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STUDIES ON STOMATAL INDEX (S.I) AND ASSOSIATED FEATURES AMONGST TEN DIFFERENT *Medicago* L. Spp. FOR ASSESSING THE SPECIES INTER-RELATIONSHIPS

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Abstract : The foliar epidermal and stomatal features of all the ten *Medicago L* species included in this investigation have presented the same general patterns. Amphistomatic leaves, frequency of stomata appreciably higher at the abaxial surfaces as compared to adaxial ones and typical anomocytic stomatal types in all the taxa tend to indicate that the species selected for studies here form a natural assemblage within the genus *Medicago.*

However,the quantitative data pertaining to Stomatal Index (S.I) and other associated features have thrown quite useful light on the species inter-relationships. The data recorded co-ordinately in terms of mean stomatal length and total stomatal frequency / mm^2 in the form of pictorial graph have clearly indicated at the very first glance, formation of different clusters amongst the species. More valuable informations in terms of species inter-relationships appear to have been provided by the Stomatal Index (S.I) data. The S.I values have indicated closest affinity amongst *M. interexta* (21.90 %), *M. rugosa* (21.85 %) and *M .polymorpha* (21.75 %) and almost a closer affinity of this group of species with *M. scutellata* (22.00 %). The S.I values have suggested that *M. sativa* (19.80 %) stands closest to *M. orbicularis* (19.39 %).The increase in ploidy level of some of the selected taxa, did not seem to affect the stomatal counts / mm^2 as well as their size.

Key words : Frequency of epidermal cells / mm^2, Stomatal frequencies / mm^2, Mean stomatal lengths, Stomatal Index (S.I), pictorial graphic representation, species inter-relationships.

INTRODUCTION :

Popularly known as Medick, Lucerne as well as Alfalfa the genus *Medicago L.* of the tribe Trifolieae happens to be an agronomically as well as economically very important Fabaceous member. Capable of growing under diverse agro-climatic conditions, the species are mainly herbaceous perennials as well as annuals (Lesins and Lesins, 1979). They are said to be native of Iran and foot hills and mountains of middle Asia. Ladakh is designated as one of the centres of origin of *Medicago* by De Candole (1967). Medics are widely cultivated in USA, Argentina and India. The plants are used particularly as pasturage. However, during last 2-3 decades, the importance of this legume has been widely realised as a protein rich fodder for all kinds of livestocks.

The annual *Medicago* group is said to be represented in nature by about 30 species (Lesins and Lesins, loc. Cit.). In their review of breeding annual medics for Australian conditions, Crawford, Lake and Boyce (1989) have emphatically referred to only 13 important species including *M. aculeata ,M. arabica, M. intertexta, M. littoralis, M. rugosa, M. scutellata, M. tornata, M. truncatula, M. turbinata.* Though India is a home of about eight species (Wealth of India-1962), including M. *falcata, M. polymorpha*, (Syn. *M. denticulata*) and *M .lupulina, M. sativa* happens to be the most popular, presently widely adapted species. Mehra(1971) has opined that *M. sativa* has been one of the important forage crops introduced into India.

Heyn (1963) observed that amongst members of genus *Medicago,* one finds great difficulties in identification, classification and elucidation of their phylogenetic relationships due to extreme degree of variations in their vegetative as well as pod characters and more importantly due to occurrence of intermediate forms within the species. Ahmed and Taia (1974) using numerical taxonomic parameters have also revealed partial agreement with Heyn's concept.

At this juncture, it becomes very important to mention here the observations made by Munir et.al. (2011). While studying the "taxanomic potential of foliar epidermal anatomy among ten wild culinary vegetables", including only two from *Medicago* species (*M. falcata* and *M. polymorpha)*, they have observed that "as far as we know, no literature is available on the quantitative analysis of foliar epidermal features of *Medicago* species" and their efforts on both the above mentioned species in this direction is probably the first attempt. Encouraged with above observation of Munir

et.al.(loc.cit.),the present quantitative studies on altogether ten *Medicago* species have been taken up here in the minutest details and some extremely valuable taxonomic and phylogenetic data have been tried to be obtained.

