Chapter – II

Morphological and anatomical features of different mulberry varieties with feeding response

2.1 INTRODUCTION

Silkworm (*Bombyx mori* L.) is a monophagous insect, depends on mulberry leaves for their growth and development. Besides environment and technology adaptation, nutritive value of mulberry leaves is the most important factors for larval growth, silk production and fibre quality (Purohit and Pavankumar, 1996; Nagaraju, 2002; Seidavi *et al*., 2005). Mulberry cultivars with superior quality leaves help to enhance good cocoon crop (Ravikumar, 1988). Different morphological and physiological attributes are considered to develop high yielding mulberry varieties (Susheelamma *et al.,* 1988; Sahu *et al.,* 1995). Through literature survey it is evident that leaf biomass production and yield of different crops depend on the CO_2 assimilation through photosynthesis (Menon and Srivastava, 1984). Photosynthesis is a foremost physiological attribute that is directly correlated with stomatal frequency of the leaves. Susheelamma and Jolly (1986) reported that adaptation of mulberry in tropical region of India is dependent on stomatal size and stomatal frequency of mulberry leaves. In the recent past, stomata are a prime morphological feature of the leaves that have been studied from the taxonomical view point (Fagundez and Izco, 2011; Kaya *et al.,* 2011). Southwood (1986) focused on mulberry leaf surface which determine its adaptation in various eco-climates.

Another major micro-morphological attributes of leaves are trichomes. Trichomes are the unicellular or pluricellular outgrowths found in different plant parts namely leaves, shoots and roots. Pattern of the trichome and idioblasts have been used for identification of mulberry genotypes in Japan (Fujita and Uchikawa, 1986). Baur *et al.* (1991) reported that the presence of trichome on leaf surface constitute a mechanical barrier that hinders herbivory insects feeding. Better acceptability by silkworm larvae and effective rearing performance are influenced by type of trichomes, the density of trichome and its distribution on mulberry leaf surface (Kesavacharyulu *et al.,* 2004). Kesavacharyulu *et al.* (2004) also stated that leaf acceptability by larvae was decreased with high density of trichomes present on the mulberry leaf surface. Several kinds of plant trichomes have different physiological and ecological functions (Johnson, 1975). It has long been recognized that trichomes have significant defence mechanism against herbivory (Navasero and Ramaswamy, 1991). Non-glandular trichomes cause obstruction for silkworm larval feeding. The exudates of glandular trichomes fabricate toxic effects for phytophagous insects. Kesavacharyulu *et al.* (2004) established a relationship between ratio of glandular and non glandular trichomes present in mulberry leaves and economical attributes of silkworm rearing. Sericulture in West Bengal, a humid sub-tropical region of India, suffers a major problem due to lack of adequate quantity of mulberry leaves with high foliar nutrition throughout all season. There is an opportunity to increase silk production from present, but is possible only when more mulberry cultivars are explored for rearing to meet overall leaf requirement in sericulture. Therefore, it becomes essential to select mulberry cultivars, which can sustain normal growth of larvae during all season for better yield.

The present study was undertaken to evaluate the relationship of micro-morphological attributes of mulberry leaves with their acceptability by silkworm larvae. To increase cocoon production, it is becoming essential to choose superior mulberry cultivars for silkworm strains. For determining the importance of mulberry cultivars for silkworm rearing, micro-morphological features of leaves of seven mulberry cultivars were evaluated by light and scanning electron microscopy (SEM). This study might assist superior mulberry cultivar selection for commercial exploitation suitable for different agro-climatic regions in India.

2.2 MATERIALS AND METHODS

In present study, seven popular mulberry cultivars, mostly used in West Bengal for silkworm rearing, was selected namely S1, V1, K2, S1635, Kosen, Bombay local and Dudhiya. These were obtained from Malda Sericulture Farm, Malda, West Bengal, India. Herbarium specimens of *Morus alba* L. var. S1, S1635, V1, K2, Kosen, Dudhiya germplasm and Bombay local (cultivars) were submitted bearing accession numbers (Acc. No. 09837-09843) in NBU Herbarium (University of North Bengal).

2.2.1 Study location

The study area, Malda district of West Bengal is located at 25˚ 32′ 08″ N and 88˚ 28′ 10″ E longitude and situated at the left bank of Ganga river (Chowdhury and Mukherjee, 2012). The weather is usually extremely humid and tropical. Temperatures can reach as high as 46˚C during the day in May and June and fall as low as 4˚C overnight in December and January. The winter season arrives in Malda district in the middle of November and continues till the last of February. Winter is succeeded by summer in the months from March to May. After the summer season, the city witnesses a rainy season that begins in the month of June and ends by the middle of September. The rains in this city are the result of the south-west monsoons. Normally, the rainfall in the area is 1453.1 mm. The brief season after rains and before the arrival of winter is the period referred to as the post-monsoon period. This season lasts for about one and a half month and is characterized by cool weather.

Another study place, Siliguri is located at 26.71˚ N and 88.43˚ E. Siliguri city, the gateway of North-East India is situated on the foothills of Himalayas and banks of Mahananda River. The temperatures of subtropical region of Siliguri can reach 32˚-34˚C during the month of May to June and fall as low as 2˚C overnight in December to January. The monsoon season generally starts from middle of the July and last till the September. The average rainfall was recorded up to 200 mm/day of the said months.

2.2.2 Study methods

2.2.2.1 Rearing of silkworm larvae

According to well established method (Krishnaswami *et al.,* 1978), the disease free layering (DFL) of 5^{th} instar silkworm larvae were reared in the laboratory. F1 hybrid (Nistari × bivoltine) was reared under an optimal temperature (27˚-29˚C) and humidity (70 ± 5%). Fresh and healthy leaves of these seven selected mulberry cultivars were used for larval feeding. Prior to initiation of rearing, the total rearing room and all ingredients used in this rearing process were carried out as preventive measure against pathogens. The trays were placed under adequate ventilation. Disinfection of the rearing room was strictly maintained at rearing time. Hands were sterilized with dettol solution before handling the worms during the rearing time. To maintain room temperature and humidity, one thermo-hygrometer was used near the larval bed. The grass of larvae was continuously discarded from the tray. Dead larvae if found, during the rearing period were immediately removed. According to Gangwar, (2010), different economical attributes such as larval weight, mortality percent, single cocoon weight, single shell weight were calculated separately. The weight of larvae in each tray was monitored by weighing them on weighing balance daily and the growth rate pattern of caterpillar was calculated. When larvae started spinning they were left uninterrupted for four to five days to form the cocoon. After complete cocoon formation, the weight of cocoon of each set was measured. Cocoon shell weight was also measured after release of the moth from cocoon shell. Weight of male and female moth was recorded separately. Number of eggs laid by per pair of moth in each set was noted. Growth index, shell ratio, effective rearing rate (ERR%) were calculated by formulae (given below). The collected data was subjected for graphical and statistical analysis (Bailey, 1955).

Shell ratio (%) = $\left[\left(\frac{Single\ \ shell\ weight\ (gm)}{Single\ \ cocoon\ \ weight\ (gm)}\right)\times 100\right]$

ERR (%) = $\left[\left(\frac{Total\ \ no.of\ cocoons\ \ harvested}{Total\ \ no.of\ larvae\ \ brushed}\right)\times 100\right]$

Weight of single cocoon (WSC) = $\frac{(Weight\ of\ 10\ male\ \ cocoons\ \ +weight\ of\ 10\ female\ \ cocoons\ )}{No.of\ \ cocoons\ \ taken\ \ (20\ nos.)}$

Growth Rate (%) = $\left[\left\{Average\left(\frac{Final\ \ weight\ of\ larvae\ -Initial\ \ weight\ of\ larvae}{Initial\ \ weight\ of\ larvae}\right)\right\}\times 100\right]$

2.2.2.2 Light microscopic and scanning electron microscopic analysis

Small portion of leaves had been slashed between the apex and base of leaves equidistant between the margin and midrib (Funmilola Mabel *et al.,* 2014). To remove chlorophyll from leaves, the sizeable portions were boiled in 80% alcohol for 30 min. After taking it out from alcohol, it was washed thoroughly with water to remove the alcohol and cleared with brush. If leaves remained unclear after brushing, it was boiled again in lactic acid. To investigate qualitative and quantitative micro-morphology of mulberry leaves, the desired samples were mounted in glycerine and observed under light microscope (Olympus trinocular microscope). Number of the stomata was determined from an average of ten counts on abaxial surface of the leaves. The area of the stomata and trichome were determined by measuring the length and width of each parameter. Stomata Index was determined by using the following formula:

$$SI = \left(\frac{S}{S+E}\right)\times 100$$

Where, S stands for the number of stomata and E for number of epidermal cell.

A camera lucida was used for drawing of stomata with epidermal cell, which helps to determine stomatal frequency. Dendrogram analysis was performed on the basis of similarity co-efficient with the help of XLSTAT 2015 software.

