Genetic Divergence and Gene Source Studies in *Terminalia arjuna* Bedd.

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Studies on genetic divergence for 19 parameters were conducted with 50 accessions of *Terminalia arjuna.* Analysis of variance showed difference among accessions for all the traits studied. Based on the relative value of the magnitude of divergence value all the accessions were grouped into 9 clusters. Intra cluster distance was maximum for cluster VI (3.27) followed by 3.21 in cluster IV. The maximum inter cluster distance was 24.60 between cluster V and VI indicating high genetic divergence among accessions of these two groups. Maximum contribution towards the divergence was 44.73% by proline followed by protein (38.94%), reducing sugar (5.31%) and non-reducing sugar (3.76%). Gene sources for higher lamina length (Acc. 225); lamina width (IC 382333); low internodal distance (IC 410837), high protein (IC 560422) and low phenol (IC 3263888) were identified for future improvement programme.

Key Words: Cluster, D^2 analysis, Gene source, *Terminalia arjuna*

Introduction

Terminalia arjuna Bedd. commonly known as *Arjun* is a multi-purpose tree. It is of immense economic importance in various industries like pharmaceutical, timber, paper, soap, match industry, food, fodder and fuel. Besides, the foliage of the tree is primary food for tropical tasar silkworm *Antheraea mylitta* D. It is extensively distributed in tropical forests all over India on the banks of river, streams and dry water course (Anonymous, 1982). Considering the diverse uses of plant, it is essential to develop superior clones of this species through breeding. Genetic improvement of any species is primarily depends on the extent and magnitude of genetic variability among collection for different important traits. Based on the requirement it is essential to identify genetically divergent genotypes among the collected germplasm to select suitable parents for breeding programme. Studies on divergence analysis in *T. arjuna* germplasm are not reported so far. The present study was aimed to determine the genetic divergence in 50 germplasm accessions available in the field gene bank at Central Tasar Research and Training Institute (TR&TI), Ranchi. This will help in arranging the accessions into homogenous groups for further utilization in breeding programme to develop clones with desirable characteristics and identification of gene source for different traits.

Material and Method

The present study was carried out in 50 accessions of *T. arjuna* established under *ex situ* condition at clonal gene bank of CTR&TI, Nagri, Ranchi, Jharkhand, India (123^o 4’ N and 85^0 88’ E, altitude-708 m SL). Data was collected from 4 plants in each accession of uniform age of 5 years. Stomatal studies were conducted by applying a thin layer of quick fix on the abaxial leaf surface. The dried layer was peeled out from the surface containing the impression of stomata. The thin layer was then kept on the slide and covered with the cover slip and observed under Olympus microscope. The size and number of stomata was calculated with the help of occulometer pre-calibrated with stage micrometer. Chloroplast number/stomata was counted of 3rd and 5th leaf of a twig in descending order from the fully opened leaf at the top using ventral surface peeling of freshly collected leaf sample. The peels were stained and mounted in 2% potassium iodide-iodine solution following the method of Dwivedi *et al.* (1986). Estimation of phenol, chlorophyll a, b and total chlorophyll, reducing and non-reducing sugar, proline and protein was done as per the methods given by Mahadevan and Sridhar (1974).

Data on growth traits *viz*., lamina length, lamina width, petiole length, internodal distance leaf weight, moisture content and moisture retention capacity(%) was recorded from 5 branches of 4 clones from the leaf located at 6, 7 and 8th node. For internodal distance, length of longest shoot from each clone was measured and the total number of nodes was counted. Thereafter, shoot length was divided by the number of nodes for calculating the internodal distance. Leaf moisture and moisture retention capacity in harvested leaves were calculated following the method reported earlier (Suryanarayana *et al.,* 2006). Multivariate analysis for

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Table 1. Range, mean, standard error and co-efficient of variance for different characters in *Terminalia arjuna*

