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Floral Biology and Breeding System of Tea [*Camellia sinensis* (L.)]: Implications on the Tea Breeding Programme

H A C K Ariyarathna, J D K Arachchi, and M T K Gunasekare
(Plant Breeding Division, Tea Research Institute of Sri Lanka, Talawakelle, Sri Lanka)

ABSTRACT

Comprehensive knowledge in reproductive traits is a prerequisite in utilizing the existing germplasm effectively for crop improvement. A representative sample of germplasm accessions were used to study floral morphology, pollen biology and stigma receptivity in tea. The results revealed a wide qualitative and quantitative variability in floral morphology, including flower size, number and arrangement of the corolla, characteristics of the pistil, pollen viability and germination and stigma receptivity. Four main categories were identified based on size of the flower. On the basis of anther dehiscence, accessions were categorized as early maturing, intermediate or as late maturing. Significant differences were noted in percentage germination of pollen and pollen viability among the accessions. Presence of non-viable pollen was found to be characteristic to certain accessions. Stigma was receptive even in the stage of mature un-open flower buds. The present study being the first comprehensive study conducted simultaneously on various reproductive traits in tea, the results provide important insights into the varying nature of breeding systems in tea germplasm in Sri Lanka. The study also generated a wealth of information readily useful in conducting tea hybridisation programme as well as in advanced research in tea crop improvement.

Keywords: *Camellia sinensis* (L.) O. Kuntze, floral morphology, pollen biology, stigma receptivity

INTRODUCTION

Tea [*Camellia sinensis* (L.) O. Kuntze] is a popular beverage all over the world. In Sri Lanka, tea being the main agricultural foreign exchange earner, more than 90% of the production is aimed for export market, earning Sri Lankan Rs. 112 billion (Anonymous, 2008). High cost of production (COP) is a main threat to the sustainability of the Sri Lankan tea industry. The total cost involved in pest, disease and weed control in tea fields is as high as 25-33 % of the COP (Jayakodi and Shyamalie, 2002). Hence, prospects of producing cultivars with high yield, good made tea quality as well as cultivars that are adequately buffered against biotic and abiotic stresses are highly recognized in the breeding strategies of the Tea Research Institute of Sri Lanka (TRISL). In order to incorporate the above desirable traits into the existing proven cultivars, research is being continued to create new progenies through controlled hybridization among known cultivars possessing

important traits. Germplasm with a wide variability in morphological and physiological characters are identified and conserved by the TRISL (Singh *et al.* 2003) that could be effectively utilized in crop improvement to overcome many constraints associated with production aspects in tea plantations.

Utilizable genetic potential in breeding stock in crop species is influenced to a great extent by the breeding characters, such as phenology of flowering and fecundity traits, mating systems and self-incompatibility (Loveless and Hamrick, 1984). Limited amount of literature is available on tea reproductive biology *viz.* floral biology (Neog and Singh, 2003), ontology (Tsou, 1997) and phenology (Bezbaruah, 1975) and pollination (Wickramaratne and Vitarana, 1985).

Insufficient knowledge on breeding systems and floral biology is a major barrier in strategic planning of practical tea crop breeding programmes as well as in research on tea crop improvement. Hence, TRISL, has initiated an extensive study to screen the Sri Lankan tea breeding stock and the tea germplasm for reproductive characters and breeding behaviour. Breeding behaviour is expressed as genetic or organismic trait/traits and environmental phenomenon or as interactions of those two (Wright, 1921). The main objective of the present study was to examine variations present in the germplasm in relation to floral traits including floral morphology, functional and morphological characters of anthers and stigmatic surface and their implications on practical tea crop improvement.

MATERIALS AND METHODS

A representative sample from the germplasm accessions was selected (Table 1), considering seasonality and intensity of reproductive traits, flowering phenology, fecundity (Unpublished) and pedigree information (Ariyarathna and Gunasekare, 2006), to study floral biology, pollen biology and stigma receptivity in tea. The study was carried out during the peak flowering period starting from January to April 2007 in the field gene bank established at Tea Research Institute of Sri Lanka (TRISL) in Talawakelle (Lat. 6° 54' N, Long. 80° 42' E, 1382m amsl; average minimum temperature 14.3 °C, average maximum temperature 24.8 °C, average number of sunshine hours 4.8 per day, total annual rainfall 1918 mm). Three vegetatively propagated, thirty-year old bushes were sampled for each of the accession for the study.

