Tea Q. 45, (3 & 4), 91-100. 1975. Printed in Sri Lanka.

PRELIMINARY OBSERVATIONS ON POLLEN TUBE INCOMPATIBILITY IN SOME TEA CLONES

S. Rogers

(Department of Biology, Queen Elizabeth College, London, UK)

The present trend towards the replacement of seedling tea by clonal plantings, allowing the selection of single genotypes and their large scale propagation, is taking place at a time when attention is also being directed towards the utilization of the seed, which tea bushes produce in considerable quantities. The edible seed oil is comparable in quality to olive oil, for which it may be used as a substitute.

The reliable production of seed from clonal plantings would require knowledge of the incompatibility system operating and genotypic identification of the clones used enabling compatible clones to be interplanted. Further steps would be the search for or production of self fertile clones and investigations into the seed setting potential and compatibility of induced tetraploid and synthetic triploid clones.

MATERIALS AND METHODS

Clonal material from the Tea Research Institute of Sri Lanka was used as follows

(1) Diploid clone TRI 2020 for all self pollinations and as the female parent in cross pollinations.

(2) Diploid clone TRI 2024 as the pollen parent in cross pollinations.

(3) Tetraploid clone of TRI 2025 because of the small quantity available only a limited range of self pollinations was attempted with this material.

Squash preparations and transverse and longitudinal sections were prepared for each pollination regime. Styles for squash preparations were softened and stained according to the technique of Martin (1959), then dehydrated and mounted in Euparal using the technique given by Ramanna (1973) with some minor modifications. Wax embedded material, sectioned at 10 and 15 μ was dewaxed, hydrated, stained in aniline blue as for squashes, then dehydrated and mounted in Euparal. Callose fluorescence was examined using Uv exciter filter BG12 in combination with barrier filter 41 and 65. The aniline blue preparations were also used for transmitted light studies. In squash preparations individual tubes may be followed easily due to their width and dark colour and they can also be identified in both transverse and longitudinal sections.

RESULTS

1—*Diploids*

Data obtained for pollen tube development in the diploid clone is summarized in Table 1 and Figure 1. Within this clone much variation was observed in the stigma and style, in the length of the expanded stigmatic lobes, in the point at which the styles become connate and in the total length of stigma and style (mean 8.23 $\pm$ 1.02 mm) so that the data are more meaningful expressed as the tube lengths rather than as the distance traversed within the style.

No useful measurements could be made after the two hour germination period. In this time tubes had emerged from the germ pores and begun to penetrate between the stigmatic papillae. After a four hour period there was penetration into the top of the stylar canals. The mean length of the self tubes at this stage was somewhat greater than that of the cross tubes but the high variance and coefficient of variation particularly in the self tube lengths made this difference non significant. Measurements after 6 and 12 hours showed unequivocally the faster growth of the self tubes, but this was not subsequently maintained and by 18 hours post pollination the cross tubes were significantly longer, having maintained the same growth rate throughout. Morphological change was becoming apparent in some self tube tips with the first indications of swelling and distortion (Fig. 2a & b). By 24 hours cross tubes were seen to have entered the ovary and had probably penetrated as far as the ovules, though preparations to observe fertilization were not made. The apparent decline in growth rate of the tubes between 18 and 24 hours arose from measurements made on stylar squashes cut off at the top of the ovary. It is more probable that tube growth maintained a uniform rate to the micropyles (Fig. 1).

The cessation of self tube elongation was accompanied by deposition of appreciable amounts of callose around the tube tips resulting in a brilliant fluorescence. The tube apices were also highly distorted and callose plugging appeared to occur closer behind the tips than in compatible tubes. Measured distances from the tube tip to the first plug were 194.3 $\mu \pm$ 48.40 for incompatible tubes and 317.6 $\mu \pm$ 60.02 for compatible tubes but insufficient measurements were obtained to establish whether this difference was significant.

Previous authors (Linskens & Esser 1957; Tupy 1959) have shown that incompatible tubes may have more or larger callose plugs than compatible ones. In this material measurement showed that some plugs in incompatible tubes were much larger than those in compatible tubes (Table 2) but the size variation within the groups was so great that any difference between them was non-significant. The distance between plugs in individual tubes was also highly variable but in spite of this, the difference between self and cross tubes was significant. Callose production was thus greater in the self tubes this being reflected in the larger size of many of the plugs and their closer spacing.

