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Study on foliar micro morphology of *Gmelina arborea* Roxb. : A clonal identification against *Craspedonta leayana*

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Abstract
Present investigation is describes with leaf micro morphological study of *Gmelina arborea* clones available in Naharoni, Experimental field of Rain Forest Research Institute, Jorhat, Assam. Non glandular uniseriate multicellular filiform hairs and capitate glandular hairs were intermingled in the mesh and vein areas of the lamina. Non glandular uniseriate, 2-celled multicellular filiform hairs were dominant in majority of the clones. Dominant occurrence of anomocytic stomata in about all the clones gave the genus *Gmelina* as an advanced entity. Low density of trichome (30.68 ±1.90), presence of short unicellular or bi-celled glandular trichome (71.2 ±15.14), thin epidermal cell wall, hypo-stomatic leaves and low frequency of stomata leads the clone GA 006 is most susceptible to leaf defoliator insect *Craspedonta leayana.* Vein islet number of the clone was observed minimum and size of the areoles was large with thin wall thickness too. Otherwise**, c**lone GA 023 comprises high density of trichome (171.52 ±3.14) with maximum length (510.88 ±62.62), polygonal- arched and thick epidermal cell wall provide the clone less susceptible to the insect.

Keywords: Foliar micro morphology, Clonal identification, *Gmelina arborea*, resistance, *Craspedonta leayana*

Introduction
Gmelina arborea Roxb commonly known as white teak, is a tropical medium sized deciduous forest species under the family Verbenaceae. It is cultivated commercially as a timber, fodder, industrial wood and has high medicinal value of leaf, root and flower. The species occurs in different geographical and ecological conditions of Nepal, Bangladesh Thailand, Laos, Cambodia, Vietnam and southern provinces of China. It is distributed naturally throughout India extensively in sub-Himalayan tracts including Assam and west Bengal to Orissa up to 1,500 meters altitudes. Previous research work on genetic improvement of *G.arborea* was initiated by the Indian Council of Forestry Research & Education (ICFRE) institutes through assembling divergent populations in the gene banks from different parts of the country. Rain Forest Research Institute has initiated the work on genetic improvement during 2003 and assembled divergent genotypes by using various conventional methods in gene bank clonal seed orchard at Naharoni, Assam. *Craspedonta leayana* the mostly attacked insect pest of the species have constantly been a hurdle in a successful plantation. From the month of December to March the adult is being hibernation and feeds on leaves during the high growth period and completely defoliate the plants or plantation with a very short period of time. Therefore, screening of resistant clone would be extremely necessary to manage the insect pest.

Owing to significant constancy, the leaf micro morphological characters viz. epidermal cell features, trichome character, stomatal behaviour, foliar venation pattern etc. can provide important additional evidences for genetic and specific delimitation of plants. During the last century attention is being paid by different scientist to sustained use of these characters at systematic level (Banerji and Das, 1972; Kumar and Jain, 1986; Thakur, 1988; Rao *et al* 1987; Sastry and Kannabiran, 1994) [1, 11, 17, 20, 14]. A few attempt was made by Singh and Barua (1995), Singh, Barua and Nath (1995), Barua and Singh (2001) [15, 16, 2] to add more information by providing epidermal, trichome and venation characters for Indian forestry species. These characters have been used as means of recognizing the hybrids, clones, germplasms and cultivars. Gunaga and Surendran (2002) [5] study the leaf morphological variations of Teak clones. However, the study confined only the qualitative and quantitative

morphological variations of the leaves. Leaf morphological variations of *G. arborea* genotypes were very much scanty apart from a few works of Kumar *et al* 2005 [10] and Barua and Singh 2003 [3]. The present investigation was conducted as a part of confirmatory study to identify the resistant clone for an improved planting stock in the future plantation programmes based on leaf micro- morphological characters.