According to Bozzola and Russell (1992), the leaf samples were prepared for scanning electron microscopy (SEM). Leaf samples was cut into 3 mm^2 and dehydrated in an alcohol-acetone series at room temperature. The abaxial surface of the dried samples was mounted on the stub with the help of double sided carbon tap. Sample was coated with gold in ionic coater and examined under scanning electron microscope (ZESIS EVO 18 SEM, Germany). The morphometry of stomata and trichome were measured by using the software attached with SEM.

2.3 RESULT AND DISCUSSION

Mulberry cultivars were categorized on the basis of leaf yield (kg/ha/season) and micro-morphological attributes. S1 and S1635 were categorized as high yielding cultivars, whereas Dudhiya, Bombay local and Kosen formed another group with lowest yield (Figure 2.1). V1 and K2 had moderate leaf yielding capacity with 5691.44 kg / ha / season and 5032.63 kg / ha / season respectively. All cultivars follow a distinct trend in case of micro-morphological attributes of leaves. Stomata are fundamental micro-morphological characters responsible for diffusion of gases and transpiration (Kumar *et al.,* 2012). Adaxial leaf surface of all mulberry cultivars were observed without stomata. Kumar *et al.,* (2012) also reported similar kind of observation on adaxial surface in various mulberry cultivars. Distribution, shape and size of stomata were widely varied among the cultivars (Figure 2.2 and 2.3). Camera lucida drawing helps to count number of stomata with respect to number of epidermal cells, thus determining stomatal index (Figure 2.4). Maximum stomatal index was recorded in S1635. High Stomatal frequency was found in abaxial surface of Kosen followed by Bombay local and Dudhiya. Stomatal number per unit area was low in case of S1 followed by K2 and S1635 (Table 2.1). V1 had moderate stomatal density (209.26/mm^2) with small stomatal size (58.86 µm^2). It was already reported that there was a correlation between stomatal size and leaf moisture retention capacity in mulberry plants having high water potential (Susheelamma and Dutta, 1993, Singhal *et al.,* 2010). The large pore sized stomata leads to reduce leaf biomass production due to excess loss of water through higher transpiration rate. In case of Dudhiya, pore size of stomata was higher than other cultivars. S1 had stomata with approximately 63-65 µm^2 pore size, whereas Dudhiya had average 181.03 µm^2. Rest of cultivars showed pore size of stomata in between these two ranges. Yield of leaves was correlated with rate of photosynthesis. On the other hand, photosynthesis is dependent on stomatal frequency and size of stomata present on respective leaf surface (Maghsoudi and Maghsoudi moud, 2008). As S1635 and S1 showed lower stomatal frequency with small stomata, they had high leaf yielding capacity. Correlation matrix (Figure 2.5) revealed that leaf yield was negatively correlated with stomatal frequency and size. Therefore, Dudhiya, Kosen and Bombay local had low yielding capability due to excess loss of water through larger stomata. In this regards, Susheelamma and Datta (1993) also reported that small stomatal size was correlated with moisture retention capacity of mulberry leaves. Mulberry leaf acceptability by larvae was also negatively correlated with stomatal frequency and size.

Present study also revealed that mulberry cultivars had significant variation in trichome and idioblast density. Light microscopic study showed that number of the idioblast per unit area was high on adaxial surface of Bombay local and Kosen leaf. S1635 had lowest idioblast density (Table 2.2 and Figure 2.6).

Light microscope and SEM study exposed that trichome density was high on abaxial surface rather than adaxial surface (Figure 2.7). Light microscopic and scanning electron microscopic study revealed that two kinds of trichomes were found in all mulberry cultivars; namely glandular (GT) and non-glandular (NGT) trichomes (Figure 2.8 and 2.9). Non-glandular trichome density was high in Kosen, whereas, high GT density was recorded in V1 cultivars (Table 2.2). S1635 and S1 exhibited lowest density of both GT and NGT. Lowest GT and NGT ratio was recorded in Kosen followed by Bombay local, S1635, Dudhiya, S1, K2 and V1. Though the Dudhiya and Bombay local showed moderate trichome density, but the length of trichome of these two cultivars was much longer in comparison with others. Trichome density and the length of the trichome were negatively correlated with silkworm feeding preference and other economical attributes of silkworm rearing (Figure 2.5). Similar observation was recorded by Kesavacharyulu *et al*., (2004). According to this report, mulberry genotypes with highest foliar production might have poor acceptability by silkworm larvae.

2.3.1 Feeding preference

Acceptability of different mulberry cultivars by larvae was measured by different economical attributes of silkworm rearing. Larval weight and cocoon production depends on foliar nutrition which ultimately differs with variation of cultivars (Subhan *et al.,* 2013).

