EVIDENCE OF LATE ACTING SELF INCOMPATIBILITY IN TEA

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Summary

To develop sound breeding strategies, it is necessary to understand the reproductive biology and breeding systems of a species. The consequences of inbreeding are particularly important as they influence the choice of progenitors, breeding population size and seed orchard design. Though the tea species (*C. sinensis* (L.) O. Kuntze) is thought to have evolved a pre-zygotic gametophytic self-incompatibility system to reduce incidences of selfing, upto 20% selfing was determined in some Kenyan tea germplasm. Using the aniline blue fluorescence assay to study incompability, successful "self" pollen penetrations were observed though most did not result in successful fruit set. This may indicate that tea has a late acting self-incompatibility system (LSI) or ovarian sterility (OS) type of control of selfing. Data on fruit set indicated huge differences among the test germplasm indicating that self-fertility varies among tea genetic resources and that tea may best be regarded as a facultative out breeder.

Key words: Tea, *Camellia sinensis*, self-incompatibility.

Introduction

Though most flowering plant species including tea have hermaphrodite flowers, most have evolved self-incompatibility mechanisms (SI) to constrain selfing which may reduce the species fitness. These mechanisms involve the inhibition of "self" pollen carrying identical S alleles. Tea is thought to be completely self-incompatible (is an obligate out breeder). Incompatibility in the species is thought to be characteristically that of a multiallelic S-locus pre-zygotic gametophytic type (Rogers, 1975). It is thought that this mechanism of incompatibility is based on specific interaction between the haploid pollen genome and the diploid pistil genome with the SI reaction involving two stages; pollen-pistil recognition, and subsequent rejection.

Three major genetically controlled physiological mechanisms of SI have been characterized in flowering plants; homomorphic gametophytic (GSI), homomorphic sporophytic (SSI) and heteromorphic SI. These mechanisms either prevent germination of self pollen tubes on stigmatic lobes or prevent the successful growth of self pollen tubes to the ovary. More recently, another mechanism of control of SI has been described in which selfed flowers fail to produce fruits despite the fact that the self pollen tube grows successfully in the style and successfully penetrates an ovule. This mechanisms termed "Late-acting self-incompatibility-LSI" or "ovarian sterility" has been described in members of the family *Bignoniaceae* (Gibbs and Bianchi, 1999) and *Heuchera micrantha* (Rabe Andrea and Soltis, 1999).

Studies of the biochemical basis of SI have revealed the production of signal chemicals, the S proteins (S gene products) that are responsible for the irreversible inhibition of pollen tube growth. These proteins have nuclease activity and have been demonstrated to cause DNA fragmentation in incompatible pollen tubes (Jordan *et al.*, 2000). An increase in Ca^{2+} has also been related to DNA fragmentation indicating a probable role of these ions in the signaling process. Two sugars N-acetylglucosamine and Chitobiose also play a role in recognition of self pollen (Ishimizu *et al.*, 1999). The mechanism of rejection in tea has not yet been fully explained though earlier studies (Rogers, 1975) had shown that deposition of the polymer callose (accumulated as glucopyranose) at pollen tube tips plays a key role. This has also been observed in other plant species (Cresti and van Went, 1976; Vishnyakova, 1991; Viti *et al*, 1997).

This paper reports on some new insights on self-incompatibility in tea.

Materials and Methods

Field studies were carried out at the Tea Research Foundation of Kenya, Kericho, Kenya (0.4^{0}S, 35.4^{0}E altitude, 2180m a.m.s.l) over a two-year period (2000 – 2001).