Floral morphology

Twenty freshly bloomed (*i.e.* bloomed on the same day) flowers collected, during 9.00-10.00 a.m., from each of the accession were used for scoring morphological attributes. Based on the Tea Descriptors (IPGRI, 1997), the following observations and measurements were made on the flowers: (1) diameter of the corolla, (2) number of sepals, (3) number of petals, (4) number of whorls in the corolla, (5) position of stigma relative to the androecium,

(6) position where the style splits (7) number of stylar arms, (9) length of stylar column (mm), (10) length of stylar arms (mm) and (11) number of locules. Vernier calliper and hand lenses were used to take the measurements. Number of locules was counted on cross sections of the ovary taken manually. The sections were observed under light microscope.

Table 1. Accession identities of the representative samples used to study floral morphology, stigma receptivity and pollen biology in tea

Aspect of study		Sample size	Accessions
Floral morphology		27	ASM 4/10, CY 9, DN, DT 1, DT 95, KEN 16/3, MT 18, N 2, PK 2, TRI 62/9, TRI 777, TRI 2023, TRI 2024, TRI 2025, TRI 2026, TRI 3013, TRI 3016, TRI 3018, TRI 3019, TRI 3020, TRI 3025, TRI 3035, TRI 3041, TRI 3047, TRI 3055, TRI 4052, TRI 4061
Stigma receptivity		15	ASM 4/10, DK 2, DN, PK 2, TK 42, VHMOR, WY, TRI 62/9, TRI 2025, TRI 3013, TRI 3015, TRI 3016, TRI 3019, TRI 3025, TRI 3037
Pollen biology	Anther dehiscence	16	ASM 4/10, B 275, DK 2, DN, DG 39, GF 7/6, PK 2, VHMOR, WY, TRI 2024, TRI 2025, TRI 3013, TRI 3015, TRI 3016, TRI 3019, TRI 3025
	Pollen viability	9	B 275, DN, GF 7/6, PK 2, TRI 2025, TRI 3013, TRI 3015, TRI 3016, TRI 3019
	Pollen germination	11	DG 7, DT 95, MT 18, PK 2, TRI 2016, TRI 2025, TRI 4067, TRI 4071, TRI 4078, *C. sasanqua*, Yabukita

Pollen biology

Studies on pollen biology were made on three progressive developmental stages of floral buds identified as bulb stage, *i.e.* just before blooming (stage I) and different stages of flowers such as just after blooming (stage II) and flowers just before withering (stage III). Sampling was carried out on 12 occasions at weekly intervals. From each of the different developmental stage five buds/flowers were sampled per accession on each occasion. Hence altogether 60 individual buds/ flowers were studied per accession per each developmental stage. Samples were collected in the morning between 9.00-10.00 a.m.

Number of anthers split was counted in order to determine the development stage that corresponds to anther dehiscence. Anthers were mounted on microscopic slides from

freshly collected floral buds/ flowers. Two slides were prepared from each floral bud/ flower. Number of anthers split and intact was counted separately, under dissecting microscope.

Pollen germination at each of the different development stages was studied *in vitro* as described by Thirukkumaran and Gunasekare, (2000). Pollen were germinated in 10% sucrose solution on microscopic slides by hanging drop method. After incubating for three hours under humid conditions, the slides were observed under the light microscope and percentage pollen germination was recorded.

Pollen viability was indirectly determined using the Alexander stain as reported by Alexander, (1969). Mature non-dehisced anthers were collected on microscopic slides and a drop of Alexander stain was placed on the anther lobes. The slides were incubated under humid conditions at room temperature (approximately 25 °C) for 10 minutes and squash preparations were made. Slides were observed under light microscope. Counts were made on 100 pollen and pollen stained in dark purple and those stained in turquoise blue were counted separately as viable and non-viable pollen respectively. Presence and absence of pseudo-pollen that are distinguishable from healthy pollen by their morphology and staining (relatively large, less rigid, transparent with ribbed walls and do not stain, as reported by Iqbal and Wijesekara, 2002 for other *Camellia* species) were also recorded separately.