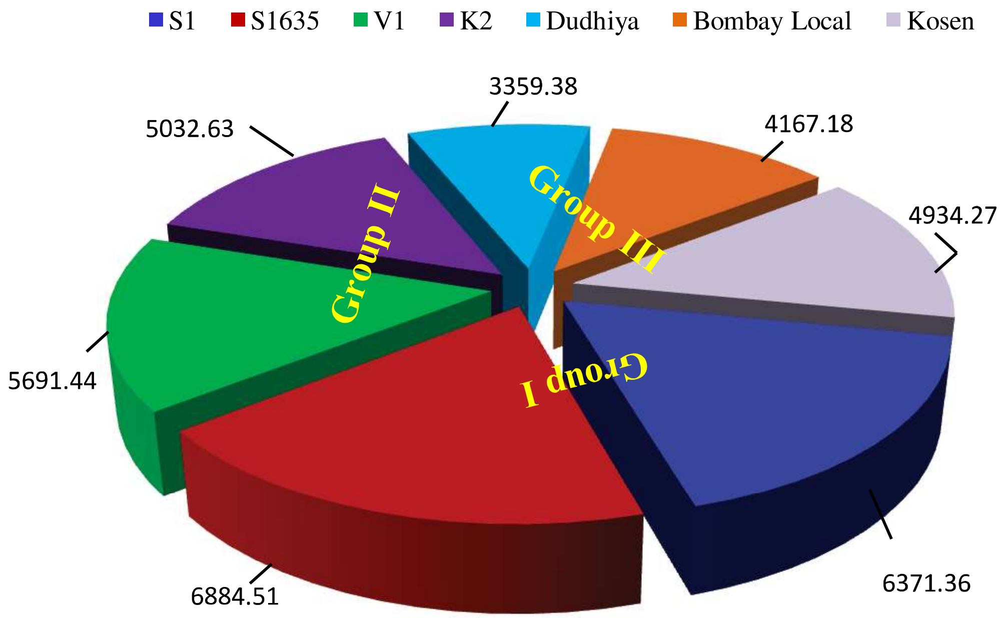

Figure 2.1: Leaf yield (kg/ha/season) of seven mulberry varieties

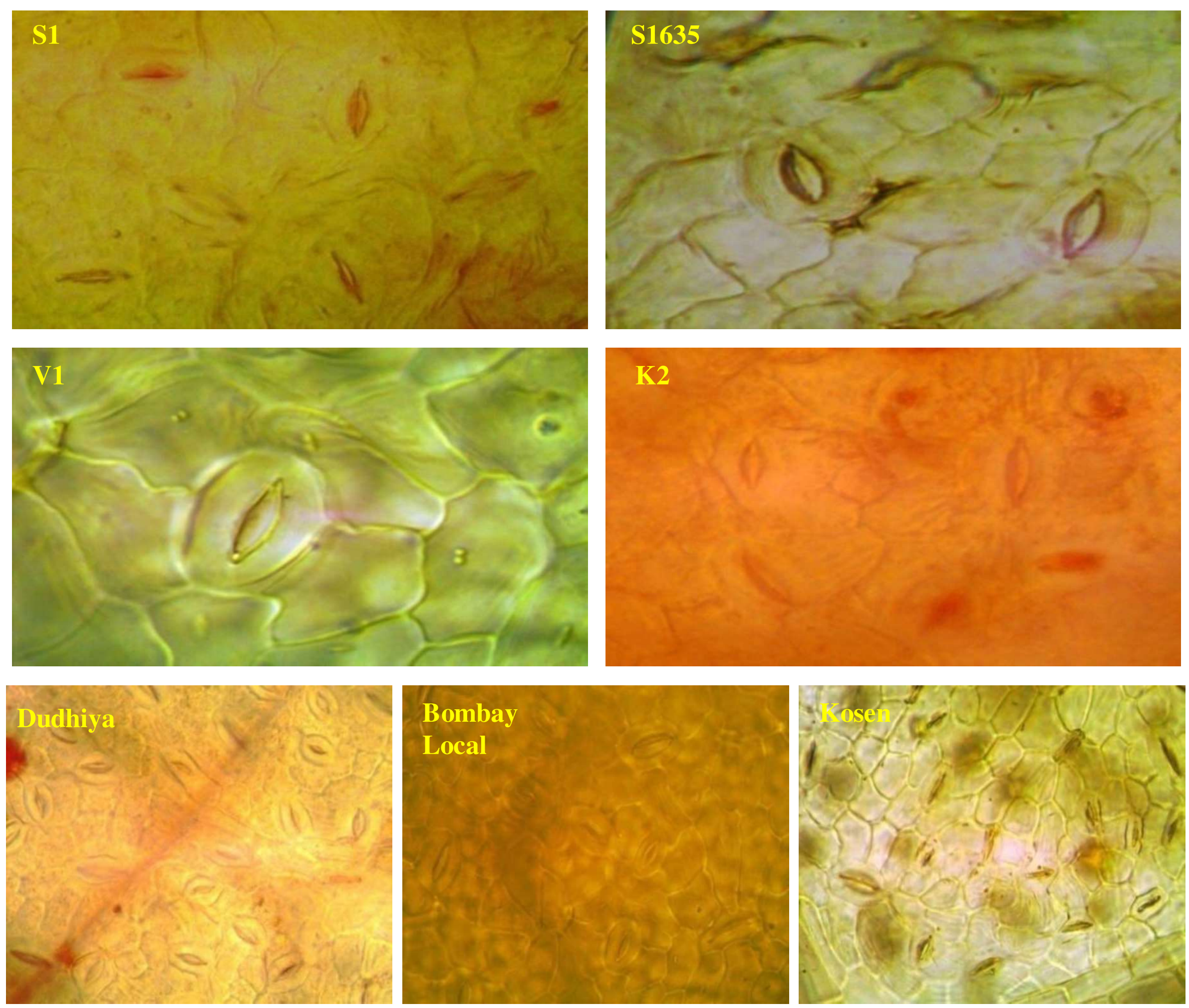

Figure 2.2: Light microscopic photograph of seven mulberry genotypes showing the comparison of stomata present on the abaxial surface of mulberry leaf (Under 40× resolution)

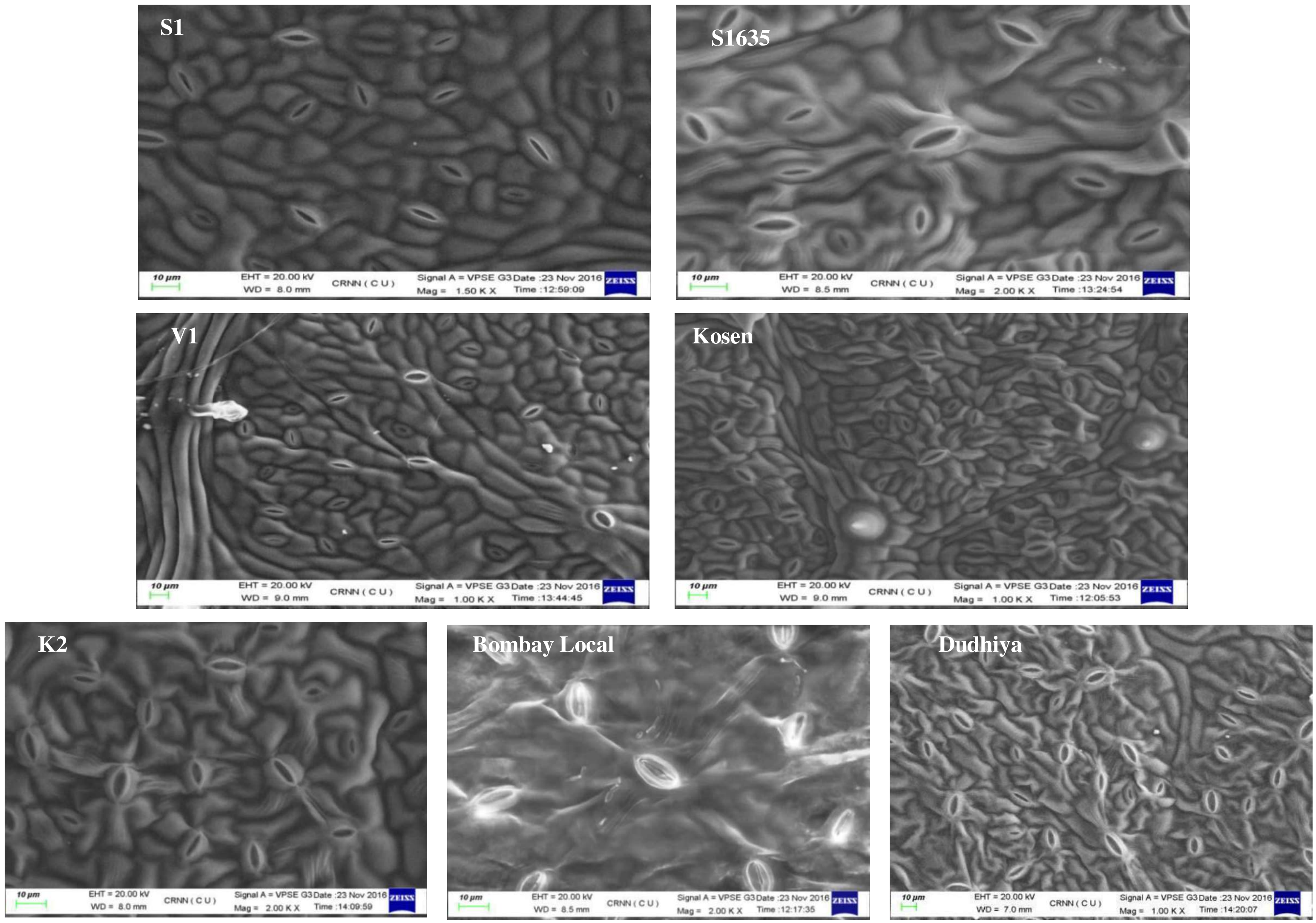

Figure 2.3: Scanning electron microphotograph (SEM) of seven mulberry genotypes showing the comparison of stomata present on the abaxial surface of mulberry leaf

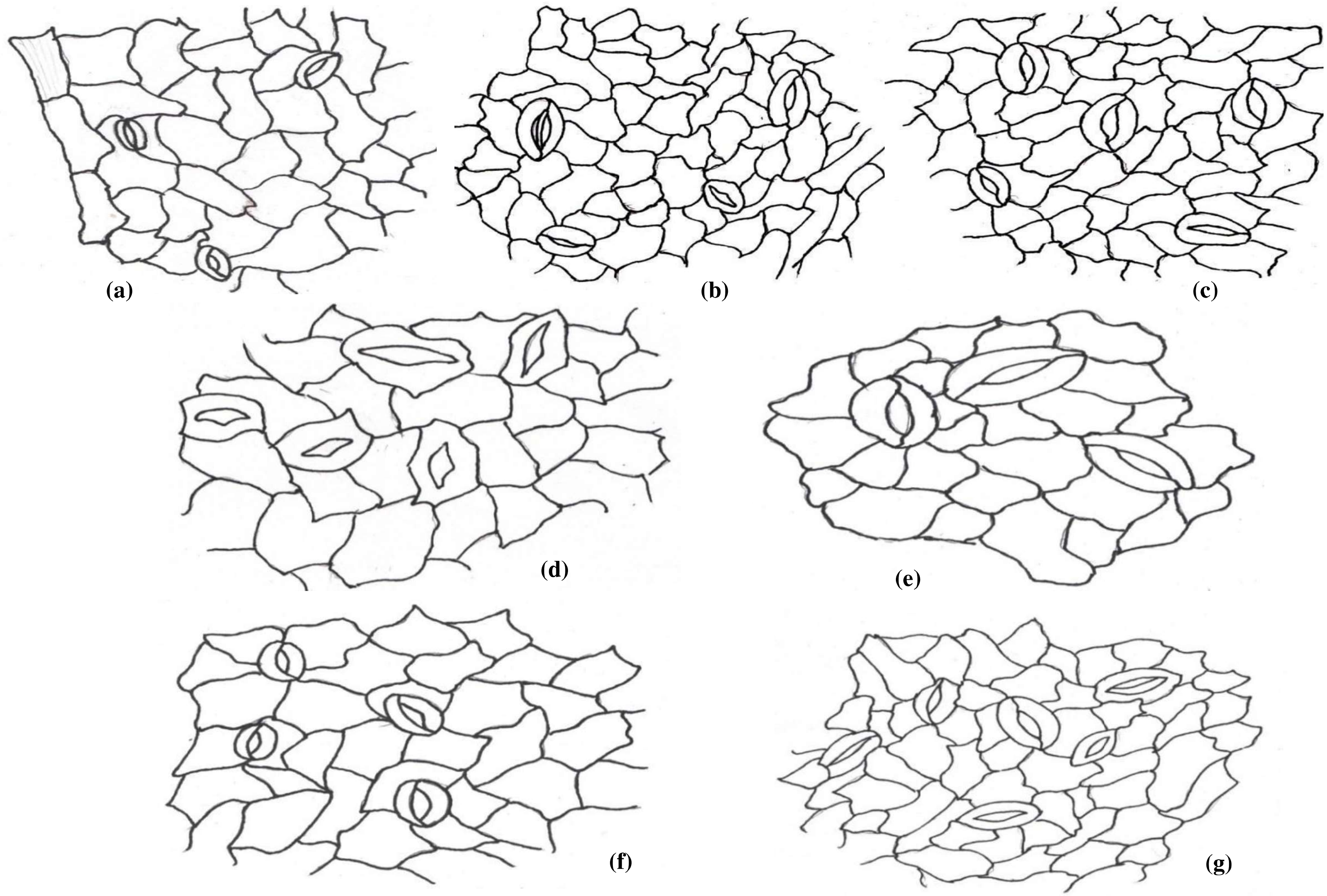

Figure 2.4: Camera Lucida drawing of stomata with epidermal cell of seven mulberry cultivars (a: S1; b: S1635; c: V1; d: K2; e: Dudhiya; f: Bombay local and g: Kosen)

