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STUDIES ON THE PHENOTYPIC VARIATION IN LEAF ARCHITECTURE OF *TINOSPORA CORDIFOLIA* (WILLD.) EX HOOK. F. & THOMAS

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The paper deals with phenotypic variation in the leaf architecture of *Tinsopora cordifolia*. Nineteen characters were studied of which ten are qualitative and nine are quantitative. The plant has two varieties bitter and non-bitter. Both male and female plants have been recognized in both the varieties. These plants exhibit similarity in leaf shape, apex, margin, texture, marginal ultimate venation, areole shape, development and arrangement. However, there is lot of variation in leaf base, size of the leaf, number and size of areole and number of vein endings per areole. All these quantitative characters are statistically significant. These two varieties can be identified on the basis of leaf size, leaf shape and other quantitative characters.

Keyword: Taxonomy; *Tinospora cordifolia*.

Introduction

Tinospora cordifolia is a plant of high medicinal value. Lot of work has been done on its medicinal values[1,2] and chemical composition[3-7]. It has two very conspicuously different phenotypes i.e. one with a very bitter tasting stem with smaller leaves and the other almost non-bitter with broader (larger) leaves. Further both the varieties (phenotypes) have male and female plants. No work has been done on its phenotypic variation. Hence the present study was undertaken.

The purpose of this work is to attempt to gain insight into variation pattern within these two phenotypes of the plant using numerical technique. The paper deals with the results of statistical/numerical analysis of leaf architecture of *Tinospora cordifolia*.

Materials and Method

The two phenotypes of the plant considered in this study are named as non-bitter and bitter and their codes are NB and B respectively. For the study of leaf architecture the leaves were cleared with the Trichloro acetic acid and phenol and stained with crystal violet. At least fifty individuals of each phenotype were observed. The measurements of quantitative characters were taken in square mm and the mean, standard deviation, variance, coefficient of variation percent were calculated for each of the character of the sample. 't' test was also applied to find out whether the results are statistically significant or not.

Results and Discussion

The leaves of both the investigated varieties of *Tinospora cordifolia* are simple, symmetrical, alternate, exstipulate and petiolate; shape of leaves is cordate, margins are entire, apices are acute, bases of the laminae are slightly cordate in bitter variety and deeply cordate in non-bitter variety (both male and female plants). The texture of leaf is smooth (Table 1.1).

The veins of 1^0, 2^0 and 3^0 constitute the major venation of the leaf and the subsequent veins constitute the minor venation. The major venation is reticulate multicostate type. The major venation includes seven major veins originating from the base of the laminae. The primary vein is weak and straight. The secondaries arise along both sides of the primary veins. The angle of 2^0 and 3^0 veins are

Table 1.1 Various Features of Leaf Architecture in the Two Varieties of *Tinospora cordifolia*.

S. No.	Name of the variety	Leaf shape	Leaf apex	Leaf base	Leaf margin	Texture of leaf	Size and course of 1^0 vein	Angle of 2^0 vein	Angle of 3^0 vein	Marginal ultimate venation	Areole shape	Areole development	Areole arrangement
1.	Non Bitter Female	Cordate	Acute	Deeply cordate upto 25 mm	Entire	Smooth	weak and straight	20-60^0	30-60^0	Looped	Rectangular to Polygonal	Well developed	Random
2.	Bitter Female	"	"	Slightly cordate upto 15 mm.	"	"	"	20-40^0	40-50^0	"	"	"	"
3.	Non-bitter Male	"	"	Deeply cordate upto 20mm	"	"	"	20-60^0	40-45^0	"	"	"	"
4.	Bitter Male	"	"	Slightly cordate upto 8mm	"	"	"	20-60^0	30-35^0	"	"	"	

Table 2.1 Basic data of quantitative characters of Leaf Architecture in the two varieties of *T. cordifolia*.

S. No.	Name of the variety	Area of leaf	Average size of areole (mm^2)	No. of areole per sq. mm	Veinlets entering into areole per sq. mm	Vein endings per sq.mm	Absolute islet number	Absolute vein ending number
1.	Non Bitter Female	1247.67 ± 26.06	0.858 ± 0.05	1.16 ± 0.01	1.74 ± 0.10	8.624 ± 0.15	1454.17	10760.85
2.	Bitter Female	397.01 ± 13.63	1.196 ± 0.06	0.836 ± 0.02	1.17 ± 0.06	7.52 ± 0.13	331.94	2986.78
3.	Non Bitter Male	912.751 ± 28.90	1.179 ± 0.05	0.848 ± 0.01	1.27 ± 0.05	7.12 ± 0.29	774.17	6503.06
4.	Bitter Male	325.26 ± 6.67	0.297 ± 0.008	3.366 ± 0.06	9.42 ± 0.19	31.64 ± 0.57	1095.15	10294.42

Table 2.2 Statistical analysis of quantitative characters of leaf architecture in the two varieties of cordifolia.

