919	0.478	0.673	0.882	1.419	0.917	0.655	0.775	0.712	0.666
	CV	12.689	7.711	9.059	14.057	19.721	17.967	9.205	12.071	9.734	11.405
INL (cm)	MEAN	27.68^{ab}	26.14^{abc}	23.17^{abc}	20.65^{bc}	28.89^{a}	24.30^{abc}	25.96^{abc}	23.53^{abc}	29.47^{a}	20.07^{c}
	SE	1.146	2.007	1.582	1.197	2.225	1.704	1.873	1.106	1.720	1.864
	CV	13.097	24.282	21.596	18.327	24.363	22.1176	22.818	14.859	18.460	29.373
LtR	MEAN	0.359^{abcd}	0.391^{ab}	0.341^{bcd}	0.348^{bcd}	0.38^{abc}	0.376^{abc}	0.375^{abc}	0.334^{cd}	0.315^{d}	0.406^{a}
	SE	0.011	0.018	0.013	0.006	0.013	0.011	0.012	0.012	0.008	0.146
	CV	9.742	14.403	12.103	5.089	10.745	9.284	10.299	10.943	8.328	11.398
LtA (sq cm)	MEAN	12.081^{a}	9.807^{ab}	8.997^{ab}	8.292^{b}	9.167^{ab}	7.821^{b}	9.754^{ab}	10.756^{ab}	12.128^{a}	10.398^{ab}
	SE	0.934	0.703	0.665	0.924	0.752	0.858	0.770	0.803	0.578	0.408
	CV	24.472	22.673	23.382	35.258	25.971	34.699	24.971	23.617	15.073	12.408

Traits means with the same superscript letters are not significantly different at $P = 0.05$ (Tukey's HSD test).

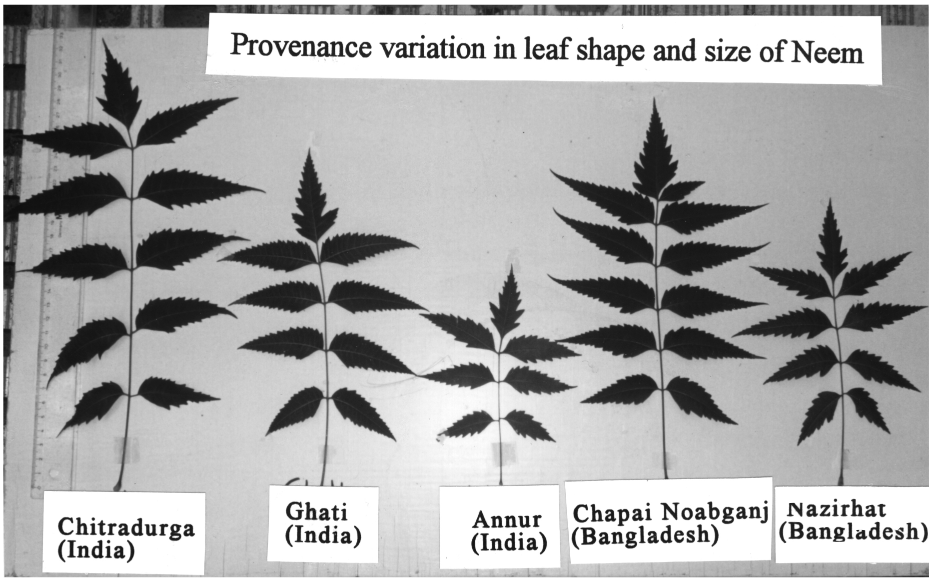

Figure 3a. – Variation in leaf size in Bangladesh and Indian provenances of *A. indica.* Mean leaf lengths of provenances S5 and S6 are significantly (P = 0.05) different from each other (*Table 2*).