2—*Tetraploids*

Tetraploid flowers showed a marked difference between those having apparently 'normal' styles with a diameter similar to that of the diploids, or slightly larger and those in which the styles were collapsed and angular with a diameter about half that of normal styles. In these, the stylar canal was almost obliterated the biggest cavities being only about 1/20 of the stylar diameter rather than at least 1/3 which is typical of diploid styles. The 'collapsed' styles showed a premature degeneration of the cells either prior to pollination or early in tube development. Pollen tube growth in the tetraploid styles also showed much variation. A considerable proportion of self tubes ceased to elongate at an early stage. During germination and early growth the tubes fluoresced strongly but during penetration into the stylar canal the callose appeared to disintegrate so that when elongation ceased the tubes were no longer fluorescing. In the 'normal' styles some self tubes were found to have penetrated to the style base within 24 hours, thus showing a growth rate comparable to diploid self tubes.

TABLE 1—*Lenth of pollen tubes at different times after pollination*

Hours post pollination	Pollination method	Mean tube length (in mm)	Coefficient of variation	Level of significance
2	Self	Tubes too short to measure	—	—
	Cross	,,	—	—
4	Self	1.76±0.28	45.17	Difference non significant
	Cross	1.45±0.20	24.89	
6	Self	2.92±0.21	26.59	Self longer than cross
	Cross	2.24±0.20	31.00	P = 0.02
12	Self	5.34±0.33	25.16	Self longer than cross
	Cross	4.25±0.28	27.88	P = 0.01
18	Self	6.089+0.62	29.70	Cross longer than self
	Cross	7.15±0.22	13.14	P = 0.05
24	Self	6.38±0.27	14.70	Cross longer than self
	Cross	7.92±0.37	11.40	P = 0.001

TABLE 2—*Callose plugs in pollen tubes*

Ploidy level	Pollination method	Mean length of callose plugs (μ)	Coefficient of variation	Mean distance between plugs (μ)
Diploid	Cross	13.41±1.21	40.26	821.25±78.40
Diploid	Self	19.86±2.42	57.50	686.76±25.43
Tetraploid	Self	27.11±4.29	48.02	