Stigma receptivity

Stigma receptivity was also studied using the three floral developmental stages as described above. Sampling was carried out on 12 occasions at weekly intervals. Five floral buds/ flowers from each accession were sampled per each of the developmental stage. Samples were collected in the morning between 9.00-10.00 a.m.

Styles were removed from freshly collected floral buds/ flowers. Stigma receptivity was examined by an esterase test using α–napthyl acetate as a substrate in the coupling reaction with Fast Blue B as reported by Mattsson *et al.*, (1974) with few modifications. One mg of α–napthyl acetate was dissolved in 0.025 ml of acetone and 2 ml of 0.1 M phosphate buffer was added to the solution. Once the initial cloudiness disappeared 5mg of Fast Blue BB salt was added and mixed thoroughly by shaking. The solution was filtered through Whatman No. 1 filter paper to remove any un-dissolved particles. Specimens were stained, with freshly prepared stain for 10 minutes. Excess stain was washed off with distilled water and the specimens were observed under light microscope. Stigmatic area stained in deep purple colour was used as a criterion to identify the receptive area of the stigma.

The data gathered as described above were analysed statistically using SAS PROC GLM procedure (SAS, 1985). Graphical illustrations were made on quantitative measurements.

RESULTS

Floral morphology

Generally flowers are perfect (or combined) and actinomorphic. The calyx consisted of two whorls whereas corolla had two to four whorls. Number of sepals was on an average 5-6 in all accessions whereas number of petals varied within a wide range (6-12). Frequently, very small and underdeveloped petals were observed as rudimentary structures. Qualitative and quantitative variation observed in floral morphology among the accessions are summarized in the Table 2.

Four class intervals could be identified in size of the flower among the accessions, such as (<3.00 cm); (3.00-4.00 cm); (4.00-5.00 cm) and (>5.00 cm). Numerous basally connate stamens that occur in two whorls formed the androecium. Filaments of the inner whorl of stamens were shorter compared to those of the outer. The syncarpous gynoecium comprised of three, four or five carpals and occurred inferior or superior to the other floral whorls or was buried in the receptacle. Among the 27 accessions studied, a wide variation was found in relation to stigma position and style splitting (Table 2) revealing a proper representation to capture the available variability in the germplasm.

Pollen biology

Three behavioural categories on anther dehiscence were identified among the accessions studied (Figure 1) *viz.* (1) early maturing accessions where dehiscence occur in the floral

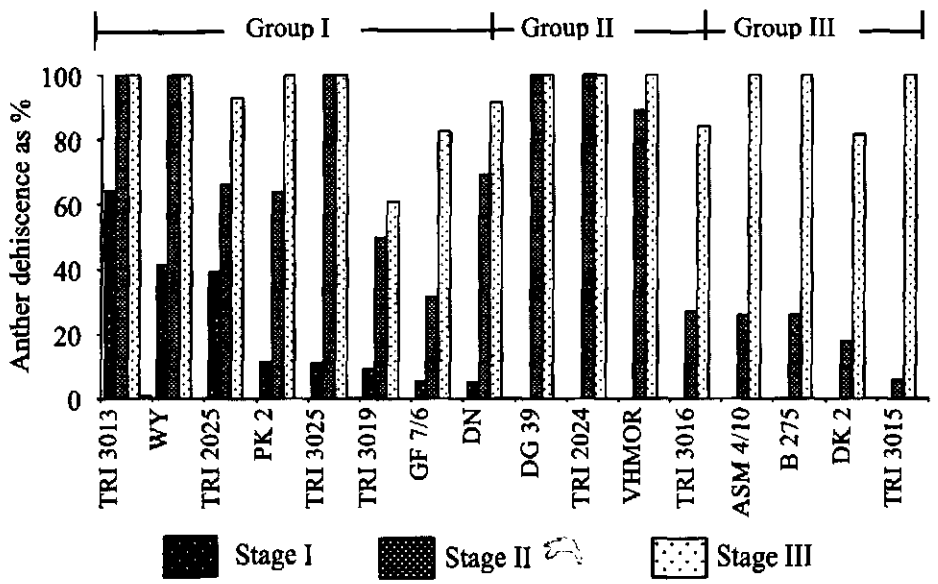

Figure 1. Percentage anther dehiscence as affected by the floral development stages and variation observed among accessions.