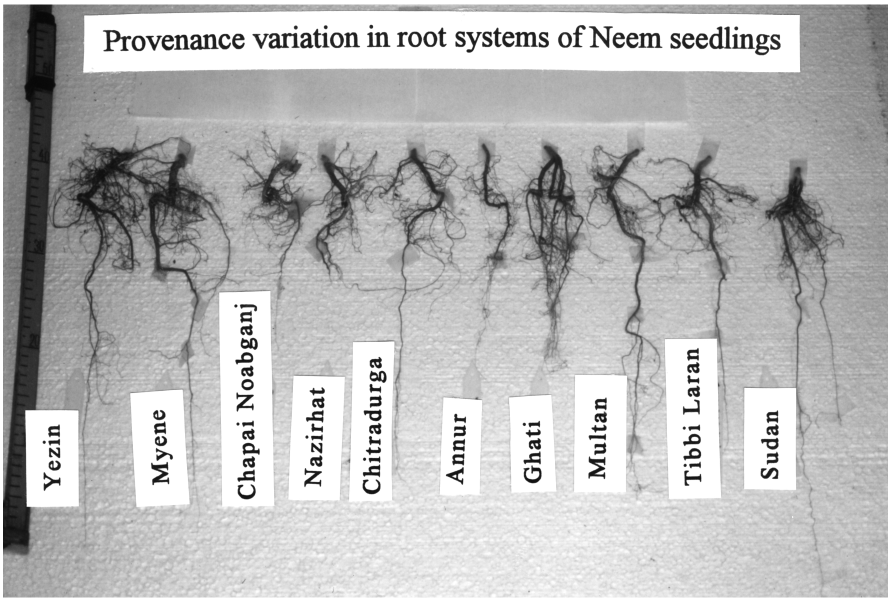

Figure 3b. – Variation in root systems of *A. indcia* among 10 provenances grown in a growth chamber. The lowest mean of shoot:root ratio of the Sudan provenance (S10) is significantly (P = 0.05) different (*Table 2*) from the provenances of Chapai-Nawabganj (S3) and Nazirhat (S4), respectively. Shoot:root ratio and MAR are highly significantly correlated (P < 0.0073) indicating adaptation to arid conditions.

and respective longitude of origin of the provenances on the other. A negative significant ($P < 0.05$) correlation was also observed between leaflet ratio (LtR) and respective latitude of origin. There is a suggestion of a separation between the northern and southern populations, those from the north had narrower leaflets than those from the south. Results of regression analyses and relationship of important traits are shown in *figures 6*, *7* and *8*. Generally, provenances from low rainfall areas showed comparatively low height growth and small leaf area, except S5.

Principal component analysis

The first 3 eigenvectors extracted in the PCA are presented in *table 5*. In the first, second and third principal components, the largest absolute loading values were MAR (0.931704), TLA (0.937487) and Alt (0.984495), respectively. The first principal component accounted for 72% of the variation and the second for 20%. A plot of the scores of the first and second principal components is illustrated in *figure 5*. The figure shows 3 distinct groups of provenances. Provenance S4 falls in group 1, provenances S1, S3 form a second group and provenances S2, S5, S6, S7, S8, S9 and S10 make up a third group. MAR was considered as the most important variate in the first principal component and the TLA in the second.

Cluster analysis

The phenogram resulting from the distance matrix calculated on the basis of morphometric data is illustrated in