MATERIAL AND METHODS:

The seeds of majority of the ten different *Medicago* L. species namely *M. intertexta* (L)Mill (2n=2x=16), *M. littoralis.* Rhode (2n=2x=16), *M .lupulina* L (2n=2x=16), *M. orbicularis* (L) Bartal (2n=2x=16),, *M. polymorpha* L (2n=2x=14), *M. rugosa* Desr. (2n=4x=30), *M. sativa* L (2n=4x=32), *M. scutellata*(L.)Mill (2n=4x=30), *M. tornata* (L.)Mill (2n=4x=32), *M. truncatula* Garten (2n=2x=16),.,were obtained through the courtsey of NBPGR , Pusa complex, New Delhi as well as Birsa Agriculture University, Kanke, Ranchi, but for *M .lupulina* and *M.polymorpha,* which were collected locally from Patna University Campus. The seeds were grown in the experimental garden of Department of Botany; Patna University for the detailed investigation .It will not be out of place to mention here that the somatic chromosome counts as well as detailed karyomorphological studies for the above mentioned species have also been undertaken in this laboratory, which will be published in a separate communication.

Foliar epidermal and stomatal studies were made from the fresh materials. Epidermal peels were prepared from both the surfaces of middle portion of an average-sized leaf, approximately half way up the leafy portion of the stems. These epidermal peels were directly transferred to the separate specimen tubes containing about 5 c.c. of freshly prepared fixative mixture of acetic-alcohol in the proportion of 1:3.The peels were then stored for a week to allow the softening of tissues. Finally, a homogenous piece of the peel was selected from the storage fluid and mounted in glycerol for study under the microscope. The quantitative data were collected from these temporarily mounted slides.

The measurements are based on an average of 10 reading and have been recorded in the tabular form. Terminology used here is the same as suggested by Metcalfe & Chalk (1950).

Stomatal Index (S I) : The S I was calculated using formula given by Salisbury (1972) i.e

$$S\,I = \frac{S}{S+E} \times 100$$

Where **S** denotes the number of stomata per unit area and
E the number of epidermal cells in the same unit area.

RESULT AND DISCUSSION :

Typical anomocytic type of stomata has been uniformly observed on both the surfaces of all the species. The detailed data pertaining to the frequency of epidermal cells / mm^2, stomatal frequency /mm^2 as well as stomatal length in μ amongst different selected taxa have been summarized in Table 1.

The values of mean stomatal frequencies vs. mean stomatal lengths of all the species under reference have been tried to be depicted through pictorial graph (Fig 1) and quite useful informations have been retrieved regarding interaffiliation of the species. The Stomatal Index (S.I) values of the different species have been presented here in **Table 1**. The S.I values have shown a range of variations from a minimum of 17.00 in *M. tornata* to the minimum of 7.84 in *M. truncatula* .

TABLE - 1

Foliar Epidermal and Stomatal Features in *Medicago* Spp..

Sl. No.	Name of Species	Frequency of Epidermal Cells / mm Sq			Stomatal Frequency/ mm Sq			Stomatal Length in µ			Stomatal Index (S I) i.e $\frac{S^*}{S+E^*} \times 100$
		Lower	Upper	Total	Lower	Upper	Total	Lower	Upper	Mean	
1.	*M. intertexta*	43.40	38.56	81.96	16.20	6.80	23.00	29.65	25.40	27.52	**21.90**
2.	*M. littoralis*	36.40	38.20	74.60	15.80	6.30	22.10	27.03	15.07	26.05	**23.50**
3.	*M. lupulina*	18.40	31.40	49.80	14.15	4.07	18.22	30.51	26.31	28.41	**26.78**
4.	*M. orbicularis*	42.00	34.20	76.20	12.41	5.90	18.31	31.27	27.33	29.30	**19.39**
5.	*M. polymorpha*	52.52	42.78	95.30	16.96	8.57	25.53	31.00	25.02	28.01	**21.75**
6.	*M. rugosa*	30.70	49.32	80.02	16.01	5.49	21.50	30.24	25.90	28.07	**21.85**
7.	*M. sativa*	29.40	46.20	75.60	12.97	5.03	18.00	28.19	27.00	27.59	**19.80**
8.	*M. scutellata*	36.56	41.44	78.00	16.53	5.47	22.00	30.10	26.04	28.07	**22.00**
9.	*M. tornata*	32.19	61.10	93.29	13.29	5.32	19.24	25.94	25.10	25.52	**17.00**
10.	*M. truncatula*	25.36	39.15	64.51	17.36	7.54	24.90	27.28	25.38	26.33	**27.84**