Pollen from the selected test tea clones were initially tested for vitality and germinability using both the acetocarmine staining method (Fernandez-Munoz *et al*, 1995) and modified *in-vitro* germination method of Tuinstra and Wedel (2000) (i.e. in 30% W/V sucrose with 1.5mgl^{-1} borate as sodium tetraborate). Using 3 bushes each of the three clonal varieties (TRFK K/purple – China variety; TRFK 301/4 – Cambod variety; TRFK 303/577 – Assam variety), 150 immature flower buds (with anthers at the late binucleate stage of development) were selected and enclosed in cotton cloth pollination bags for a further 72 hrs until they were about to open (i.e. when they had attained the optimum receptivity stage). The buds were then emasculated by removal of the male parts and rebagged for 72 hours. The buds were then treated as follows and immediately rebagged; (i) Self pollinated (S) using the emasculation method and (ii) Cross pollination (HP) using pollen from another clone at least 10 m distant. A third set of unemasculated buds was left to open pollinate (OP). After pollination, the flowers were sampled at different intervals (24 hrs, 36 hrs, 48 hrs and 96 hrs), fixed, stained and examined for pollen tube growth in the style and for ovule penetrations using a modified ABF (Aniline Blue Fluorescence) method as described by Gibbs and Bianchi (1999). The method which is based on callose fluorescence allowed observations of pollen tube elongation to be made and any sites of rejection to be identified based on thickening and development of callose plugs as well as arrested pollen tube development. Some flowers were left to monitor fruit set which was censured at approximately 6 months.

Results and Discussions

The pollen used in this study were highly viable (Figures 1a & b). The self as well as cross pollen germinated prolifically within the stigma and produced a mass of pollen tubes which grew down the style (Figure 1c). Since no pistils were fixed prior to 24 hrs, it was not possible to determine whether cross pollen tubes arrived at the ovary in a shorter time interval compared to the self ones. It was however evident that both the cross as well as self pollen tubes had reached the ovary at 24 hrs and some ovule penetrations from all the treatments had already taken place as at this time. Presence of pollen tube "tails" was used as a positive score for ovule penetration (Figure 1d). This score may however have underestimated the numbers of penetrated ovules since whilst all ovules with a tail were clear positives, those lacking a pollen tube remnant may have lost it during dissection of the ovary. Any such false scores were nevertheless assumed to be more or else equal for all the treatments. Growth of self pollen tubes to the embryo sac was observed (Figure 1e). No distorted growth of the self-tubes was observed and no deposition of callos was noted.

Data on the proportion of penetrated ovules is presented in Table 1. There was an initial difference in the proportion of penetrated ovules by self and cross pollen tubes at 24 and 36 hrs. The lag in self penetrations was not attributed to slow growth of self pollen tubes for as many such pollen tubes reached the ovary as those from cross pollination (data on number of pollen tubes that reached the ovary is not presented). Ovule penetrations increased with time. By 48 hrs, self pollen tubes had achieved a significantly higher ovule penetration compared to the other crossing treatments. However, during the overall testing period (24 – 96 hrs), the OP (Open pollinated) treatment had marginally higher mean ovule penetrations compared to the other treatments though this was not statistically significant.

Earlier studies on SI in tea had shown that the mechanism of rejection of self-pollen was predominantly a pre-fertilization incompatibility system similar to the S-Z incompatibility system (Roger, 1975). Based on the results of fruit set presented here (Table 2) which show that self fertility in tea varies with genotype, tea could be considered a facultative out breeder but with a homomorphic gametophytic self incompatibility system (GSI). However, the successful growth of the pollen tube and penetration of ovules accompanied by the generally low percent fruitset in the selfing treatment indicates that tea may have a late acting self incompatibility (LSI) type of selfing control, which may also be