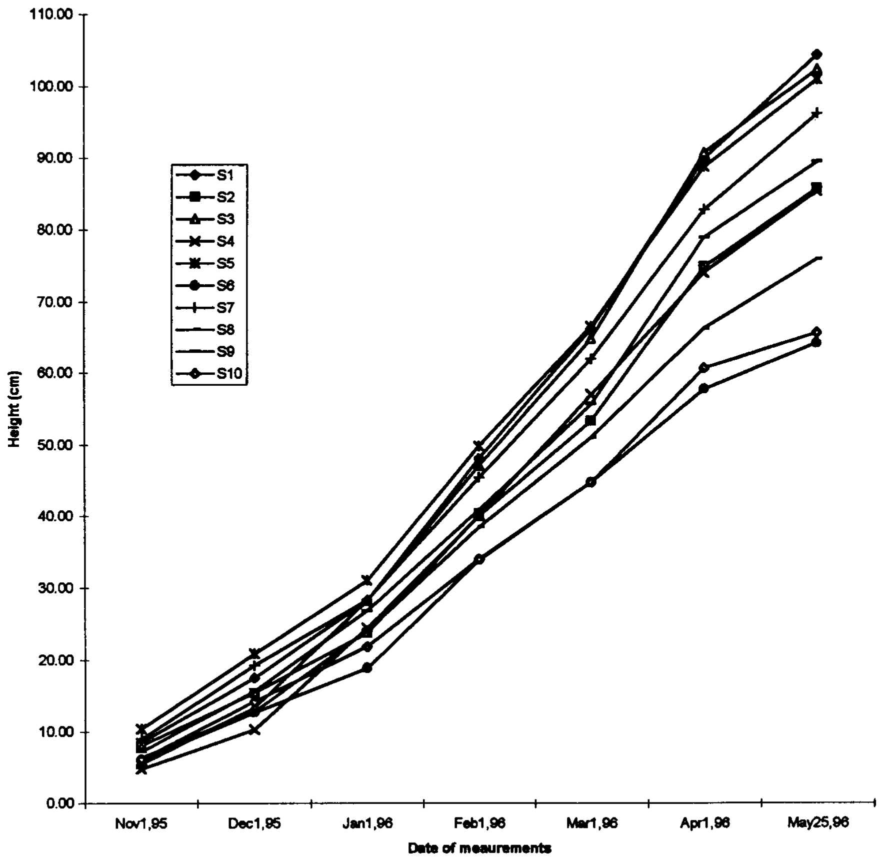

Figure 4. – Differences in seedling height growth of 10 different seed sources (S1 to S10) of *Azadirachta indica* grown in a growth chamber.

Table 4a. – Correlation coefficients (*r*) among the seed and seedling traits (provenance mean basis) of *Azadirachta indica*.

Traits	SL	SD	SW	CL	CW	CT	HT	CD	DCL	FW	DW	VOL	St/Rt	TL	TLA	LL	INL	LtR
SD	0.51																	
SW	0.46	0.31																
CL	0.76**	0.42	0.71*															
CW	-0.20	-0.22	0.47	0.29														
CT	0.44	0.36	0.43	0.35	-0.29													
HT	0.77**	0.37	0.15	0.45	-0.18	0.13												
CD	0.66*	0.51	0.12	0.30	-0.26	0.06	0.93***											
DCL	-0.08	-0.22	-0.67*	-0.38	-0.46	-0.24	0.34	0.29										
FW	0.73**	0.46	0.18	0.38	-0.22	0.06	0.96***	0.98***	0.29									
DW	0.75**	0.46	0.22	0.42	-0.20	0.06	0.96***	0.98***	0.24	0.99***								
VOL	0.75**	0.45	0.14	0.39	-0.26	0.04	0.95***	0.97***	0.24	0.99***	0.99***							
St/Rt	0.78**	0.25	0.67*	0.64*	0.16	0.41	0.71*	0.61	-0.31	0.66*	0.69*	0.67*						
TL	0.79**	0.41	0.45	0.47	-0.21	0.33	0.73**	0.73**	-0.24	0.76**	0.78**	0.81**	0.88**					
TLA	0.76**	0.47	0.26	0.40	-0.21	0.09	0.91**	0.97***	0.13	0.97***	0.98***	0.98***	0.72**	0.85**				
LL	0.58	0.46	0.08	0.33	-0.02	-0.02	0.91**	0.93**	0.34	0.92**	0.90**	0.88***	0.52	0.53	0.86**			
INL	-0.25	-0.29	-0.39	0.02	-0.14	-0.19	0.56	0.42	0.84**	0.45	0.42	0.41	0.03	-0.06	0.31	0.55		
LtR	-0.20	0.08	-0.29	-0.19	-0.40	0.12	-0.29	-038	0.15	-0.35	-0.37	-0.33	-0.36	-0.25	-0.44	-0.47	-0.23	
LtA	-0.23	0.06	-0.60	-0.54	-0.36	-0.38	0.25	0.48	0.61	0.36	0.33	0.35	-0.32	-0.08	0.33	0.48	0.45	-0.32

* Significant, ** highly significant, and *** very highly significant.

Table 4b. – Simple correlation (*r*) between characters studied (provenance mean basis) and geoclimatic data of 10 provenances of *Azadirachta indica*.