Table 2. Observations and measurements on floral morphology of tea accessions. Figures represent average of twenty flowers ± standard error.

Accession	Corolla diameter (cm)	Length of style column (mm)	Length of style arms (mm)	Stigma position	Style splitting	No. of sepals	No. of corolla whorls	No. of petals	No. of style arms	No. of locules
KEN 16/3	2.71±0.04	1.11±0.01	0.19±0.01	same	united	5-6	3	6-9	3-4	3-4
TRI 777	2.85±0.05	0.98±0.02	0.11±0.01	same	united	5	2	6	3	3
TRI 3013	3.15±0.05	1.23±0.01	0.19±0.01	same or androecium high	united	5-6	3	6-9	3-4	3-4
TRI 3035	3.29±0.02	0.86±0.02	0.27±0.01	same or androecium high	ascending	5-6	2	6	3	3
DN	3.30±0.05	1.05±0.01	0.51±0.01	same	ascending	5-6	2	6-7	3-4	3-4
CY 9	3.32±0.06	1.08±0.04	0.41±0.01	same androecium high	ascending	5-6	2	6-7	3	3
MT 18	3.36±0.05	0.91±0.03	0.30±0.01	androecium high	ascending or united	5-6	3	6-7	3	3
TRI 3020	3.39±0.02	1.00±0.01	0.10±0.00	same	united	5-6	2	6	3	3
TRI 2023	3.42±0.03	1.15±0.01	0.18±0.01	same	united	5-6	2	6	3-4	3-4
TRI 2025	3.60±0.05	1.09±0.01	0.19±0.00	same or gyminocium high	united	5-6	2	6	3	3
TRI 3018	3.63±0.05	0.74±0.04	0.31±0.01	same or androecium high	ascending	5-6	3	6-9	3	3
TRI 4061	3.70±0.04	1.11±0.01	0.35±0.01	gymonocium high	ascending	5-6	3-4	9-10	4-5	4-5
TRI 3025	3.85±0.04	1.05±0.02	0.18±0.00	androecium high	united	5-6	2-3	6	3-4	3-4
TRI 3055	3.85±0.05	0.89±0.01	0.34±0.01	same or androecium high	ascending	5-6	3	6-9	3	3
TRI 2026	3.91±0.05	1.04±0.03	0.30±0.01	same	united	6	3	6-9	3	3
PK 2	4.09±0.05	1.26±0.01	0.40±0.01	same	ascending	5-6	3	6-9	3-4	3-4
TRI 2024	4.15±0.05	1.02±0.01	0.62±0.02	androecium high	ascending	5-6	3	6-8	3-4	3-4
DT 1	4.19±0.05	1.08±0.01	0.26±0.02	androecium high	united	5-6	2	6	2-3	2-3
TRI 3019	4.40±0.06	1.33±0.02	0.96±0.03	same or androecium high	geniculate	5-6	3	9	5	5
DT 95	4.46±0.06	1.35±0.01	0.37±0.01	same	united	5-6	3	7-8	3	3
ASM 4/10	4.47±0.05	1.20±0.02	0.65±0.01	same	ascending	5-6	3	9	3-4	3-4
TRI 3016	4.48±0.07	1.67±0.02	0.62±0.02	same or gyminocium high	ascending	5-6	3	6-9	3-5	3-5
TRI 3047	4.55±0.04	0.99±0.02	0.89±0.13	androecium high	ascending	5-6	3	9	3-5	3-5
TRI 62/9	4.60±0.04	1.05±0.01	0.33±0.01	same	united	5-6	4	9-12	3-4	3-4
TRI 3041	4.63±0.04	1.07±0.02	0.57±0.03	androecium high	united	5	3	7-9	3-4	3-4
N 2	4.64±0.12	1.33±0.02	0.55±0.01	same	united	5-6	3	6-9	3	3
TRI 4052	5.15±0.05	1.13±0.03	0.47±0.01	androecium high	ascending	5-6	3-4	7-10	4-5	4-5

development stage I (bulb stage), (2) intermediate maturing accessions where anthers dehisced during the floral development stage II (freshly bloomed flowers), and (3) late maturing accessions in which anther dehiscence is initiated during the floral development stage II though it does not reach its maximum until one day after blooming. Stage of anther dehiscence was found to be dependent on accessions as well as the development stage of the bud/ flowers (Table 3).