Parameter	Range	Mean	SE±	CV%
Lamina length (cm)	9.22-16.32	13.10	0.53	7.02
Lamina width (cm)	3.70-6.72	4.84	0.07	2.53
Petiole length (cm)	0.23-0.72	0.54	0.05	18.06
Internodal distance (cm)	1.55-2.68	2.08	0.02	2.00
Single leaf weight (gm)	1.09-2.08	1.64	0.07	7.45
Moisture content	62.58-78.57	67.51	0.15	0.40
Moisture retention capacity (%)	61.44-76.64	65.23	0.03	0.09
Frequency of stomata (mm^2)	193.93-524.18	381.34	7.07	3.21
Stomata length (µm)	22.73-30.83	26.08	0.85	5.70
Stomata width (µm)	12.36-17.46	15.46	0.66	7.42
Number of chloroplast in guard cells	7.66-11.00	8.63	0.54	10.90
Phenol (µg/mg)	0.34-4.51	1.89	0.04	4.41
Chlorophyll a (mg/g)	0.58-1.56	1.08	0.04	7.95
Chlorophyll b (mg/g)	0.39-1.68	0.86	0.03	7.74
Total Chlorophyll (mg/g)	0.88-3.49	1.93	0.02	1.98
Reducing sugar (µg/mg)	2.64-14.41	7.40	0.03	0.75
Non- Reducing sugar (µg/mg)	4.27-14.62	8.24	0.04	0.96
Proline (µ mol/g)	14.55-71.11	43.73	0.08	0.35
Protein (mg/g)	10.11-35.93	20.59	0.04	0.37

the estimation of genetic distance among all the accessions was carried out by using Mahalanobis D^2 statistic analysis as suggested by Mahalanobis (1936) and clustering of genotypes was done by the method of Tocher (Rao, 1952).

Results and Discussion

Analysis of variance showed significant differences among accessions for all the traits studied. It indicated existence of genetic variability in the accessions (Table 1). Based on the relative value of the magnitude of divergence of the 50 accessions were grouped into 9 clusters (Table 2). The maximum genotypes were grouped in cluster I (17) followed by cluster II (14); VI (7); IV (6); V (2) and one each in cluster III, VII, VIII and IX. Intra cluster distance was maximum for cluster VI (3.27) followed by 3.21 in cluster IV. However, cluster III, VII, VIII and IX had no intra cluster distance as they were represented by a single genotype. The maximum inter cluster distance (24.60) was observed between cluster V and VI indicating high genetic divergent accessions of these two groups. Minimum inter cluster distance (3-21) was observed between cluster III and IV showing their close proximity. However, in all cases inter cluster distances were greater than the intra cluster distances indicating the presence of genetic diversity among the accessions between different clusters (Table 3).

The cluster means indicated substantial variation among 9 clusters (Table 4). Maximum lamina width was in cluster V which represents 2 accessions collected from Andhra Pradesh and Uttar Pradesh. Frequency of stomata and width of stomata was minimum in cluster III representing only 1 accession (IC 382306). Accessions in cluster IX showed minimum internodal distance. Cluster V showed lower phenol content in the leaf whereas, cluster VI had maximum chlorophyll b and non-reducing sugar content in the leaf. Moisture content and moisture retention capacity was higher in cluster

Table 2. Cluster composition in *T. arjuna*

Cluster No.	No. of accessions	Accession number and source
I	17	382334, 382330, 410840; Uttarakhand 410797, 326375, 410795; Maharashtra 210, 214, 225, 212, 410830, 410779, 410780; Jharkhand 410748, 382303, 382300; Andhra Pradesh 410863; Chhattisgarh
II	14	560439 ,560451; Uttar Pradesh 410781, 355582, 410827, 381646, 381644, 381639, 118; Jharkhand 560423; Maharashtra 410752; Andhra Pradesh 382333; Uttarakhand 410859, 410865; Chhattisgarh
III	01	382306; Andhra Pradesh
IV	06	410806, 560424; Maharashtra 410838; Uttarakhand 410866; Chhattisgarh 410755; Andhra Pradesh 381641; Jharkhand
V	02	560452; Uttar Pradesh 410754; Andhra Pradesh
VI	07	135, 381635; Jharkhand 560422, 560418, 326382, 326388; Maharashtra 560436; Uttar Pradesh
VII	01	410858; Chhattisgarh
VIII	01	410806; Maharashtra
IX	01	410837; Jharkhand