Traits	SL	SD	SW	CL	CW	CT	HT	CD	DCL	FW	DW	VOL	St/Rt	TL	TLA	LL	INL	LtR	LtA
Rainfall	0.51	0.14	0.62	0.50	0.03	0.51	0.27	0.23	-0.62	0.25	0.30	0.33	0.78**	0.77**	0.39	0.03	-0.36	-0.18	-0.45
Altitude	-0.27	-0.15	-0.15	-0.13	0.32	-0.14	0.05	-0.14	0.32	-0.09	-0.13	-0.17	-0.09	-0.31	-0.27	0.06	0.22	0.45	-0.15
Longitude	0.67*	-0.20	0.12	0.40	-0.18	0.18	0.59	0.43	0.04	0.50	0.53	0.58	0.69*	0.70*	0.57	0.31	0.35	-0.32	-0.14
Latitude	0.22	0.17	-0.03	0.07	-0.00	-0.05	0.20	0.37	-0.10	0.28	0.29	0.31	0.09	0.19	0.40	0.40	0.13	-0.82**	0.40

* Significant, ** highly significant.

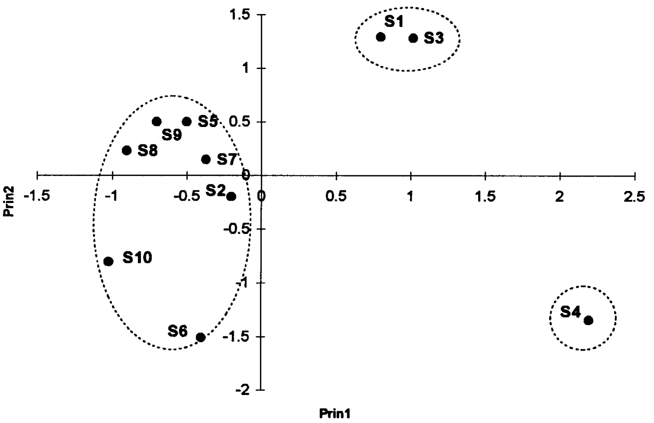

Figure 5. – Relationship between first and second principal components showing the distribution of provenances on the basis of PCA.

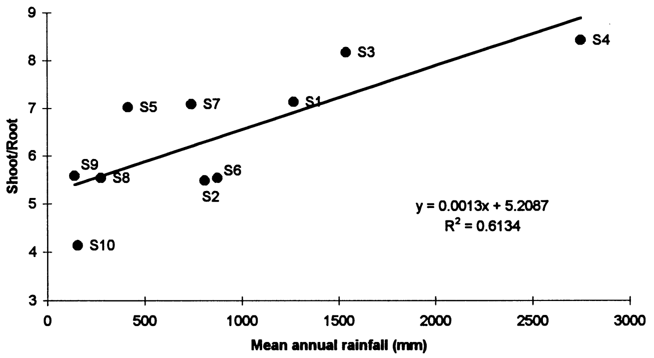

Figure 6. – Relationship between mean annual rainfall and shoot:root ratio of *A. indica* seedlings of 10 provenances.

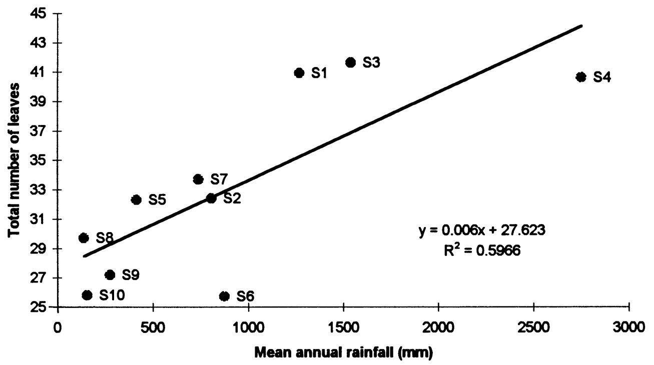

Figure 7. – Relationship between mean annual rainfall and number of leaves (provenance mean basis) of 10 provenances of *A. indica*. The correlation coefficient '*r*' is highly significant ($P < 0.0084$).

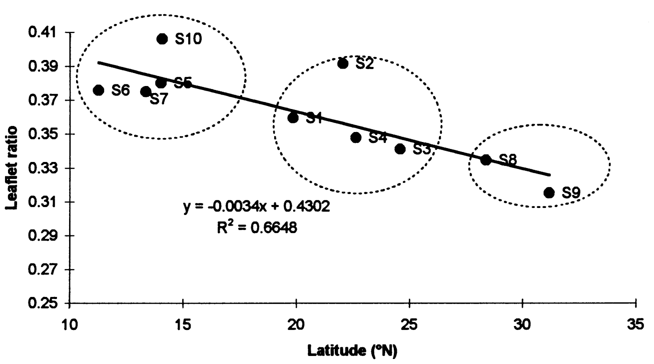

Figure 8. – Regression showing relationship between latitude of origin and leaflet ratio of 10 provenances of *A. indica*. The value of '*r*' is highly significant ($P < 0.004$).