Difference significant difference at P 0.05, 0.02

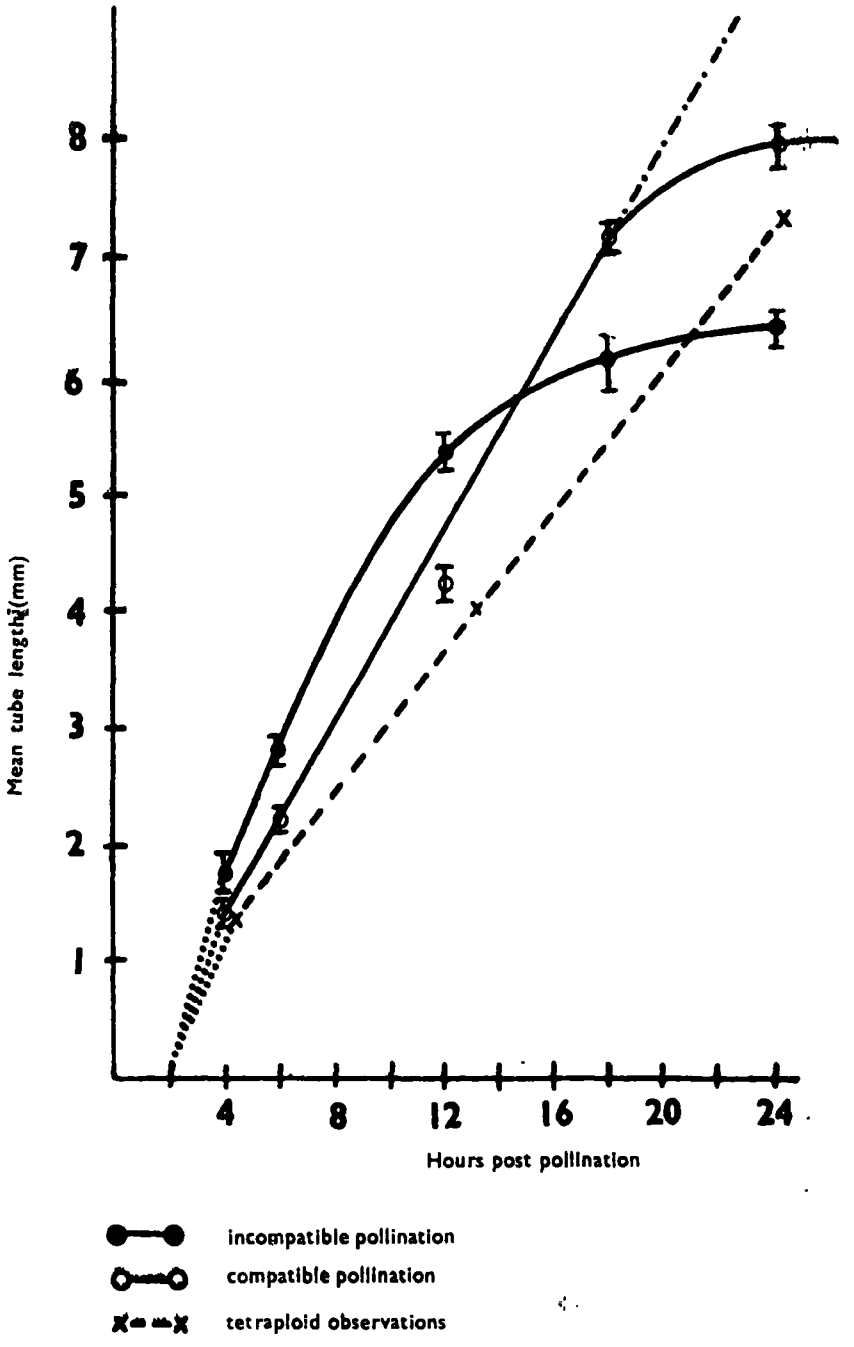


FIG. 1—*Pollen tube development*

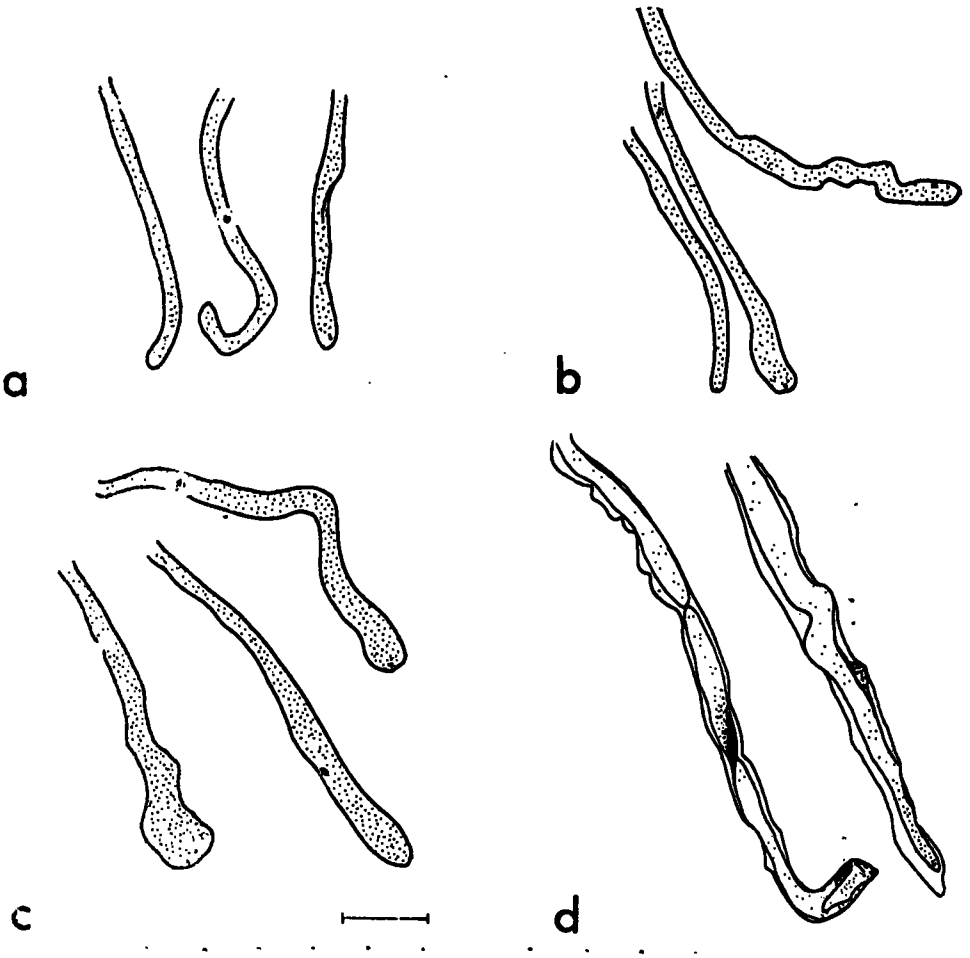
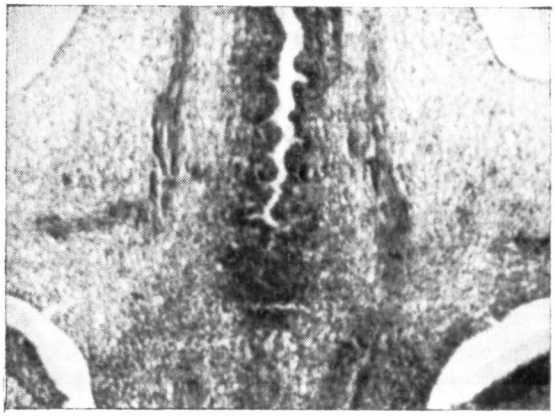