Table 2.1: Comparison of stomatal fractures of the seven mulberry genotypes

Cultivars	SI	SF	SL (µm)	SB (µm)	SL+EL (µm)	SB+EB (µm)
S1	23.23 ± 0.045^{b}	127.78 ± 0.33^{a}	12.59 ± 0.07^{b}	5.06 ± 0.009^{d}	21.37 ± 0.045^{b}	17.46 ± 0.061^{c}
S1635	24.35 ± 0.06^{a}	174.07 ± 0.41^{c}	12.03 ± 0.03^{a}	4.11 ± 0.008^{a}	21.04 ± 0.052^{a}	16.33 ± 0.047^{a}
V1	16.40 ± 0.027^{d}	209.26 ± 0.41^{d}	13.47 ± 0.09^{c}	4.37 ± 0.006^{c}	23.43 ± 0.061^{d}	17.76 ± 0.044^{d}
K2	20.09 ± 0.061^{c}	162.96 ± 0.29^{b}	23.37 ± 0.05^{e}	6.89 ± 0.008^{f}	37.69 ± 0.068^{e}	23.32 ± 0.054^{e}
Dudhiya	15.03 ± 0.059^{e}	224.07 ± 0.42^{e}	23.51 ± 0.08^{f}	7.7 ± 0.007^{g}	46.34 ± 0.055^{f}	26.09 ± 0.029^{f}
Bombay local	16.36 ± 0.048^{d}	281.48 ± 0.38^{f}	27.16 ± 0.07^{g}	5.53 ± 0.007^{e}	51.26 ± 0.047^{g}	29.7 ± 0.051^{g}
Kosen	13.53 ± 0.046^{f}	322.22 ± 0.71^{g}	19.55 ± 0.04^{d}	4.34 ± 0.003^{b}	22.53 ± 0.036^{c}	17.15 ± 0.032^{b}

Results are represented as mean±SEM, n=3. Values with different letters (a, b, c, d, e, f & g) are significantly ($p < 0.05$) different from each other by Duncan's Multiple Range Test (DMRT).

Abb. Used: SI: stomatal index; **SF:** stomatal frequency; **SL:** length of stomata; **SB:** breadth of stomata; **EL** and **EB:** epidermal cell length and breadth respectively.

	LY	SI	SF	SL	SB	SL+GL	SB+GB	GT/NGT	NGTL	GTL	NGTD	GTD	TTD	ID	GR	ERR%	WSC	WSS	SR	FL	NBFL
SI	**0.782**																				
SF	-0.556	**-0.834**																			
SL	**-0.876**	-0.574	0.512																		
SB	-0.729	-0.219	-0.129	0.672																	
SL+GL	**-0.839**	-0.404	0.287	**0.909**	0.750																
SB+GB	**-0.809**	-0.383	0.282	**0.903**	0.707	**0.994**															
GT/NGT	0.228	0.011	-0.377	-0.419	-0.101	-0.279	-0.281														
NGTL	**-0.939**	-0.698	0.570	**0.934**	0.627	**0.934**	**0.928**	-0.242													
GTL	-0.488	-0.503	0.108	0.250	0.549	0.138	0.058	0.319	0.245												
NGTD	-0.448	-0.635	**0.870**	0.468	-0.087	0.167	0.166	-0.694	0.399	0.020											
GTD	-0.446	**-0.759**	0.358	0.119	0.168	0.009	-0.024	0.559	0.272	0.750	0.121										
TTD	-0.582	**-0.885**	**0.887**	0.441	0.009	0.142	0.125	-0.303	0.458	0.376	**0.880**	0.578									
ID	**-0.779**	**-0.797**	**0.893**	**0.755**	0.218	0.589	0.589	-0.570	**0.786**	0.076	**0.866**	0.222	**0.818**								
GR	**0.959**	0.751	-0.575	**-0.780**	-0.686	**-0.763**	-0.711	0.294	**-0.859**	-0.525	-0.474	-0.404	-0.583	**-0.762**							
ERR%	**0.925**	**0.806**	-0.745	**-0.780**	-0.489	-0.731	-0.703	0.423	**-0.876**	-0.293	-0.655	-0.329	-0.696	**-0.914**	**0.948**						
WSC	-0.076	0.095	0.098	0.020	0.053	0.005	0.008	**-0.767**	-0.030	-0.331	0.470	-0.423	0.184	0.333	-0.171	-0.289					
WSS	**0.890**	**0.837**	-0.695	**-0.802**	-0.519	-0.709	-0.663	0.106	**-0.861**	-0.582	-0.461	-0.530	-0.632	-0.732	**0.891**	**0.843**	0.221				
SR	**0.938**	**0.822**	-0.736	**-0.838**	-0.557	-0.738	-0.693	0.355	**-0.879**	-0.485	-0.617	-0.401	-0.700	**-0.855**	**0.963**	**0.952**	-0.089	**0.952**			
FL	**0.957**	**0.787**	-0.694	**-0.838**	-0.597	**-0.776**	-0.736	0.401	**-0.895**	-0.416	-0.604	-0.345	-0.662	**-0.867**	**0.981**	**0.981**	-0.202	**0.903**	**0.987**		
NBFL	**0.949**	**0.780**	-0.697	**-0.850**	-0.548	**-0.835**	**-0.810**	0.339	**-0.948**	-0.293	-0.543	-0.314	-0.597	**-0.871**	**0.948**	**0.978**	-0.134	**0.894**	**0.958**	**0.978**	
FS	**-0.951**	**-0.846**	0.594	0.739	0.655	0.638	0.596	-0.220	**0.799**	0.616	0.545	0.583	0.727	**0.769**	**-0.945**	**-0.906**	0.171	**-0.844**	**-0.917**	**-0.930**	**-0.875**

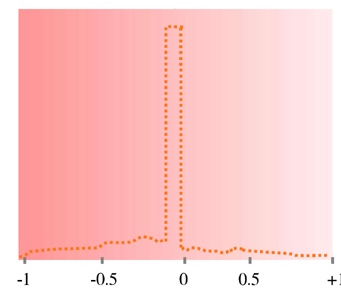

Abb. Used: GT: glandular trichome; **NGT:** non-glandular trichome; SI, SF, SL & SB: stomatal index, frequency, length and breadth respectively; ID: idioblast density, TTD: total trichome density; GR: growth rate; WSC & WSS: weight of single cocoon and shell respectively; SR: shell ratio; FL: filament length; NBFL: non-breakable filament length ;FS: filament size; ERR: effective rearing rate.

Figure 2.5: Correlation matrix between morphological attributes of mulberry leaves with different economic parameters of silkworm rearing

Table 2.2: Different attributes of trichomes and idioblast of seven mulberry genotypes

Name of the cultivars	Ratio of GT/NGT	NGT density	GT density	Total trichome density	Idioblast density
S1	1.67 ± 0.008^{c}	14.42 ± 0.056^{b}	24.04 ± 0.056^{b}	38.46 ± 0.082^{b}	27.78 ± 0.069^{b}
S1635	1.33 ± 0.004^{e}	14.42 ± 0.049^{b}	19.23 ± 0.037^{a}	33.65 ± 0.075^{a}	22.22 ± 0.059^{a}
V1	4 ± 0.007^{a}	9.62 ± 0.062^{a}	38.46 ± 0.051^{e}	48.08 ± 0.084^{d}	33.33 ± 0.08^{c}
K2	2 ± 0.007^{b}	14.42 ± 0.062^{b}	28.85 ± 0.048^{c}	43.27 ± 0.063^{c}	38.89 ± 0.063^{d}
Dudhiya	1.56 ± 0.005^{d}	21.63 ± 0.067^{c}	33.65 ± 0.054^{d}	55.29 ± 0.071^{f}	88.89 ± 0.081^{e}
Bombay local	0.83 ± 0.004^{f}	28.85 ± 0.072^{d}	24.04 ± 0.048^{b}	52.88 ± 0.066^{e}	116.67 ± 0.077^{g}
Kosen	0.78 ± 0.006^{g}	43.27 ± 0.086^{e}	33.65 ± 0.043^{d}	76.92 ± 0.071^{g}	111.11 ± 0.082^{f}

Results are represented as mean ± SEM, n=3. Values with different letters (a, b, c, d, e, f & g) are significantly ($p < 0.05$) different from each other by Duncan's Multiple Range Test (DMRT).

Abb. Used: GT: glandular trichome; **NGT:** non-glandular trichome.