Table 5. – Eigenvectors extracted from the data by principal component analysis.

Variables	PRIN1	PRIN2	PRIN3
SL	0.000817	0.001286	0.000238
SD	0.000069	0.000243	-.000012
SW	0.003872	-.001233	0.002537
CL	0.000289	0.000127	0.000223
CW	-.000020	-.000257	0.000514
CT	0.000209	-.000177	0.000076
HT	0.007157	0.026417	0.018317
CD	0.000342	0.001480	0.000349
DCL	-.000242	0.000427	0.000189
FW	0.006522	0.026793	0.009042
DW	0.002008	0.007158	0.002301
VOl	0.008162	0.026801	0.007484
St:Rt	0.001354	0.000887	0.001761
TL	0.006355	0.006101	0.002887
TLA	0.324633	**0.937487**	0.113365
LL	0.000533	0.004929	0.002217
INL	-.001036	0.003954	0.001989
LtR	-.000009	-.000024	0.000034
LtA	-.000565	0.002065	-.001435
Lat	0.001355	0.006395	-.016085
Long	0.014807	0.010098	-.000090
Alt	-.161542	-.063842	**0.984495**
MAR	**0.931704**	-.338564	0.130906

figure 9. The provenances were separated into 3 major groups. Provenances S1 and S3 fall into the first group, S2, S4, S5, S7, S8 and S9 are closed together in the second, while S6 and S10 form a third group. Provenances S4 and S5 are relatively remote from the rests in the second group.

Discussion

In the present study of *A. indica*, significant amounts of provenance variation were found in a wide array of traits. Strong correlation between the morphometric traits and geoclimatic data shows that there is probably a cline in distribution of neem populations. Seed weight seedling height, volume, leaf area, leaflet ratio, number of leaves, and shoot:root ratio provided good discriminating characters for comparing provenances. Variation between populations larger than within populations in this study suggests a good opportunity for selection and breeding among neem populations.

Strong correlations between number of leaves, shoot:root ratio with MAR indicate adaptation to water availability. Genetic differentiation of drought tolerance has been found within tree species with extensive geographic or habitat range (BONGARTEN *et al.*, 1986; ABRAMS *et al.*, 1990; PARKER and PALLARDY, 1991). Since all life requires water and since water is unevenly distributed, its abundance or scarcity in an environment is reflected in striking vegetational characteristics (BILLINGS, 1964). According to DAUBENMIRE (1947), a difference in rainfall, either in annual or total or in the seasonal distribution, was almost invariably accompanied by differences in natural plant populations. Plants display a wide range of mechanisms of adaptation to water deficits (TURNER, 1986); reduction in leaf area, increased root density and depth are important adaptive features. The results of the present study supports Turner's investigation. A lower shoot:root ratio may indicate drought tolerance in a provenance, as is also suggested by EKANAYAKE *et al.* (1985), and PULKKINEN and TIGERSTEDT (1991).

The northern populations are distinct from the southern populations by having narrower leaflets. Other features which

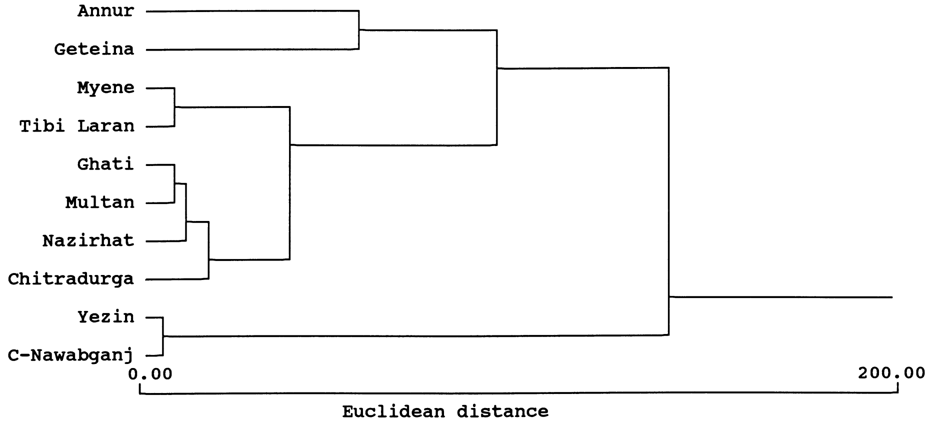

Figure 9. – Phenogram of provenance groups based on morphometric data showing relationship among 10 provenances of *A. indica.* Distance metric is Euclidean distance (PEARSON) single linkage method (nearest neighbour) [1]Chapai-Nawabganj.