Materials and Methods

Out of 70 numbers established clones total six numbers of clones were selected as most susceptible (03) and less susceptible (03) against the infestation of C. *leayana* after confirmation of field and laboratory study. For the present leaf micro morphological study, 4-5 mature leaves were collected randomly from each clone in replicated manner from the experimental station, Nahorani, Golaghat, Assam. Small leaf discs of 1 sq. cm were cut and gently boiled in 5% KOH solution for 1-5 minutes meant for de pigmentation. De pigmented leaf discs were kept in aqueous solution of chloral hydrate for more clearness. The samples were stained with 2% aqueous safranin solution and mounted in glycerine. Characterization of epidermal cell peeling was taken from adaxial and abaxial surfaces. Mean values of 20 observations of stomatal, epidermal, trichome and venation characters were recorded from10 different leaf discs for each clone along with standard deviation. Terminology of Kidwai (1981) [9] was followed for stomatal explanation. Classification of the leaf architecture was followed by Hickey (1971) [7] and terminology of trichome was go after Ramayya (1962, 1964) [18, 19]

Results and Discussion

Qualitative features of the leaves of *Gmelina arborea* clones showed that the leaves are simple, opposite and decussate, ovate in shape with acute apex, base cordate, margins are entire. Two glands present at the base of the lamina in the dorsal side. The pattern of major venation is pinnate camptodromous (brochidodomous). Primary vein is moderate to stout and runs straight into lamina. The secondaries arise alternate to sub opposite manner. The angle of divergence of secondary vein is uniformly acute. Secondary vein extend towards margin and bending to form smooth arch. Kumar *et al* (2005) [10] discussed about the leaf size and areas of twenty five *G. arborea* clones from established in clonal trial of Nahorani experimental station, Golaghat, Assam. They recorded 8% genotype were highly resistant to *C. leayana*. The present investigation deals with the study of foliar micro-morphology which is essential owing to limitation of macro-morphological features in phylogenetic classification for clonal identification. Based on a range of leaf micro morphological characters, the present study delimited the six selected *G. arborea* clones according to susceptibleness of most devastating leaf defoliator insect *C. leayana*. Application of trichome morphological traits has some importance to developed promising genotypes for higher productivity. Trichome diversity of *G. arborea* clones observed that non glandular uniseriate multicellular filiform hairs and capitate glandular hairs were intermingled in the mesh and vein areas of the lamina. Trichome density was found more in the abaxial surface of the leaves. Hair density and length is the most significant character for categorization of different clones. Inamdar (1969) [8] in his observation reported hooked trichome with terminal silicified cell in *G. arborea*, but during the course of present study this kind of hair was not observed. Trichome pattern of *G. arborea* clone and their measurement was reported by Barua and Singh (2003) [3]. However, in their study the other leaf micro morphological characters were not covered up.

Non glandular uniseriate multicellular filiform trichome: Non glandular uniseriate, 2-celled multicellular filiform hairs were dominant in majority of the clones except clone 006 (Plate1: 2b & c, plate 2 d-f). Variations of the length and breadth were prominent. Origin of the hairs was single or double celled. Hair apical straight or curved, lateral wall smooth, wall thickness fine or thick.

Non glandular uncellular simple filiform trichome: Non glandular uncellular simple filiform trichome observed only in clone GA 006 (Fig 1a), body slightly curved, lateral wall thin and smooth

Capitate Glandular trichome: Capitate Glandular trichome bears unicellular globose head with medium or short stalk. (Table-1)