S. No.	Microanatomical character		Standard deviation		variance		Coefficient of variation %	
			NB	B	NB	B	NB	B
1.	Leaf Area	Female	184.31	96.40	33970.67	9293.40	14.77	24.28
		Male	204.37	47.16	41770.69	2224.97	22.39	14.50
2.	Area of areole	Female	0.42	0.47	0.180	0.229	49.48	40.07
		Male	0.36	0.06	0.130	0.004	31.29	23.15
3.	No. of areoles per sq.mm.	Female	0.09	0.149	0.008	0.022	7.86	17.88
		Male	0.13	0.452	0.010	0.204	15.49	13.43
4.	No. of veinlets entering into areole per sq.mm.	Female	0.74	0.47	0.550	0.221	42.62	40.259
		Male	0.39	1.37	0.150	1.89	30.98	14.61
5.	No. of free vein endings per sq. mm.	Female	1.09	0.98	1.190	0.969	12.68	13.09
		Male	2.12	4.10	4.51	16.88	29.83	12.98

Table 2.3 Statistical analysis of quantitative characters in *Tinospora cordifolia.*

S. No.	Microanatomical character	Difference of mean (a)		Standard error of difference (b)		t = a/b	
		Female	Male	Female	Male	Female	Male
1.	Leaf Area	850.66	587.49	29.41	29.66	28.22***	19.80***
2.	Area of areole	0.338	0.882	0.087	0.053	3.885***	16.64***
3.	No. of areoles per sq. mm.	0.329	2.518	0.024	0.065	13.708***	38.738***
4.	No. of veinlet enterings into areole per sq. mm	0.578	8.155	0.124	0.196	4.66***	41.607***
5.	No. of free vein endings per sq. mm.	1.01	24.529	0.207	0.654	5.318***	37.506***

(98 Degree of Freedom)*** = very highly significant.
Distribution of t for 0.050 P = 1.9828, 0.010 P = 2.6338, 0.001 P = 3.3876

Table 3.0 Degree of Variability of antomical characters of the two varieties of *T. cordifolia.*

S.No.	Name of the taxa with variety	Leaf Area	Area of areole (mm2)	No. of areole/mm2	Veinlet enterings into areole per sq. mm.	Vein endings per sq. mm.
1.	T. cordifolia Non-Bitter Female					
	Coefficient of variation %	14.772	49.58	7.86	42.62	12.65
	variability grade	III	V	I	IV	II
2.	Bitter Female					
	Coefficient of variation %	24.28	40.07	17.88	40.25	13.09
	Variability Grade	III	IV	II	V	I
3.	Non-Bitter Male					
	Coefficient of Variation %	22.39	31.29	15.49	30.98	29.83
	Variability Grade	II	V	I	IV	III
4.	Bitter Male					
	Coefficient of variation %	14.50	23.15	13.43	14.61	12.98
	Variability Grade	III	V	II	IV	I

I - Most stable, II - stable, III - Less stable, IV - Very less stable, V - Variable

variable and overlapping in both the varieties. Further 4^0 and 5^0 veins also occur. These are randomly oriented and form the reticulum. Three to four celled bundle sheath covers the 1^0 vein/midrib. The smallest areas formed by the veinlets are known as vein islet or areole. These are of variable shape, rectangular to polygonal and contain one to many vein endings. Size of the areole differs in both the varieties and the statistical results are significant between both the varieties. In bitter male plants the areoles are very small. The number of veinlets entering in an areole and vein-endings per areole is also variable and are statistically significant. Male bitter plant stand distinct in having the maximum number of vein-endings per square mm and in the number of veinlets entering in an areole (Table 2.1, 2.2 & 2.3). Table 3 exhibits the degree of variability of anatomical characters in both the varieties.

On the basis of the classical work of Ettinghausen[8], Foster[9], Hickey[10], Carlquist[11,12] and many others have stressed that the venation

pattern of the leaves has potential significance in taxonomy. Gupta[13], Levin[14], Varghese[15] are of the view that number of vein-islets and vein-endings are constant for a species and can be used as a valuable specific character. In the present investigation it has also been observed that bitter and non-bitter varieties can be distinguished on the basis of these characters. In addition to this they also differ in leaf size and leaf base. However, in contrast to this view Sehgal and Paliwal[16], Singh *et al.*[17], Gupta and Bhambie[18] are of the view that the number of vein-islets and vein-endings can not serve as an aid in identification either at the specific level or at varietal level. According to Wallis[19,20] the modern science of quantitative analytical microscopy is a very important tool in the identification of medicinal plants. Kshetrapal *et al.*[21] have been emphasized the importance of quantitative microscopy in distinguishing the varieties of *Crotalaria medicagenia*. Choudhary[22] also holds a similar opinion for varietal differentiation in *Tylophora indica*. Kshetrapal *et al.*[23] provided a diagnostic key utilizing characters of analytical microscopy for the identification of certain species of Helianthoideae. Present study concluded that the leaf architecture can be an important tool in the identification of varieties. The above conclusion is based on the results of statistical analysis of microanatomical characters of leaves as all the results are statistically significant. However, other aspects including cytology, biochemistry, embryology, palynology etc. are to be investigated so as to establish varietal differentiation in *Tinospora cordifolia*.

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