Table 3. summary of statistical analysis of pollen biology and stigma receptivity

Source of variance		d.f.	Pr > F
Pollen biology	**a. Anther dehiscence**		
	Accession	15	0.0329**
	Developmental stage	2	<0.0001**
	b. Pollen germination		
	Accession	10	0.0148**
	Developmental stage	2	0.0695
	c. Pollen viability		
	Accession	8	<0.0001**
	Developmental stage	2	0.6711
Stigma receptive area	Accession	14	<0.0001**
	Developmental stage	2	0.3628

** significantly different

Presence of non-viable pollen was characteristic to certain accessions such as GF 7/6 and B 275. In some other accessions (TRI 3013, 3015 and 3016) non-viable pollen were totally absent. Hence, presence of non-viable pollen was accession dependent but not a developmental stage depended character (Table 3).

Percentage pollen germination varied among the tested accessions. In general pollen germination was highest in the floral development stage II and III (Figure 2). However, in accessions, MT 18 and PK 2, highest pollen germination was observed in floral developmental stage I. In contrast, in TRI 4071 maximum pollen germination was observed in floral developmental stage III. It was interesting to notice that in certain accessions germination ability of pollen last longer than in others. Of the 11 accessions studied, accessions DG 7, MT 18, PK 2, TRI 2016, TRI 2025 and *C. sasanqua* pollen obtained from all the developmental stages were able to germinate, whereas in others the pollen germination was restricted to floral development stages II and III.

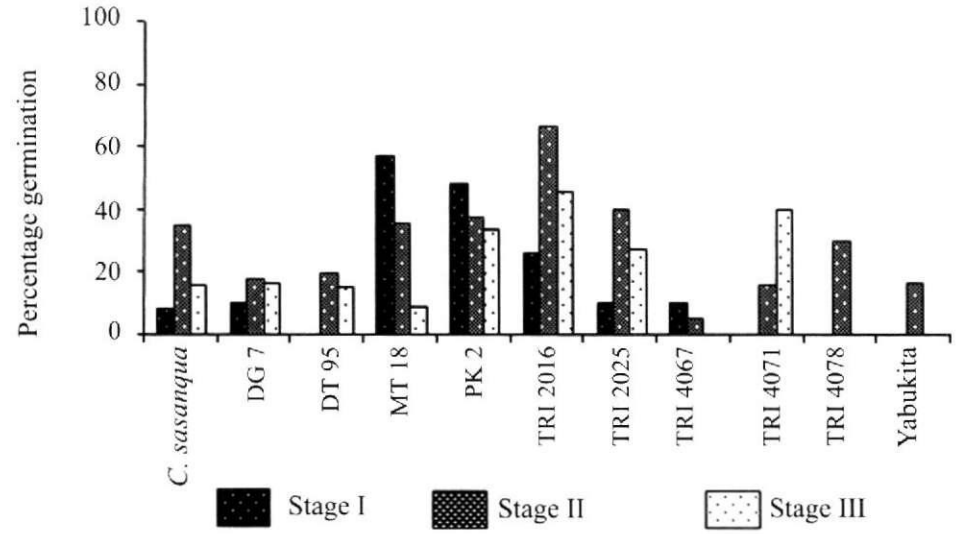

Figure 2. Percentage of pollen germination at different stages of floral development in tested accessions

Stigma Receptivity

Irrespective of the accessions studied, stigma of all floral development stages was found to be receptive. However, significant variation was observed among the accessions in the receptive area of the stigma (Figure 3). The receptive area was restricted to the apex of the style (apical stigma) in certain accessions whereas in others stigma was extended linearly along the stylar arms (linear stigma) (Plate 1). It was noticed that accessions