Stomatal and epidermal characters are considered as secondary key character for identification of the clones. Majority members of the *Gmelina* family bear Ranunculaceous (anomocytic) type of stomata (Metcalf and Chalk, 1950) [12]. Dominant occurrence of anomocytic stomata in all the clones gave the genus as an advanced entity. Pant and Kidwai (1964) [13] reported mixed combination type of stomata existed in different member of verbenaceae. Present study also showed the similar observation in *G. arborea* clones. It possessed two distinct groups amphistomatic and hypostomatic. The number of stomata was very less in the upper surface of the leaf (Plate 1:4 a-c Plate 2: 4 d-f). Maximum stomatal frequency was shown in GA 095 (Table 2). Occurrence of mixed type of stomata in the clones was a source of confusion during the study of the genotypic variation. However, dominant occurrence of anomocytic stomata in about all the clones gave the genus is an advanced entity in the family. On the other hand diacytic and paracytic types too were recorded also. Anomaly of the stomatal structure was found to have degenerated stomata in clone GA095 only, contagious stomata (side to side & pole to pole) in GA 034, stomata with single guard cell in GA002, contagious stomata (side to side) and stomata with single guard cell present in GA006 (Plate1: 3a). Stomatal abnormality was not found in clone GA023 and GA081 during the present study (Plate1:3 a-c; plate2: 3d-f).

Leaf architectural character has been used as an important tool in obtaining taxonomic conclusion (Dilcher, 1974; Gupta, 1961) [4, 6]. Vein islet density was found one of the key characters of classification of *Gmelina* clones (Table-3.) (Plate 1: 2a-c & Plate 2: 2d- f). The areoles are randomly arranged and shape is polygonal, sometimes devoid of free vein ending. In GA 002 it is quadrangular to polygonal in shape (Plate 1: 2b). Usually vein let are simple, linear or curved, showing branching once. In GA 002 it was forked once to twice, equally or unequally and twice to thrice in GA081 (Plate 1: 2c). Vein islet number was observed minimum in clone GA006 (14.34 ±0.45 sq mm.). Size of the areoles was large and wall thickness was also less (223.76 ±3.70) and 9.52 ±0.14 respectively). Due to the occurrence of such characters clone GA006 is clearly stands apart from the rest of the clone (Plate 1: 2a).

Low density of trichome, presence of unicellular or bi-celled glandular hair, minimum vein islet number having large areoles, thin epidermal cell wall, hypo-stomatic leaves, low

frequency of stomata was the primary identifying characters of clone GA 006. Simple unicellular filiform hair was recorded sporadically. Hair base was 2-celled bulbous transparent, apical straight. Wall thickness of the vein was thin and islets number was less in comparison to other clones (Fig 1). Occurrence of every character leads the clone most susceptible to leaf defoliator insect *Craspedonta leayana* among the all clones (Plate 1 1a, 2a, 3a & 4a).

Clone GA 023 comprised high density of trichome (171.52 ±3.14) (Plate 2: 1d) with maximum length (510.88 ±62.62). These characters were the primary identifying characters that the clone undoubtedly stands away from the other taxa and found less susceptible to the insect. The other supporting characters were leaf amphi-stomatic and stomata anomocytic, epidermal cell polygonal and arched and cell wall thickness was wide (Table-4) (Plate 2: 3d & 4d). Hair base was single celled, rarely 2-celled bulbous and apical straight (Fig 2). Vein wall thickness is thick and islets number was found maximum (Plate 2: 2d).

Support on epidermal cell characters, stomatal behaviour, foliar trichome, venation pattern etc. a synoptic dichotomous key is constructed for categorization of various *G. arborea* clones.

Identification Key for *Gmelina arborea* clones

1a.Trichome density high, vein islet more, leaf amphistomatic
2a. Trichome medium ---------- GA34
2b. Trichome long
3a. Vein wall and epidermal wall thick, epidermal cell polygonal arched, abnormal stomata absent ---------- GA23
3b. Vein wall and epidermal wall moderately thick, epidermal cell polygonal sinuate, abnormal stomata present ------- GA95
1b. Trichome density low, vein islet less, leaf amphistomatic or hypostomatic
4a. Abnormal stomata abscent, trichome length medium
5a. Leaf amphistomatic, areoles bigger, glandular trichome abscent, epidermal cell polygonal isodiametric ---------- GA81
5b. Leaf hypostomatic, stomata paracytic and anomocytic, areoles medium, glandular trichome rarely present, epidermal cell polygonal sinuate ---------- GA02
4b. Leaf hypostomatic, stomata paracytic, trichome length short, glandular trichome present, Uni cellular simple filiform trichome rarely present. ---------- GA06