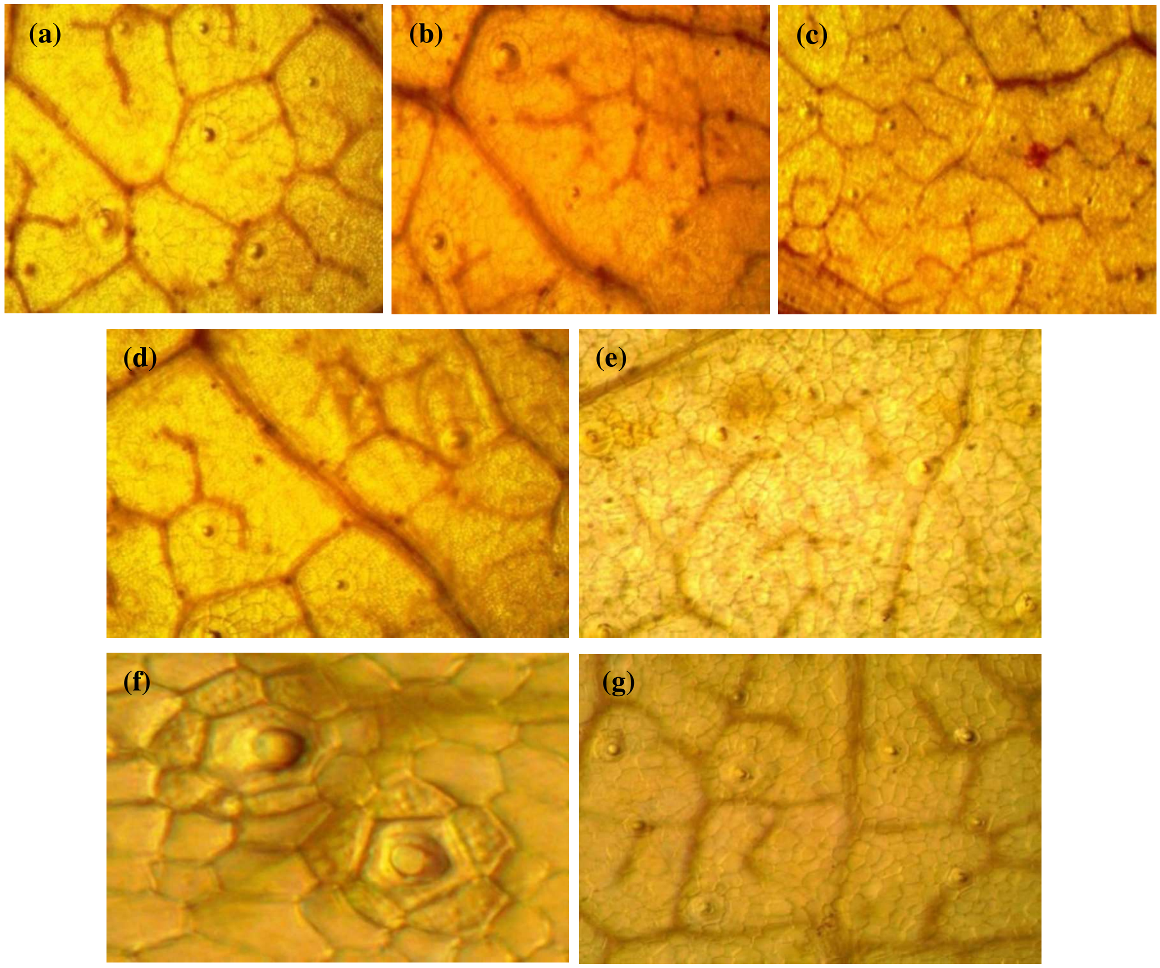

Figure 2.6: Light microscopic photograph of seven mulberry genotypes showing the comparison of idioblast present on the adaxial surface of mulberry leaf (Under 40× resolution), (a): S1; (b): S1635; (c): V1; (d): K2; (e): Dudhiya; (f): Bombay local; (g): Kosen

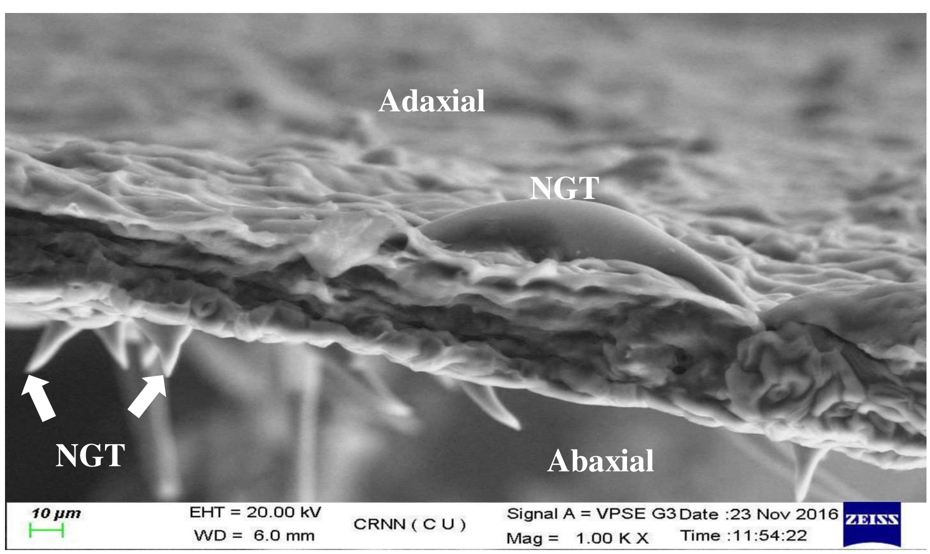

Figure 2.7: Scanning electron microphotograph (SEM) of mulberry leaf showing the comparison of trichome density on the adaxial and abaxial surface

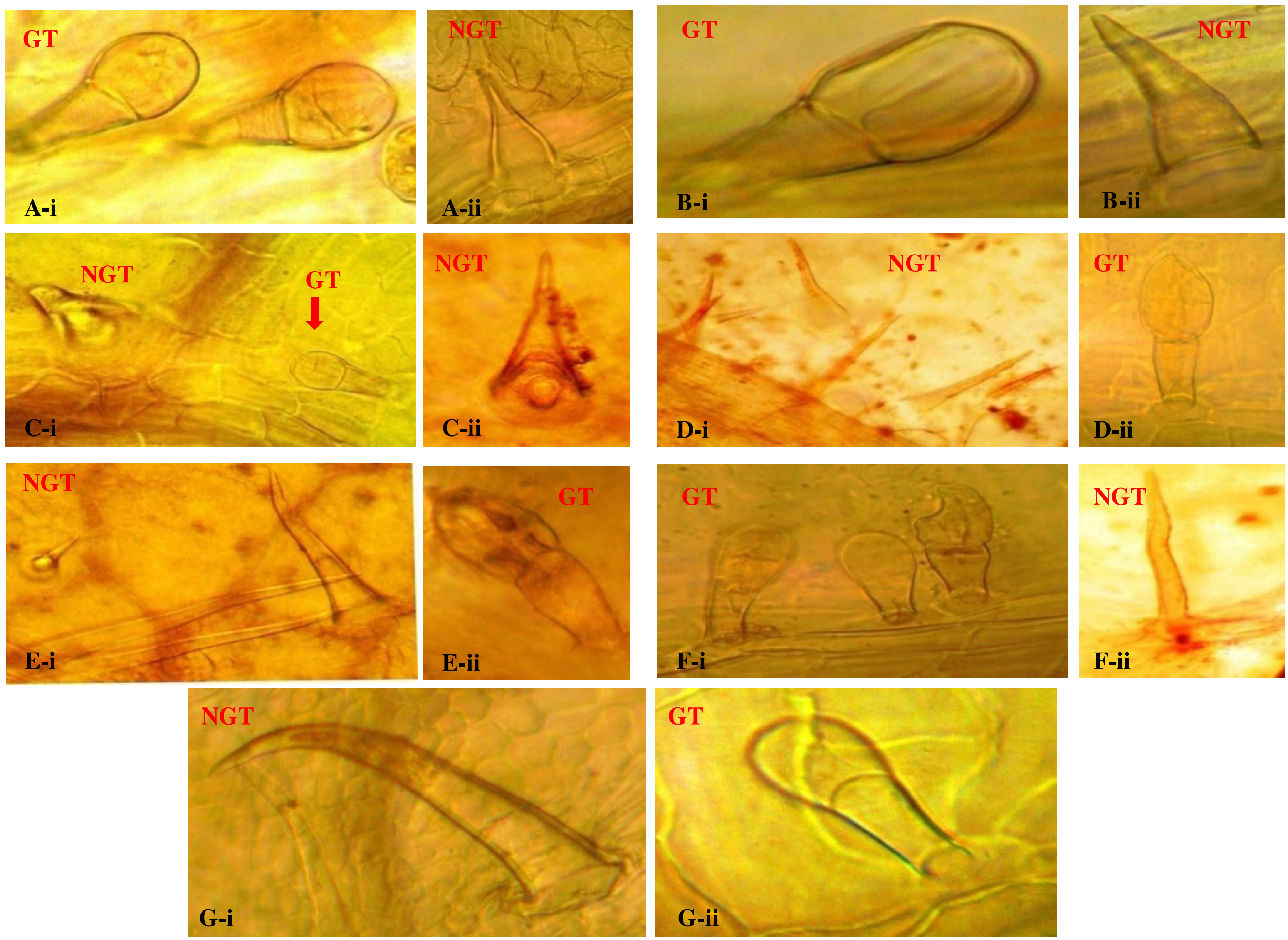

Figure 2.8: Light microscopic photograph of seven mulberry genotypes showing the comparison of trichome present on the leaf surface (Under 40× resolution); (A): S1; (B): S1635; (C): V1; (D): K2; (E): Dudhiya; (F): Bombay local; (G): Kosen