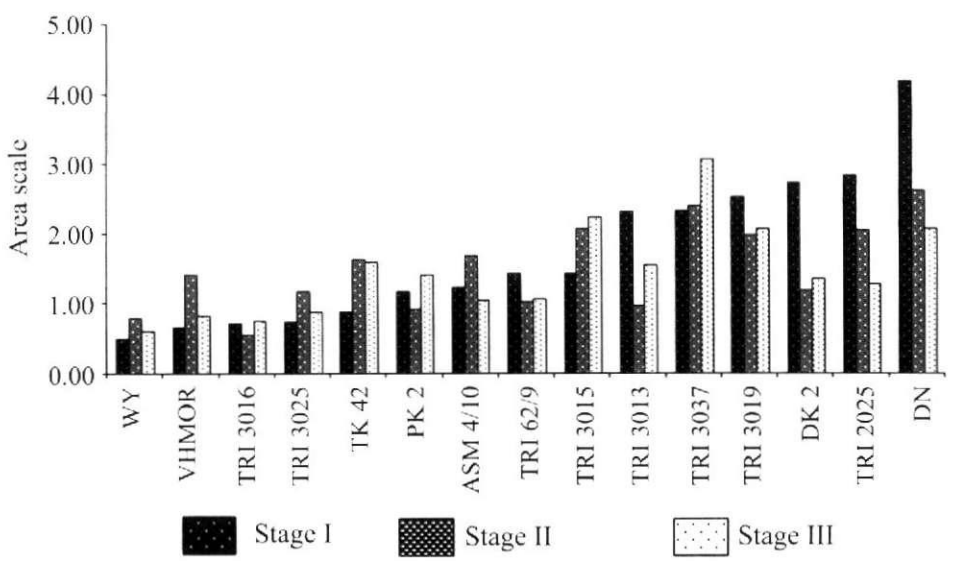

Figure 3. Variations observed among accessions in the area of stigma receptivity revealed by esterase test

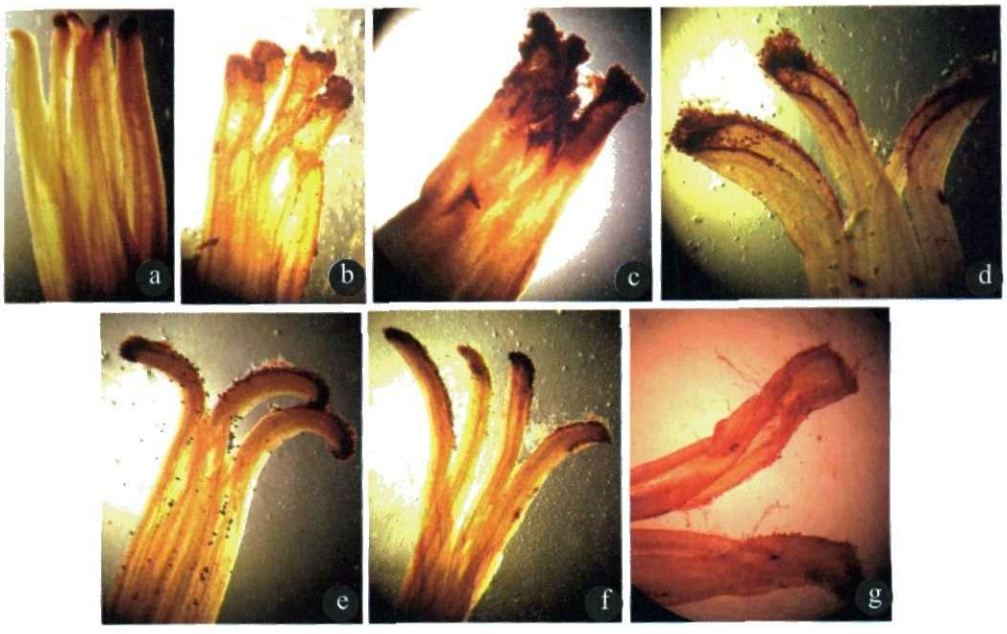

Plate I. Variability of the stigmatic receptive area observed among the accessions studied: (a), (b) and (c) - Apical stigma in cultivars TRI 62/9, TRI 3013 and TK 42 respectively; (d), (e) and (f) - Linear stigma in cultivars TRI 3037, TRI 2025 and TRI 3019 (x10); (g) - stigamitc surface of TRI 3019 showing (arrow) long to medium length stigmatic hairs (x 40)

having ascending or geniculate splitting fashion of the style tend to have relatively larger linear stigma while those having united pattern of splitting to have apical stigma. Though stigmas of all the developmental stages showed receptivity, in several accessions a noticeable increase in receptive area was observed in later stages of development. Receptive area of the stigma is found to be an accession dependent character (Table3).