Table 1: Trichome Characterization of *Gmelina arborea* clones

Number of Clones		Non-Glandular Trichome			Glandular Trichome			Characters
	Density/mm^2	Length	Breadth	L/B Ratio	Length	Breadth	L/B Ratio	
GA023	171.51 ±3.14	510.88 ±62.62	10.96 ±2.23	46.61 ±11.64	77.04 ±11.17	9.68 ±1.38	8.04 ±1.19	Non glandular, Multicellular Uniseriate filiform Trichome dominant, 2-3 celled, base bulbous, long. glandular trichome present
GA095	107.97 ±3.55h	477.68 ±45.13	9.36 ±0.39	51.02 ±4.19	-	-	-	Non glandular, Multicellular Uniseriate filiform Trichome dominant, 2-3 celled, long, glandular trichome very rarely present.
GA34	164.54 ±8.88	198 ±27.25	10 ±2.48	20.49 ±4.3	84.64 ±30.48	10.72 ±0.94	8 ±3.11	Non glandular, Multicellular Uniseriate filiform Trichome dominant, 2 celled, medium, base bulbous, transparent, single or bi-celled origin, glandular trichome present
GA02	45.42 ±2.25	308.96 ±52.45	12 ±1.13	25.91 ±4.68	73.12 ±6.47	11.36 ±1.30	6.51 ±0.98	Non glandular, Multicellular Uniseriate filiform Trichome dominant, 2 celled, long, glandular trichome rarely present
GA81	44.42 ±2.11	217.44 ±41.91	11.76 ±1.41	18.78 ±4.63	152 ±39.13	13.36 ±1.25	11.44 ±2.89	Non glandular, Multicellular Uniseriate filiform Trichome dominant, 2-3 celled, medium, base bulbous, transparent, single or bi-celled origin, glandular trichome present, non bulbous, long
GA06	30.68 ±1.90	-	-	-	71.2 ±15.14	12.24 ±1.13	5.90 ±1.58	Unicellular or bi-celled glandular trichome present, short. Unicellular simple filiform trichome rarely present.

Table 2: Stomatal characters of *Gmelina arborea* clones

Clone Number	Leaf surface	Type of stomata	Size of the stomata (µ)			Size of the aperture (µ)			Stomatal Frequency	Stomatal Index
			Length	Breadth	L/B Ratio	Length	Breadth	L/B Ratio		
GA23	U	Leaf amphistomatic, Anomocytic stomata	41.76 ±0.1	27.52 ±0.04	3.06 ±0.11	38.08 ±0.13	9.04 ±0.10	9.67 ±1.04	334 ±1.85	27.04
	L		39.73 ±0.06	25.60 ±0.35	1.55 ±0.15	45.33 ±0.15	6.13 ±0.12	7.39 ±1.03	6 ±0.75	0.74
GA95	U	Leaf amphistomatic, Anomocytic & Diacytic stomata, degenerate stomata present	29.33 ±0.12	17.60 ±0.35	1.67 ±0.20	19.47 ±0.40	1.33 ±0.06	8.80 ±5.22	12 ±0.63	0.94
	L		28.64 ±0.26	20.48 ±0.34	1.40 ±0.22	17.44 ±0.28	1.44 ±0.04	12.11 ±3.96	345 ±1.25	27.91
GA34	U	Leaf amphistomatic, contagious stomata present (side to side & pole to pole), upper stomata paracytic & Diacytic, lower Anomocytic	31.08 ±0.40	22.82 ±0.35	1.36 ±0.02	18.34 ±0.72	6.02 ±0.57	3.05 ±1.31	8 ±0.89	1.15
	L		30.58 ±0.75	19.78 ±0.74	1.55 ±0.22	21.38 ±0.89	6.85 ±0.31	3.12 ±0.76	326 ±1.49	30.87
GA02	U	Leaf hypostomatic, Anomocytic & Paracytic stomata, stomata with single guard cell present	NIL							
	L		27.04 ±0.34	17.28 ±0.47	1.56 ±0.40	16.56 ±0.45	4.08 ±0.09	4.06 ±0.71	204 ±1.40	25.16
GA81	U	Leaf amphistomatic, Anomocytic stomata	33.20 ±0.44	23.40 ±0.10	1.42 ±0.13	31.80 ±0.56	7.00 ±0.22	4.54 ±0.66	8 ±0.89	1.07
	L		34.24 ±0.65	24.08 ±0.72	1.42 ±0.40	31.36 ±1.06	7.28 ±0.31	4.31 ±1.29	334 ±1.85	30.42
GA06	U	Leaf hypostomatic, stomata with single guard cell and contagious stomata (side to side) present, Stomata Anomocytic	NIL							
	L		31.76 ±0.33	24.64 ±0.13	1.29 ±0.09	14.56 ±0.36	4.72 ±0.11	3.08 ±1.07	189 ±0.79	19.83