Abb. Used: GT: glandular trichome; **NGT:** non-glandular trichome

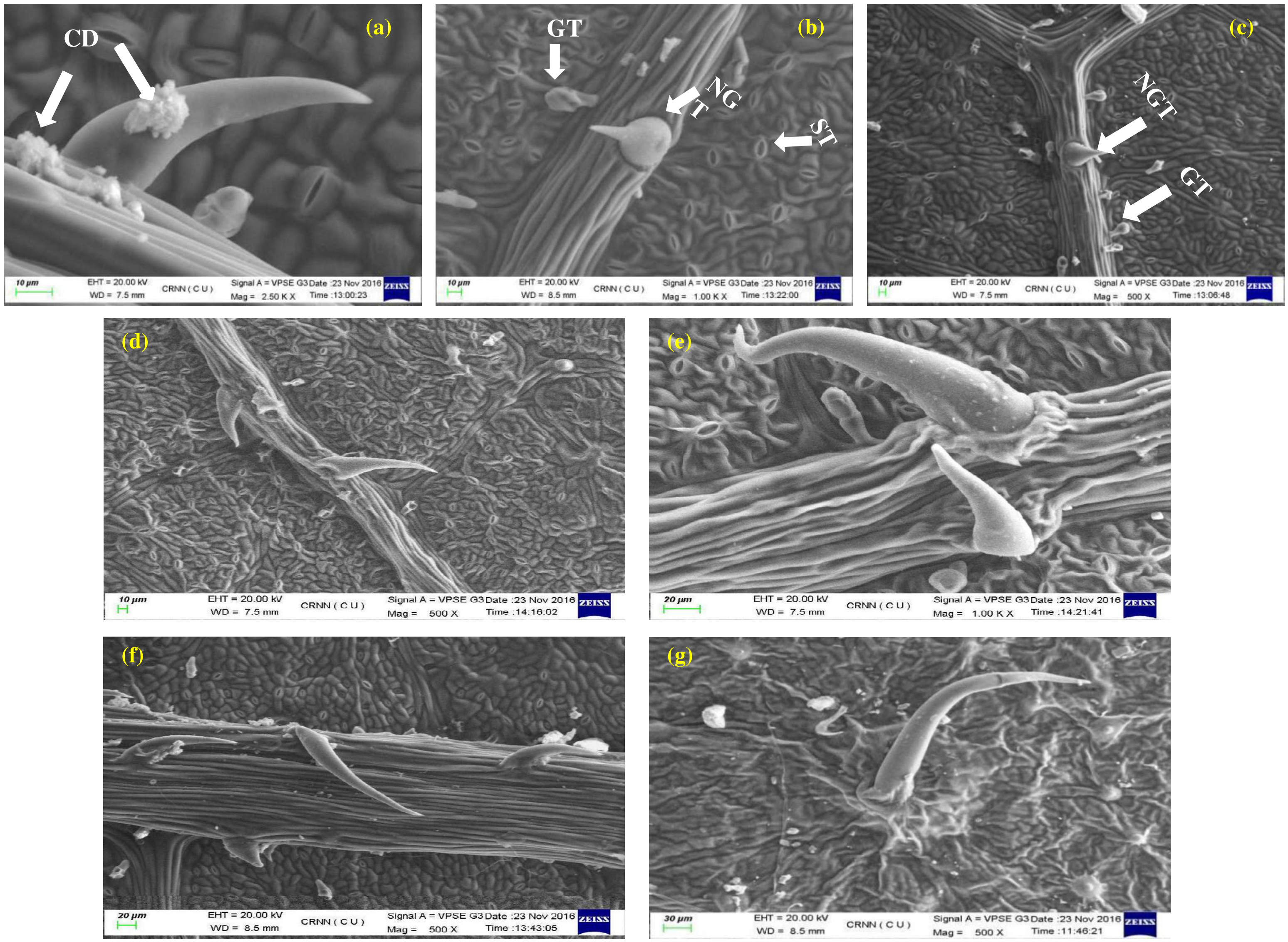

Figure 2.9: Scanning electron microphotograph (SEM) of seven mulberry genotypes showing the comparison of trichome present on the leaf surface; (a): S1; (b): S1635; (c): V1; (d): K2; (e): Dudhiya; (f): Bombay local; (g): Kosen

Abb. Used: GT: glandular trichome; **NGT:** non-glandular trichome; ST: stomata; CD: calcium deposition.

Sujathamma *et al.* (2001) evaluated different mulberry genotypes and recommended two varieties among them, namely Tr-10 and Mr-2 for commercial cultivation in Andhra Pradesh. Bohidar *et al.* (2007) reported that V1, S36, and DD were most suitable mulberry variety for high silk production in Orissa climate. Lalfelpuii *et al.* (2014) recommended S1635 and V1 varieties for silkworm rearing due to good larval growth and better commercial cocoons achieved by S1635 and V1 nourishment. Present experiment also confirmed the same as S1 and S1635 had better pre-cocoon and post cocoon attributes. Highest larval growth rate was recorded under nourishment with S1 leaves followed by S1635, V1, K2, Kosen, Bombay local and Dudhiya. Effective rearing rate is a commercial character, reflects on success rate of rearing and production rate. Larvae reared with S1 and S1635 mulberry leaves, exhibited highest EER (%) and minimal (40%) ERR was recorded by Dudhiya nourishment. Another two important commercial characters are WSC and WSS (Gaviria *et al.,* 2006). Maximum WSC and WSS were obtained by rearing with S1 leaves (Table 2.3). Post cocoon attributes reflect on fibre characters. Shell ratio (SR %) helps to determine the amount of raw silk obtained from fresh cocoons. Highest SR % was obtained with S1 nourishment followed by S1635, V1, K2, Kosen, Bombay local and Dudhiya. Post cocoon parameters, like filament length (FL), filament size (FS) or denier of fibre, non-breakable filament length (NBFL) was measured in each experimental set. FL and NBFL were longer in S1 and S1635. Similarly, Lalfelpuii *et al.* (2014) obtained longer filament length from S1635 followed by V1.

2.3.2 Statistical Analysis

Dendrogram based on dissimilarity co-efficient was generated and all seven mulberry cultivars were categorized into three distinct groups or clusters. Dudhiya and Bombay local were placed in Group I (Figure 2.10). S1 and S1635 formed another cluster, Group II. K2 and Kosen occupied the third group along with V1. On the basis of above characters, S1 and S1635 might be categorized into high yielding group with effective commercial cocoon production. V1, Kosen and K2 are considered as moderately effective group due to moderate leaf quality and moderate quality of cocoon production. On the basis of similarity co-efficient, heat map was generated, where K2 and V1 formed a cluster (group I) (Figure 2.11). S1 and S1635 were placed in another group (II) sharing a common ancestor with group I. Dudhia was placed in group III. Bombay local and Kosen took another clustering (group IV).

Table 2.3: Pre-cocoon and post-cocoon attributes of silkworm larvae under nourishment with seven different mulberry genotypes

Cultivars	Pre-cocoon attributes				Post-cocoon attributes			
	Growth Rate (%)	ERR%	WSC	WSS	SR (%)	FL	NBFL	FS (D)
S1	90.69 ± 1.23^{a}	100 ± 0.89^{a}	1.24 ± 0.07^{a}	0.26 ± 0.007^{a}	20.97 ± 0.24^{a}	679.73 ± 1.07^{a}	679.73 ± 0.99^{a}	2.27 ± 0.04^{b}
S1635	86.48 ± 1.44^{b}	100 ± 0.92^{a}	1.16 ± 0.05^{ab}	0.22 ± 0.004^{b}	18.97 ± 0.27^{b}	657.67 ± 0.99^{b}	657.67 ± 0.94^{b}	2.16 ± 0.05^{a}
V1	78.62 ± 1.16^{c}	90 ± 0.97^{b}	1.02 ± 0.03^{c}	0.18 ± 0.005^{c}	17.65 ± 0.19^{c}	631.37 ± 1.12^{c}	601.27 ± 0.96^{c}	$2.35 \pm 0.02b^{c}$
K2	71.29 ± 1.09^{d}	90 ± 0.85^{b}	1.07 ± 0.06^{bc}	0.17 ± 0.004^{cd}	15.89 ± 0.12^{d}	591.34 ± 1.04^{d}	591.34 ± 0.94^{d}	2.41 ± 0.04^{cd}
Dudhiya	32.76 ± 1.12^{g}	40 ± 0.73^{e}	1.2 ± 0.04^{a}	0.13 ± 0.005^{f}	10.83 ± 0.17^{f}	438.33 ± 0.97^{g}	411.46 ± 1.02^{g}	2.7 ± 0.03^{f}
Bombay local	56.62 ± 1.06^{f}	50 ± 0.49^{d}	1.16 ± 0.02^{ab}	0.15 ± 0.005^{e}	12.93 ± 0.23^{e}	494.76 ± 1.09^{f}	426.41 ± 0.91^{f}	2.47 ± 0.04^{de}
Kosen	61.84 ± 1.27^{e}	60 ± 0.7^{c}	1.2 ± 0.04^{a}	0.16 ± 0.004^{de}	13.33 ± 0.24^{e}	524.61 ± 1.02^{e}	524.61 ± 0.9^{e}	2.54 ± 0.04^{e}

Results are represented as mean ± SEM, n=30. Values with different letters (a, b, c, d, e, f & g) are significantly ($p < 0.05$) different from each other by Duncan's Multiple Range Test (DMRT).