DISCUSSION

The size of tea flower varies extensively among different accessions studied and four main size categories could be identified. As observed, tea flowers being bisexual, bowl shaped, actinormorphic, with large number of stamens, are unspecialised and arranged solitarily or loosely clustered. Faegri and Pijl, (1979) reported that such characteristics are typical floral syndromes among insect pollinated plant species. General pollinators, mainly dipterans contribute to pollination success in tea (Bezbaruah, 1975; Wickramaratne and Vitarana, 1985). Floral display, described by the number, size, and arrangement of open flowers on a plant, influence plant mating success since it is one of the major criteria that determine pollinator behaviour (Karron *et al.*, 2004; Vamosi *et al.*, 2006). Considering the wide variability observed particularly in floral display and morphology, it would be reasonable to expect differences in pollinator behaviour and possibly

discrimination of different tea floral syndromes by pollinator insects. Therefore, in view of the vast differences observed among the accessions in relation to floral display, differences are likely to occur in fecundity in different accessions as well. However, no information is available on the effect of floral display on pollinator behaviour in tea.

Although majority of accessions carry viable pollen, some accessions carry non-viable pollen and pseudo-pollen. Pseudo-pollen is a known phenomenon in many species of Theaceae such as *C. Hengchunensis*, *C. Tunuifolia* to attract pollinators (Tsou, 1997). However, Iqbal and Wijesekara, (2002) reported that they were unable to observe pseudo-pollen in *C. sinensis*. Thus this is the first report of observation of pseudo-pollen in *C. sinensis*. Presence of non-viable pollen is a clear indication of poor fertility. Hence, presence of non-viable pollen bears direct implications on pollination success and need to be studied carefully when selecting male parental lines for controlled hybridisation programmes in tea.

Three categorical groups were identified based on stigma position, *viz.* (1) stigma above stamens; (2) stigma aligned with the height of stamens; (3) stigma positioned below the stamens. In several other species having mixed mating systems strong correlations were observed between stigma position and autogamous fruit set (Schoen, 1982), out-crossing rate (Ritland and Ritland, 1989; Carr and Fenster, 1994) or with both autogamy and syngamy (Motten and Stone, 2000). Although it was reported that the mating system of tea is predominantly out-crossing (Wright, 1959), mixed mating systems have also being observed within the TRISL breeding stock (Anonymous, 1981; 1982; 1983; 1984). Implication of this phenomenon on pollination of tea flowers, and on the behaviours of pollinators needs to be studied in detail.

The characteristics of stigma observed among the accessions is in agreement with the observations reported by Helslop-Harrison and Shivanna (1977) that tea flower having a wet type of stigma (i.e. Group III). Among the accessions, stigma receptivity was observed to occur invariably even in the floral buds.

Although anther dehiscence occurs just after or simultaneously with blooming in a majority of accessions studied, there were few accessions where anthers dehisced in the bud stage. Furthermore, results revealed that stigma becomes receptive in un-open mature buds suggesting that tea flowers are protogynous. The fact is further substantiated by results obtained in controlled pollination of floral buds and flowers of different development stages where maximum fruit set was observed when un-open mature flower buds were pollinated (Anonymous, 1983). In protogynous species anther dehiscence prior to blooming would clearly facilitate pollination of flowers by its own pollen (autogamous self-pollination).

However, tea is known to have gametophytic late acting self-incompatibility or pseudo-self-incompatibility mechanisms (Rogers, 1975; Helslop-Harrison and Shivanna 1977; Wachira and Kamunya, 2005). Further, accession, TRI 3013, which showed early pollen maturation was found to be self-incompatible (Unpublished).

The present study being the first comprehensive study conducted simultaneously on various reproductive traits in tea, the results provide important insights into the major premises underlying the varying nature of breeding systems in the TRISL tea germplasm. This information is vital in advanced research on tea breeding as well as in developing techniques to exploit the variability in the mating systems productively in breeding programmes.

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