Table 3: Foliar venation characters of *Gmelina arborea* clones

Clone Number	Wall thickness	Vein -islet (sq.mm)	Vein entering in areoles (sq.mm)	Vein ending termination (sq.mm)	Nature of vein ending	Size of the Areoles (µ)		
						Length	Breadth	L/B Ratio
GA23	12.24±0.33	19.42±0.50	4.8	8.3	Branch once	187.44±3.71	147.68±1.31	1.27±0.17
GA95	10.48±0.07	19.42±0.50	3.6	7.9	Branch once	188.64±3.89	147.36±3.21	1.30±0.36
GA34	11.36±0.34	20.92±0.58	5.4	10.2	Branch once	177.28±3.49	134.08±3.82	1.32±0.27
GA02	9.44±0.13	14.94±0.58	9.2	18.0	Branch once or twice	201.84±1.57	141.68±0.42	1.38±0.22
GA81	9.04±0.14	14.44±0.50	7.9	14.7	Branch once to thrice	226.72±3.61	167.84±4.21	1.35±0.38
GA06	9.52±0.14	14.34±0.45	3.0	5.8	Branch once	223.76±3.70	154.4±2.78	1.42±0.15

Table 4: Epidermal features of *Gmelina arborea* clones

Clone Number	Leaf surface	Shape of the Epidermal cell	Wall thickness	Size of the Epidermal cell (µ)			Epidermal cell Frequency
				Length	Breadth	L/B Ratio	
GA23	U	Polygonal, Arched, slightly sinuate	1.60 ±0.00	49.31 ±1.64	24.91 ±1.66	1.67 ±0.46	809 ±29.9
	L	Polygonal, Arched	1.36 ±0.05	41.71 ±1.03	21.60 ±1.06	2.02 ±0.57	901 ±78.17
GA95	U	Polygonal, Irregular	1.44 ±0.04	25.92 ±0.37	15.2 ±0.16	1.71 ±0.25	1266 ±27.76
	L	Polygonal, Sinuate	1.04 ±0.05	34.4 ±0.44	17.76 ±0.36	1.94 ±0.40	891 ±67.30
GA34	U	Polygonal, Isodiametric	1.28 ±0.05	61.28 ±1.36	36.64 ±0.85	1.56 ±0.22	686 ±41.08
	L	Polygonal, Iso-diametric, slightly sinuate	0.55 ±0.05	46.24 ±0.79	39.12 ±0.70	1.98 ±0.79	730 ±35.06
GA02	U	Polygonal, Sinuate	1.44 ±0.04	113.76 ±1.69	49.28 ±1.47	1.75 ±0.92	561 ±60.73
	L	Polygonal, Sinuate	1.2 ±0.05	88.48 ±0.83	37.36 ±1.69	2.31 ±0.59	595 ±38.64
GA81	U	Polygonal, Isodiametric	1.60 ±0.71	56.16 ±1.27	38.24 ±1.40	1.47 ±0.32	742 ±30.98
	L	Polygonal, Isodiametric	1.36 ±0.05	42.96 ±0.51	27.60 ±0.31	1.56 0.00	764 ±32.42
GA06	U	Polygonal, Sinuate	1.28 ±0.0	76.16 ±0.73	42.24 ±1.21	1.93 ±0.84	637.1 ±34.03
	L	Polygonal, Sinuate	1.04 ±0.05	79.04 ±0.47	47.76 ±0.65	1.65 ±0.16	676.89 ±36.55