*S = Stomatal Frequency / mm. sq.
*E = Epidermal Frequency / mm. sq.

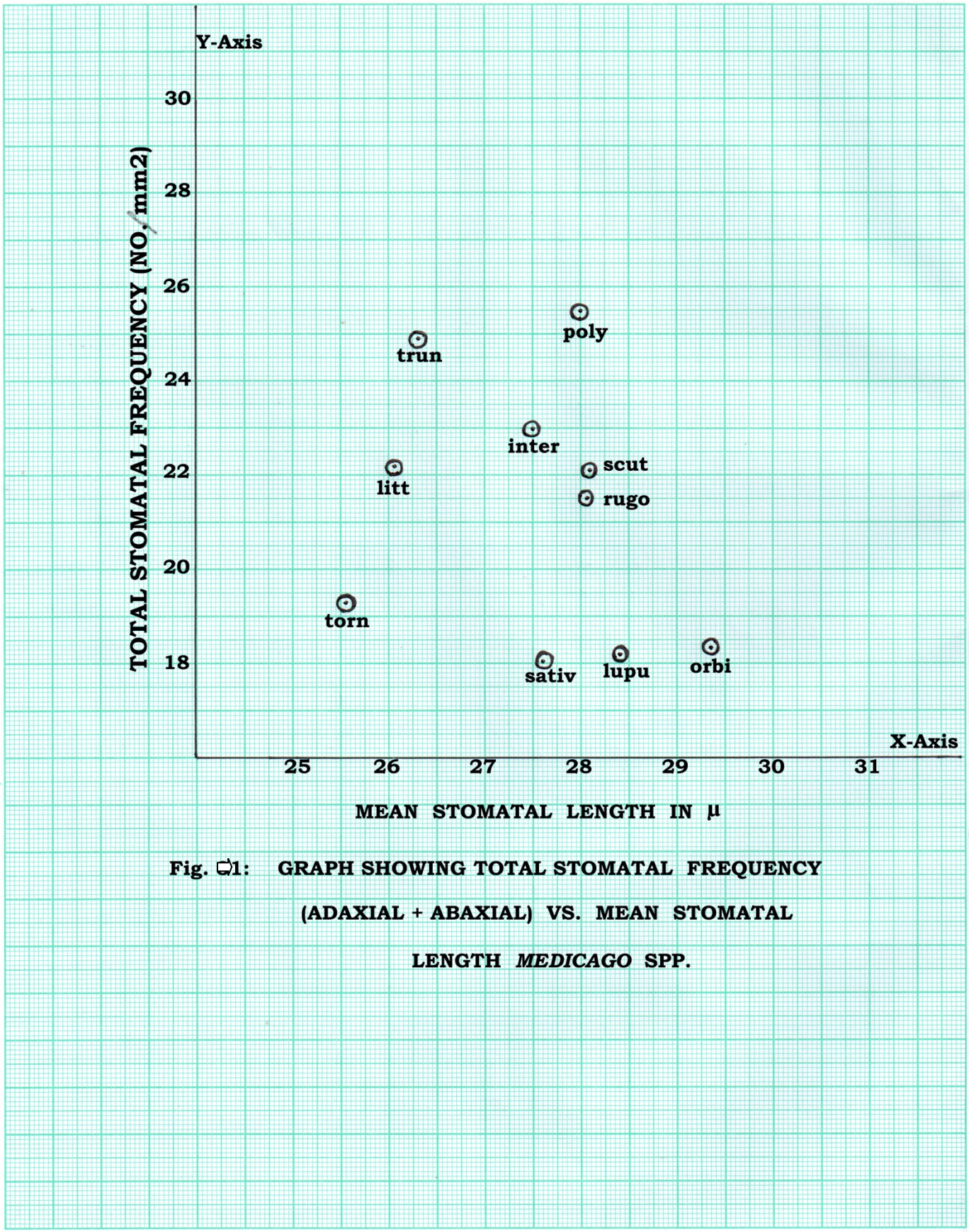

Fig. 1: GRAPH SHOWING TOTAL STOMATAL FREQUENCY (ADAXIAL + ABAXIAL) VS. MEAN STOMATAL LENGTH *MEDICAGO* SPP.

Abbreviations:- trun = *M. truncatula* , poly= *M. polymorpha* , inter = *M. intertexta* , litt = *M. littoralis* , scul = *M. scutellata* , rugo = *M. rugosa* , torn = *M. tornata* , sativ = *M. sativa* , lupu = *M. lupulina* , orbi = *M. orbicularis* .

The pictorial graph prepared from the quantitative data in **Table 1** at the very first glance indicates that six of the selected species namely *M. intertexta* ,*M. scutellata, M. rugosa*, *M. polymorpha*, *M. truncatula* and *M. littoralis* form a cluster at the upper side of the graph, while the other three species, namely *M. sativa*, *M. lupulina* and *M. orbicularis* form another linear cluster on the lower side. In this respect *M. tornata* stands apart in isolation. It is to be mentioned here that *M. scutellata*, *M. rugosa* and *M. sativa* are tetraploids and rest all other species are diploids.

In the upper association of the species *M. intertexta* appears to occupy a central position, while other species seem to encircle it peripherally. If the peripheral species of this association are again looked back, the tetraploid *M. scutellata* appears to stand not only closest to the tetraploid *M. rugosa* but it (*M. scutellata*) also shows greatest affinity to the diploid *M. intertexta* and relatively greater affinity to *M. polymorpha* situated on the same side of the pictorial graph **(Fig. 1)**