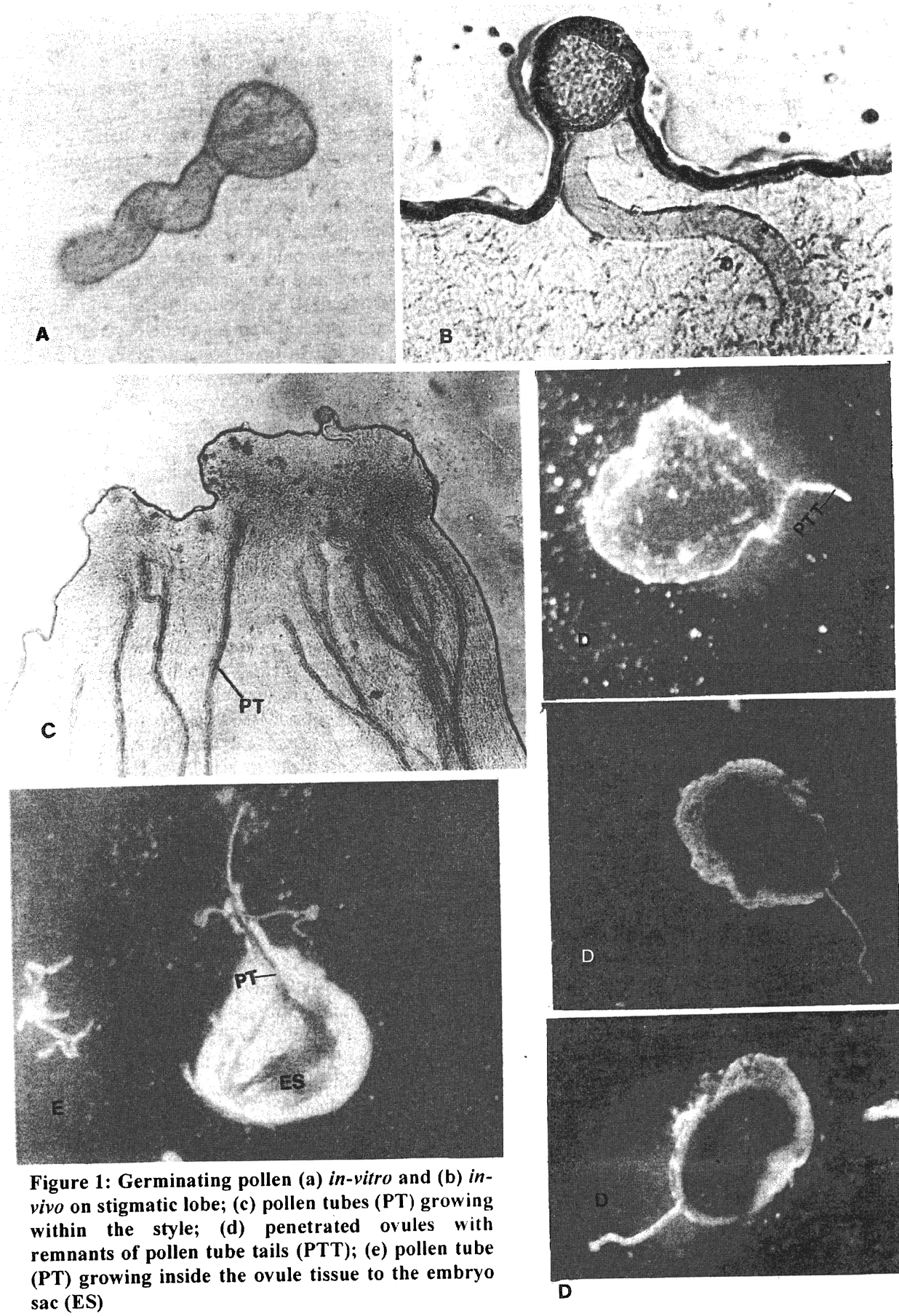

Figure 1: Germinating pollen (a) *in-vitro* and (b) *in-vivo* on stigmatic lobe; (c) pollen tubes (PT) growing within the style; (d) penetrated ovules with remnants of pollen tube tails (PTT); (e) pollen tube (PT) growing inside the ovule tissue to the embryo sac (ES)

Table 1.Proportion (arcsine transformed data) of penetrated ovules per flower at 24, 36, 48 and 96 hrs following pollination.