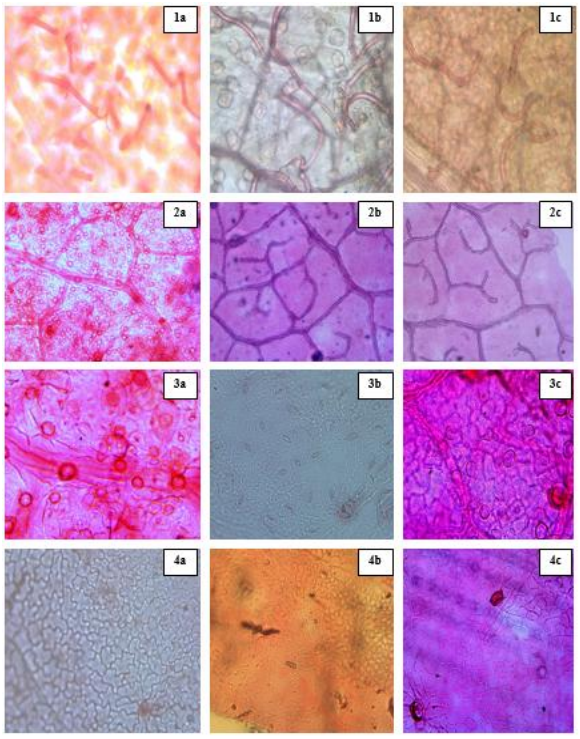

Plate 1: Foliar Micro-morphological characters of most susceptible *Gmelina arborea* clones
a: Clone GA06**, b:** Clone GA02**, c:** Clone GA81
1. Foliar Trichome **2.** Venation pattern **3.** Lower epidermis **4.** Upper epidermis

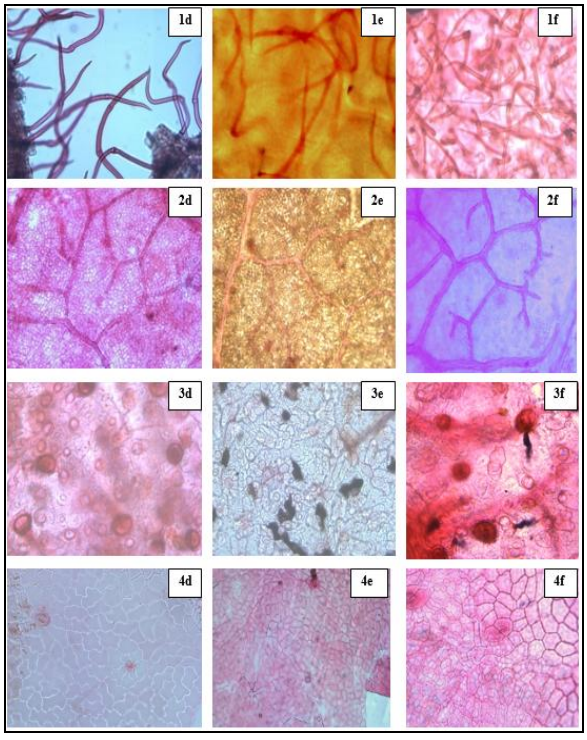

Fig 1: Foliar Micro-morphological characters of less susceptible *Gmelina arborea* clones
d: Clone GA23**, e:** Clone GA95, **f:** Clone GA34
Foliar Trichome **2.** Venation pattern 3.Lower epidermis 4. Upper epidermis

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