Abb. Used: ERR: Effective Rearing Rate; **WSC:** Weight of Single Cocoon; **WSS:** Weight of Single Shell; **SR:** Shell Ratio; **FL:** Filament Length; **NBFL:** Non-Breakable Filament Length**, FS (D):** Filament Size (Denier).

Table 2.4: ANOVA (Two-way) analysis of micro-morphological (stomata) attributes of seven mulberry genotypes

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Mulberry genotypes	26965.3	6	4494.216	2.724761	0.025111**	2.323994
Micro-morphological attributes of leaf	348957.2	7	49851.02	30.22376	1.93E-14*	2.23707
Error	69274.73	42	1649.398			
Total	445197.2	55				

*Significant at *p<0.01* and **significant at *p<0.05* level

Table 2.5: ANOVA (Two-way) analysis of micro-morphological (trichome and idioblast) attributes of seven mulberry genotypes

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Mulberry genotypes	6997.327	6	1166.221	4.235046	0.001281*	2.254053
Micro-morphological attributes of leaf	64807.8	10	6480.78	23.53447	3.32E-17*	1.992592
Error	16522.4	60	275.3739			
Total	88327.57	76				

*Significant at *p<0.01* and **significant at *p<0.05* level

Table 2.6: ANOVA (Two-way) analysis of economical attributes of silkworm rearing under nourishment with seven mulberry genotypes

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Mulberry genotypes	132056.9	6	22009.49	4.068744	0.002644*	2.323994
Economical Attributes of Silkworm rearing	4593301	7	656185.8	121.3046	9.12E-26*	2.23707
Error	227195	42	5409.406			
Total	4952553	55				

*Significant at *p<0.01* and **significant at *p<0.05* level

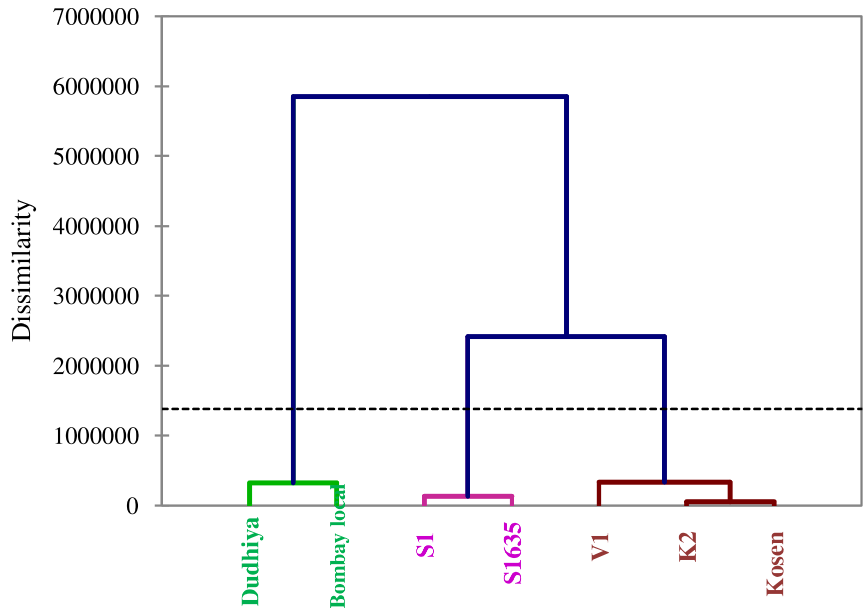

Figure 2.10: Dendrogram cluster analysis of seven mulberry varieties

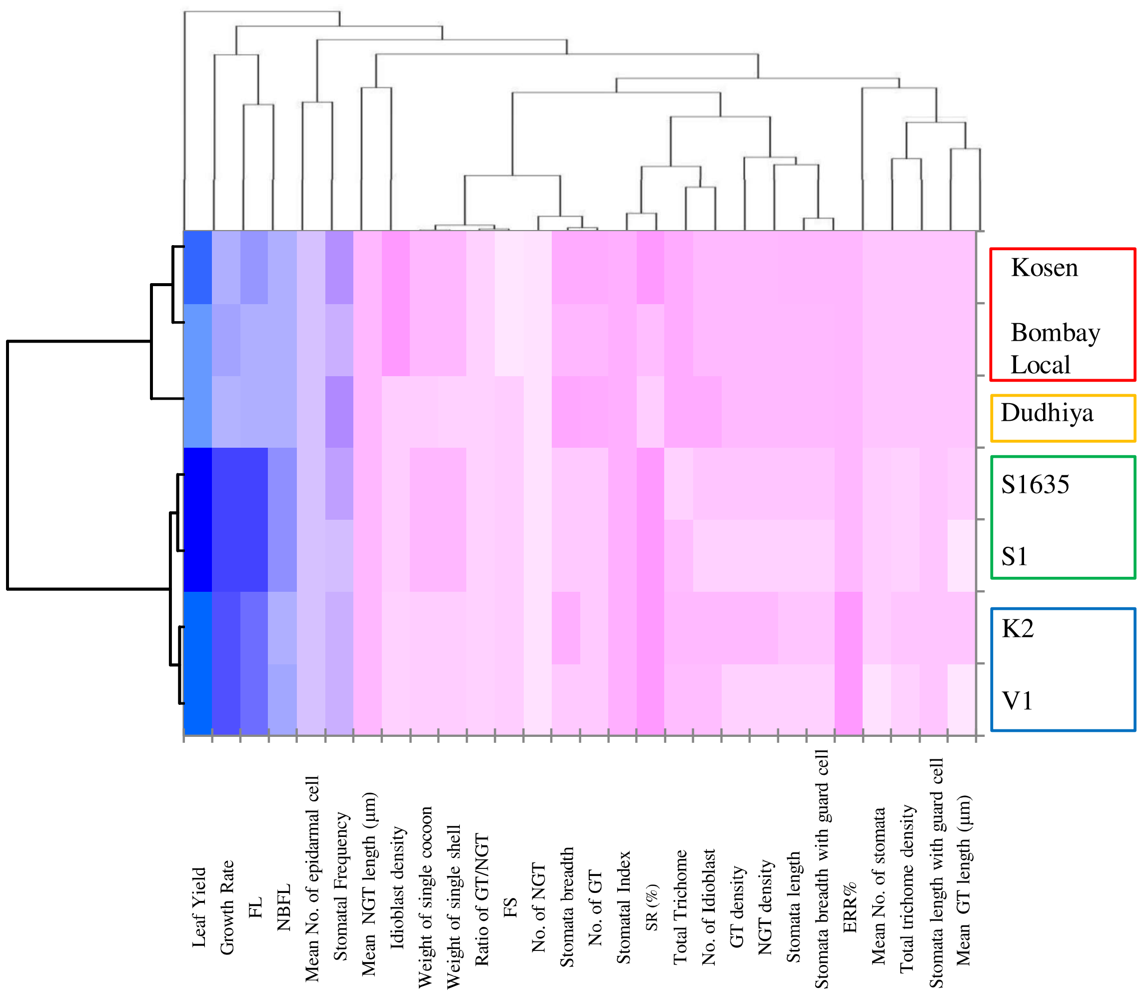

Figure 2.11: Heat map of seven mulberry cultivars on the basis of their micro-morphological and economical attributes

Abb. Used: FL: filament length, **NBFL:** non-breakable filament length, **NGT:** non-glandular trichome, **GT:** glandular trichome, **SR:** shell ratio, **ERR:** effective rearing rate