Table 3. Inter and Intra-cluster distances of accessions in gene pool

Clusters	I	II	III	IV	V	VI	VII	VIII	IX
I	1.76								
II	7.13	2.20							
III	3.08	7.68	0.0						
IV	4.39	8.64	1.51	3.21					
V	3.47	14.56	4.48	6.52	0.75				
VI	14.93	6.08	10.54	11.26	24.60	3.27			
VII	7.50	3.86	12.07	12.96	13.75	13.46	0.0		
VIII	12.69	6.28	12.18	14.58	16.88	11.60	7.64	0.0	
IX	11.29	13.75	3.57	4.58	13.34	10.67	21.52	19.39	0.0

VIII. Higher values of lamina length, petiole length, chlorophyll a, total chlorophyll, reducing sugar and total protein were recorded in cluster IX. Maximum contribution towards total divergence was 44.73% by proline followed by protein (38.94%). On the contrary the others characters have given very little or no contribution to total divergence for enabling clear discrimination of the genotypes (Table 4).

The results indicated that geographical distribution and genetic divergence did not follow the same pattern. The grouping of genotypes of different geographical locations into one cluster may be due to presence of some common genes controlling the most important characters through modifying effect of micro and macro environment affecting genetic diversity. Another feature that genotypes from same location were placed in separate cluster indicating wide diversity among genotypes from same location. This shows that the geographical diversity may not necessarily be related to genetic diversity in this species. The present findings are in agreement with Srivastava *et al.* (2007) and Dwivedi and Mitra (1996) in apricot and litchi, respectively. Thus, the complex composition of clusters indicates that geographical diversity is important but not the sole factor determining the genetic diversity (Murthy and Arunachalam, 1966). The geographical diversity has been disproved to be an index of genetic diversity in mulberry (Rajan *et al.*, 1997; Tikadar *et al.*, 2003); pearl millet (Singh *et al.*, 1981) and in black gram (Das and Dasgupta, 1984).

Based on the analysis, accessions with gene source for some of the important qualitative and quantitative parameters have been identified (Table 5). Gene source for lamina length Acc. 225; lamina width IC382333 and internodal distance in IC410837, whereas IC560422, IC326388 and IC560452 are gene source for protein, phenol and proline respectively. Since, leaf quality (sugar and protein contents, and moisture retention capacity) is the major factor in determining the silk yield, accessions