Treatments	24 hrs				36 hrs				48 hrs				96 hrs				
	TRFK 301/4	TRFK 303/577	TRFK K/purple	Mean	TRFK 301/4	TRFK 303/577	TRFK K/purple	Mean	TRFK 301/4	TRFK 303/577	TRFK K/purple	Mean	TRFK 301/4	TRFK 303/577	TRFK K/purple	Mean	Mean of Means
Self	36.1	32.9	44.9	38.0	34.7	34.6	42.1	37.1	46.4	42.8	43.6	44.3	38.9	41.3	41.1	40.5	39.9
Crossed (HP*)	41.3	37.7	42.1	40.4	41.4	44.4	43.5	43.1	41.2	40.2	39.7	40.4	42.9	40.4	42.9	42.1	41.5
OP**	41.8	36.2	46.1	41.4	38.8	37.0	46.0	40.6	37.8	38.3	40.5	38.9	50.8	45.4	51.0	49.1	42.5
Mean	39.9				40.3				41.2				43.9				
C.V.=	9.79%																
LSD													0.05%		0.01%		0.001%
Treatments													1.61		NS		NS
Duration													2.20		2.93		NS
Treatments x duration													3.81		5.07		6.60
Clones													1.91		2.54		3.30
Clones x treatments													NS		NS		NS
Clones x duration													NS		NS		NS
Treatments x clones x durations													NS		NS		NS

*H.P. – Hand pollination using immasculation technique.

**OP – Natural open pollination

Table 2: Fruit set from self and cross pollination in tea.

	% fruit set		
Treatment	TRFK K/purple	TRFK 301/4	TRFK 303/577
Self	20	3	0
Crossed	6	10	2
OP	21	0	17

post fertilization. Such a system has been described in other plants (Gibbs and Bianchi, 1999; Rabe Andrea and Soltis, 1999).

Data presented here indicates that the genotype (clone) explained a significant ($P<0.001$) amount of the total variation in the proportion of penetrated ovules and subsequently in percent fruitset. The successful fruitset from selfs in the study indicates possible breakdown of the SI system or presence of pseudo-self compatibility (PSC) with weakened SI in some genotypes e.g. clone TRFK K/purple. These results therefore demonstrate that tea exhibits variable expression of the SI trait. Such variable expression of SI has been observed in other plant species (Vogler *et al.*, 1998). The tea clones with low percent fruit set from the self-treatment thus showed stronger expression of SI than those with higher percent fruitset for the same treatment. Studies on the genetic control of SI have indeed revealed that this trait is phenotypically plastic and its differential breakdown may be related to genetic (Charlesworth and Charlesworth, 1995) and environmental factors (Hiscock, 2000), as well as the level of activity of pollinators (Vogler *et al*, 1998) and consequently the pollen transfer dynamics (Steinbachs and Holsinger, 1999). In *Lycopersicon*, it has been reported that the abundance of the putative transposable genetic element (Lyt 1) is related to differences in self-compatibility rates (Charlesworth and Charlesworth, 1995). Linkage and Quantitative Trait Loci (QTL) analysis in the same species revealed that the SI locus (S) region in chromosome 1 also haboured most major QTLs for several floral traits important to pollination biology(e.g. number and size of flowers) suggesting a gene complex controlling both genetic and morphological mechanisms of reproduction control (Bernachi and Tanksley, 1997).

Though it is not clear what factors may have contributed to the differential breakdown of SI in tea, this condition provides tea breeders with an opportunity to select stable and elite inbreds from clones expressing the pseudo-self compatibility (PSC) trait. It would be expected that progeny from self crosses of plants with stronger expression of SI would exhibit greater levels of inbreeding depression than progeny from plants with weaker expression of SI (Vogler *et al.*, 1999). Studies in Kenya on the effects of inbreeding depression (Wachira -unpublished data) have indicated variable performance of progenies from self-crosses with some far outperforming their parents. This explains why very high yielding and stable clones have been selected from inbred clone AHP S15/10 e.g. clone AHP SF186. Clone AHP S15/10 and its progeny clone AHP SC12/28 do not strongly express the SI trait (personal observation). From this data, it would therefore be expected that clones TRFK 303/577 and TRFK 301/4 would exhibit greater inbreeding depression than clone TRFK K/purple.

This study has demonstrated that the tea species may have an active late acting self incompatibility (LSI) system though some genotypes are pseudo-self-compatible.

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