differentiated the provenances from the north to the south were leaf area, leaf length and leaflet area. Climatic stress, perhaps the low MAR at the western range of the species suggest that the narrow leaflets are an adaptation to this area. The climatic classification described by RAO *et al.* (1971) showed the extent of aridity of these geographic regions. RAO's results depicted the classical determinant of weather patterns in these latitudes, in particular a gradient of increasing aridity from the east to the west. The correlation between seed size or seedling morphology with the corresponding MAR and latitude would suggest a selective environmental pressure through increasing aridity (BURLEY *et al.*, 1971).

The results of PCA distinguished 3 distinct groups of populations. PCA not only provides a visual representation of the relationship such as the one discussed above, but also indicates which characters are the most important in defining that relationship (RADFORD, 1986). Morphometric traits and geoclimatic data were used by LOPEZ-MATA (1987) and CHANG-GENG (1987) in the study of genecological differentiation among provenances. In the present study, mean annual rainfall (MAR) and leaf area (TLA) were the most important characters considered in PCA which defined drought as an adaptive feature in differentiation of neem populations. Several studies on other species (IBRAHIM, 1996; DORAN *et al.*, 1995; PINYO-PUSARERK *et al*, 1993; and MORISHIMA and OKA, 1981) support the results of the present study.

Cluster analysis of variation for morphological traits revealed the existence of three distinct major groups. The former group represents provenances from relatively low and the latter high rainfall areas. Morphological traits were used to correlate the genetic studies of VERGA (1995) and KLEINSCHMIT *et al.* (1995) to interpret the genetic relationship of different populations. In the present study, the morphometric traits showed distinct relationship among the 10 different populations.

Since the environmental deviations are negligible for the experiments conducted under controlled conditions the observed phenotypic values may be good indicators of genetic distance. The correlation of morphological characters of seed and seedling with latitude found in the present study would suggest the existence of a selective environmental pressure through increasing aridity. However, the eastern part of the natural distribution zone of neem seems to have more moist conditions than the western parts. A marker gene analysis of genetic variability and field evaluation may give greater insight into the success of improvement of *A. indica*.

Acknowledgment

The authors are grateful to the respective authorities who provided the seeds. Thanks are due to Mr. PETER JOY, for his technical assistance. Mr. KARI TUOMELA made his valuable comments.

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Vegetative Propagation of *Cupressus sempervirens* L. of Cretan Origin by Softwood Stem Cuttings[1]

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(Received 21st February 1997)

Abstract

One winter and 2 spring experiments were conducted testing the influence of genotype, auxin treatment, duration of the rooting period, time of cuttings collection and preliminary hedging on the rooting percentage and root quality of *Cupressus sempervirens* softwood stem cuttings. In total, 15 hedged and 7 nonhedged 4-year-old seedlings from 7 Cretan provenances of the species were used as cutting donors. The duration of the rooting period (3 months vs. 4 months) significantly affected the rooting and callus formation percentages. One month extension of the rooting period resulted in a 41.73% increase of rooted cuttings. The genotype was a major factor influencing the percentage of rooting, number of roots and length of the main root. Neither the treatment application, nor the time extension were able to offset the variation among the genotypes. The auxin treatment enhanced rooting and its positive effect was more pronounced during shorter rooting periods. However, the optimal IBA concentration for rooting differed among the genotypes and treatment x rooting duration interaction was present. The comparative results between the effect of the season and the preliminary rejuvenation showed that the cuttings obtained from slowly growing shoots (either from the lower part of nonhedged donors or those collected in winter) demonstrate higher capability to survive and to root than those from intensively growing shoots (from hedged donors, collected during the spring). Statistically significant genotype x season interaction was also found.

[1]) This article represents part of the senior author's M.Sc. thesis, conducted in the Mediterranean Agronomic Institute of Chania, Chania, Greece

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