FIG 2—*(a) Normal tube development in a diploid 18 hours following a compatible pollination (b) diploid incompatible tubes after the same time interval showing the begining of apical distortion (c) incompatible tubes in a tetraploid, 24 hr post pollination showing apical swelling but no distortion (d) diploid incompatible tubes 24 hr following pollination showing irregular tips of the arrested tubes and the deposition of excess callose around the tube tip.*



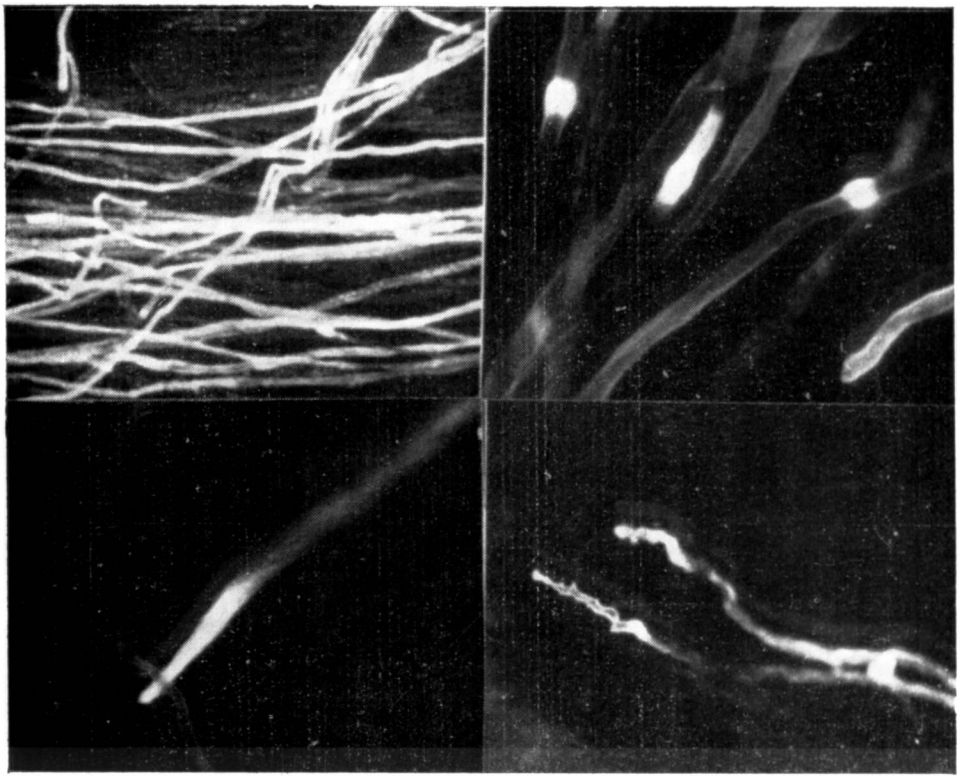


PLATE 1—*Top, LS Style base showing site of pollen tube inhibition. Centre Left, Pollen tube growth in the style. Centre right, Callose plugs in self pollen tubes. Bottom Left, brightly fluorescing tip of a self tube* 18 *hr post pollination. Bottom Right, Tips of inhibited self tubes* 24 *hr post pollination.*