Another important aspect of the present foliar studies has been the complete synthesis of the related data in the form of Stomatal Index **(S.I)**.Valuable informations in terms of species diversity vis-à-vis their interrelationships appear to have been obtained. Although the SI percentage values have presented a range of variations from a minimum 17.00 in *M. tornata* to a maximum of 27.84 in *M. truncatula* **(Table 1)**, they have clearly indicated the closest affinity amongst *M. intertexta* (21.90), *M. rugosa* (21.85) and *M. polymorpha* (21.75) and almost a closer affinity of this group of species with *M. scutellata* (22.00). This is totally in conformity with the picture of species interrelationships indicated earlier in the pictorial graph **(Fig.1)**. The S.I values of *M. sativa* (19.80) and *M. orbicularis* (19.39) also bring them closer to each other as compared to rest of the species studied here. However, the above findings on species inter-relationships observed here, need a closer scrutiny, specially at the detailed karyomorphological and meiotic levels. The synthetic data thus obtained may provide some valuable helps for the future alfalfa breeding programmes.

Although an increase in ploidy level is usually thought to result in increased stomatal counts as well as their size (Sax, 1938), the mean values of stomatal size in tetraploid species namely *M. rugosa* (28.07µ), *M. scutellata* (28.07µ) and *M. sativa* (27.59µ) show a maximum similarity with the diploid *M. lupulina* (28.41µ) and *M. intertexta* (27.52µ) and also an almost near similarity with rest of the diploid counterparts. Similar observations have also been made in terms of stomatal counts **(Table 1)**. Thus the increase in ploidy level does not seem to affect this very foliar character in the *Medicago* Species studied here.

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