Table 4. Mean performances of the clusters with respect to different characters

Parameter	Cluster									
	I	II	III	IV	V	VI	VII	VIII	IX	Contribution %
Lamina length (cm)	13.31	13.17	12.87	12.52	12.92	12.92	13.47	13.10	13.93	0.0
Lamina width (cm)	5.15	4.68	4.09	4.56	5.38	4.82	3.70	4.49	5.12	0.0
Petiole length (cm)	0.56	0.57	0.54	0.54	0.50	0.50	0.47	0.54	0.62	0.0
Internodal distance (cm)	2.22	2.10	2.25	1.88	1.89	2.01	1.91	2.32	1.55	0.0
Single leaf weight (gm)	1.66	1.61	1.17	1.64	1.74	1.80	1.10	1.65	1.49	0.0
Leaf moisture (%)	67.34	66.78	70.21	66.60	73.44	66.42	67.96	78.57	67.97	0.0
Moisture retention capacity %	64.59	64.97	66.49	64.76	69.24	64.61	65.24	76.64	66.96	6.69
Frequency of stomata (mm^2)	400.53	398.98	215.80	363.10	406.77	319.21	483.88	263.20	483.14	0.08
Stomata length (µm)	25.70	26.43	27.57	25.67	27.47	25.98	25.77	26.70	26.47	0.0
Stomata width (µm)	15.69	15.34	13.07	15.77	15.23	14.89	14.60	17.47	17.37	0.0
Number of chloroplast in guard cells	8.45	8.71	9.00	8.83	7.83	8.71	8.67	10.33	8.33	0.0
Phenol (µg/mg)	2.05	1.87	1.75	2.20	1.11	1.31	1.24	4.51	1.48	0.16
Chlorophyll a (mg/g)	1.00	1.08	1.11	1.15	1.01	1.19	1.04	1.24	1.32	0.0
Chlorophyll b (mg/g)	0.80	0.95	0.79	0.85	0.59	0.99	0.59	0.98	0.94	0.0
Total chlorophyll (mg/g)	1.73	2.04	1.96	1.98	1.58	2.21	1.45	2.20	2.44	0.33
Reducing sugar (µg/mg)	7.93	6.55	7.78	8.82	9.19	5.42	10.92	3.85	12.03	5.31
Non- Reducing sugar (µg/mg)	8.30	8.77	6.34	6.54	6.33	10.17	8.84	4.59	5.64	3.76
Proline (µmol/g)	54.83	27.25	61.88	61.30	68.38	28.62	20.17	20.63	65.78	44.73
Protein (mg/g)	14.73	20.46	24.11	24.24	12.53	32.84	11.92	20.48	35.73	38.94

Table 5. Gene donors for some important characters in *T.arjuna* accessions

Character	Potential	Accession
Lamina length (cm)	16.32	Acc. 225
Lamina width (cm)	6.72	IC 382333
Internodal distance (cm)	1.55	IC 410837
Leaf moisture (%)	78.57	IC 410806
Reducing sugar (µg/mg)	14.41	IC 381641
Non-Reducing sugar (µg/mg)	14.62	IC 326382
Proline (µ mol/g)	71.11	IC 560452
Protein (mg/g)	35.93	IC 560422
Phenol (µg/mg)	0.34	IC 326388

showing high mean values contributing in leaf quality will be utilized for future selection and breeding for superior varieties.

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References

Anonymous (1982). *The Wealth of India . Raw Materials Vol X Sp-W*. Publications and Information Directorate, Council of Scientific and Industrial Research, New Delhi, pp 161-164.

Das PK and T Dasgupta (1984) Multivariate analysis and selection of parents for hybridization in black gram. *Indian J. Genet.* **44(2)**: 243-247.

Dwivedi AK and SK Mitra(1996) Divergence analysis of litchi (*Litchi chinensis* Sonn) cultivar grown in West Bengal. *Indian J. Genet.* **56**: 486-489

Dwivedi NK, AK Sikdar, SB Dandin, R Kumar and K Giridhar (1986) Stomatal chloroplast count technique as a tool to ascertain different ploidy level in Mulberry. *India J. Sericulture* **25(2)**: 88-90.

Mahadevan A and R Sridhar (1974) *Methods in Physiological Plant Pathology*. Sivakami Publication, Chennai.

Mahalanobis PC (1936) On the genetic distance in statistics. *Proc. Nat. Ins. Sci. India* **2**: 49-55.

Murthy BR and V Arunachalam (1966) The nature of divergence in relation to breeding system in crop plants. *Indian J. Genet.* **26A**:188-198.

Rajan MV, HK Chaturvedi and A Sarkar (2003) Multivariate analysis as an aid to genotype selection for breeding in Mulberry. *Indian J. Seric*. **36(2)**:111-115.

Rao CR (1952) *Advanced Statistical Methods in Biometric Research*. John Willy & Sons, New York.

SinghYP, Awadhesh Kumar and BPS Chauhan (1981) Genetic divergence in pearl millet. *Indian J. Genet.* **41(2)**: 186-190.

Srivastava KK, MK Sharma and AS Sundauri (2007) Estimation of genetic divergence in apricot (*Prunus armeniaca* L.). *J. Plant Genet. Resour.* **20(3)**: 234236.