Ploidy	Hours post pollination		
	4	6	24
	Mean tube length (mm)		
2X	1.76±0.28	2.92±0.216	6.38±0.27
4X	1.48	1.29	7.4

In the 'collapsed' tetraploid styles there was also evidence of tube growth, thus callose plugs were found in transverse section extending to about one half the length of the style 24 hours after pollination. The tube tips would be below this but are not visible in transverse sections.

Like the diploids, tetraploid self tubes had ceased to elongate by 24 hours after pollination. The clone examined showed tubes with markedly swollen though not irregularly distorted tips which gave a much less intense fluorescence than the diploids suggesting less callose deposition around the tube tip (Fig. 2c & d).

DISCUSSION

The location and nature of the self incompatibility reaction in tea is characteristically that of a multiple allele gametophytic system; and all the morphological features of the self tube inhibition in tea have previously been reported in other species. Sears (1937) reported swollen, thick walled tube ends in *Petunia violacea* and Arasu (1967) showed that incompatible tubes are not inhibited in the hollow styles of *Ribes* but only where the tubes are forced into close contact with the cells lining the stylar canal. Other hollow styled species behave similarly (Brewbaker 1957). In tea Tomo *et al.* (1956) reported the cessation of self tube elongation at the style base, an observation confirmed in this study. However Simura and Oosone (1956) stated that self tubes in tea grow much more slowly than cross and Tupy (1959) also reported an inverse relationship between tube growth rate and intensity of callose formation in *Nicotiana* and apple from his study of callose deposition following self and cross pollination in these species.

The present observations confirm for tea, the greater amounts of callose in the incompatible tubes but also show that over the first twelve hours their growth rate is significantly faster than that of compatible tubes. The pattern thus resembles that in red clover where incompatible tubes grow rapidly for half the length of the style, followed by a sharp retardation (Silow 1931). Linskens (1955) postulated a similar sugar consumption in compatible and incompatible tubes resulting in a heaping up of glucopyranose in the latter as elongation ceases. The initially faster growth could imply a faster metabolism of sucrose in the incompatible tubes and therefore the accumulation of more glucopyranose to be deposited as polymer callose. This would accord with the observed differences in plug size and number between compatible and incompatible tubes not only near the site of the incompatibility reaction but also in the upper half of the tubes which are already empty when the inhibition reaction occurs. The heaping up of callose would therefore be proportional to tube growth rate over the pre-inhibition period but also as Tupy (1959) stated; inversely related to their final length.

ACKNOWLEDGEMENT

The author wishes to thank the Tea Research Institute of Sri Lanka for the facilities provided to carry out the field work for this investigation and Dr A. R. Sebastiampillai for helpful criticisms and suggestions during the preparation of the manuscript.

REFERENCES

ARASU, N. T. (1968). *Genetica* **39**, 1–24.
BREWBAKER, J. L. (1957). *Journal of Heredity* **48**, 271–277.
LINSKENS, H. F. (1955). *Z. Bot.* **43**, 1–44.
LINKSENS, H. F. & ESSER, K. L. (1957). *Naturwissenschaften* **44**, 16–17.
MARTIN, F. W. (1959). *Stain Technology* **34**, 125–128.
RAMANNA, M. S. (1973). *Stain Technology* **48**, 103.
SEARS, E. R. (1937). *Genetics* **22**, 130–181.
SILOW, R. A. (1931). *Bulletin of the Welsh Plant Breeding Station Series H* **12**,228–233.
SIMURA, T. & OOSONE, K. (1956). *Japanese Journal of Breeding* **6**, 111–114.
TOMO, M. FUCHINONE, Y. & YAMANE, H. (1956). *Japanese Journal of Breeding* **5**, 247–253.
TUPY, J. (1959). *Biol. Plantarum* **1**, 192–198.

Accepted for publication—12*th September* 1975.