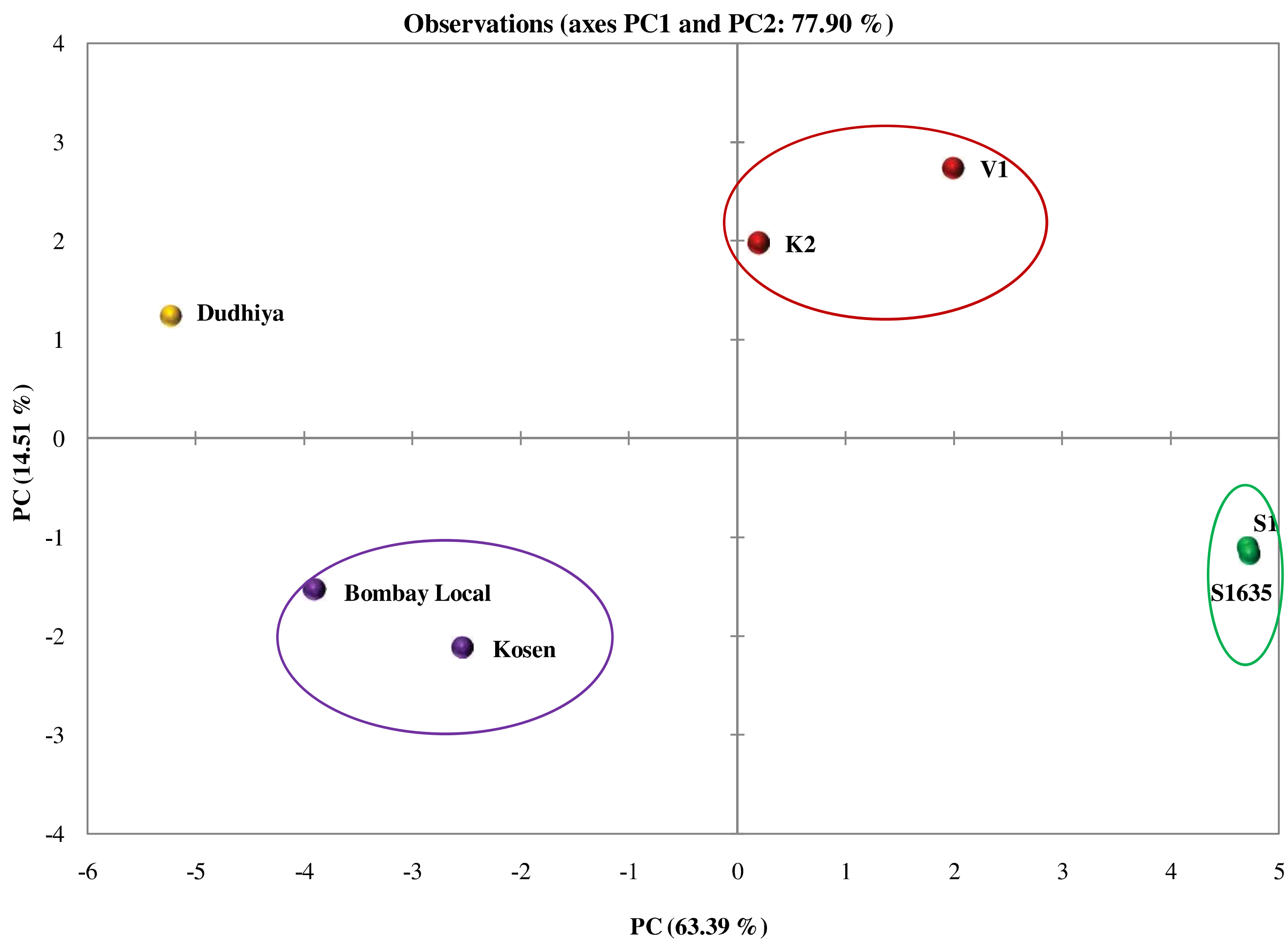

Figure 2.12: PCA analysis of seven mulberry genotypes on the basis of biochemical attributes of leaves and their performance on economical parameters of silkworm rearing.

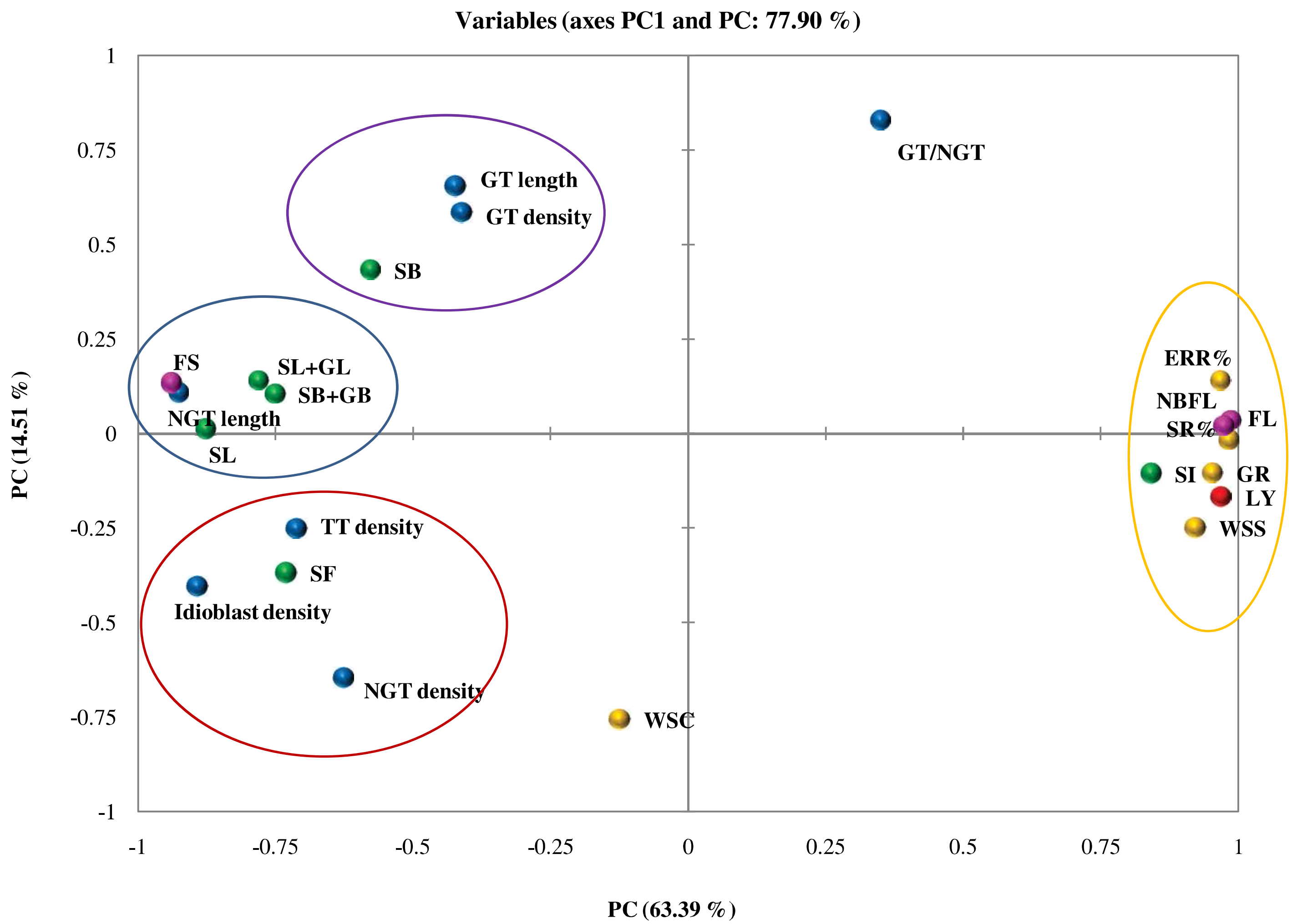

Figure 2.13: PCA analysis of morphological attributes of seven mulberry genotypes and economical parameters of silkworm rearing. Economical attributes: pre-cocoon (yellow dot), post-cocoon (pink dot); Morphological attributes: stomata (green dot), trichome and idioblast (blue dot)

Similarly, seven mulberry cultivars formed four separate clusters on PCA plot (Figure 2.12). From PCA analysis (Figure 2.13), it was found that, pre-cocoon and post cocoon attributes were formed a cluster along with stomatal index of leaf. Similar kind of clustering also was found in heat maps, where per-cocoon and post cocoon parameters were clustering with few micro-morphological attributes of mulberry leaves. These types of statistical data revealed that economical attributes of silkworm rearing was influenced by leaf micro-morphological characters. From PCA plot analysis, it can also be stated that, density of trichome and idioblast, length of trichome had negative effects on larval growth rate and other economical attributes.

Two-way ANOVA analysis was performed to find out the variation of different micro-morphological attributes of leaves and economical parameters of silkworm rearing in different cultivars. The different micro-morphological attributes (stomata and trichome characters) were significantly ($p<0.01$) varied among different cultivars (Table 2.4 and 2.5). Significance variation ($P<0.01$) was also found among different economical attributes of silkworm rearing (Table 2.6). Economical attributes of silkworm rearing was significantly influenced by different mulberry genotypes as well as micro-morphological features of leaves.

2.4 CONCLUSION

This study suggests that S1 and S1635 as the most promising mulberry cultivars with high foliar yield and higher acceptability by silkworm larvae. S1 and S1635 both had shorter trichome length with low trichome and idioblast density ultimately which help the cultivars being suitable for larval feeding. Low stomatal frequency with short stomatal size is also favourable leaf surface attribute of S1 and S1635. Suitable stomatal features of these two cultivars had an influence on leaf yield and foliar nutrition and finally silk production. The study reveals that K2 and V1 also can be selected for silkworm rearing in sub-tropical region of India.