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CHEMISTRY OF TEA *(CAMELLIA SINENSIS)* LEAF SURFACE

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The adaxial and abaxial surface waxes of first and second leaves of five drought resistant and five drought susceptible tea clones were extracted separately using chloroform in order to examine possible relationships between surface wax contents of leaves and drought resistance of the tea plant. The wax contents of the drought resistant clones were higher than that of the drought susceptible clones. One of the components of wax was identified as caffeine and the caffeine content of the adaxial and abaxial surfaces of the leaves were quantified using Gas Chromatography.

INTRODUCTION

In the field, the tea plant is subject to various forms of stress. Moisture stress occurs during periods of drought which causes a substantial loss of crop. However, those tea plants which are physiologically better adapted to withstand such stress factors would continue to yield in spite of the drought. In this respect, selection of clones which show drought tolerant properties would be of benefit in areas prone to periodic droughts.

It is known that morphological and physiological characteristics contribute to drought resistance in plants (Parker, 1969 ; Begg and Turner, 1976). Among the physiological characteristics, reduction in stomatal and cuticular transpiration contribute to drought resistance in plants ; factors like cuticle thickness, composition and structure of the cuticle could cause variations in the drying rate of leaves. Nagarajah (1979 a) showed that there were clonal differences in the drying rates of mature and old tea leaves while the drying rates of young leaves was not different. He attributed the similarity in the drying rate of fast and slow-drying clones after the cuticle was damaged to clonal differences in cuticular resistances while the absence of clonal differences in the drying rate of young leaves was attributed to the similarity of the cuticle at that early stage of foliar development. As a criterion of clonal selection for drought resistance it is desirable to select clones that have a high culticular resistance in mature leaves, because most of the transpiration takes place from these exposed mature leaves. He also showed an inverse relationship between the drying rate of mature leaves and the yield of the clones studied.

Nagarajah (1979 b) has also shown that potassium deficient leaves had a lower cuticular resistance.

The outermost layer in the leaves of plants is the epicuticular wax. Due to its unique properties and fundamental role as the barrier between the leaf and its environment, the physico-chemical properties of these have been well studied (Martin and Juniper, 1970 ; Kolattukudy and Walton, 1972).

In the study of young tea shoots the emphasis has been on the intra-cellular constituents of the leaves. Studies on the lipid content of tea have been on the whole tea leaf (Khanna, Seshadri and Seshadri, 1974 ; Roberts, 1974 ; Anan, 1983). This paper reports the results of a preliminary study on the chemistry of the tea leaf surface. The objective of the study was to examine possible relationships between surface wax contents of tea leaves and drought resistance of the tea plant.

MATERIALS AND METHODS

Surface area meter, preparative TLC (Silica Merck) and Tracor 560 Gas Chromatograph fitted with Tracor TS 10 recorder, Glass column 1.5 m × 2 mm prepacked with SE 30.

Operating parameters of the gas chromatograph

Argon flow rate	—	30 ml/min
Hydrogen flow rate	—	30 ml/min
Air flow rate	—	350 ml/min
Detector	—	Flame ionisation detector (FID) 250° C
Column temperature	—	175° C isothermal
Injection volume	—	3 μl 200° C $CHCl_3$
Attenuation	—	4
Chart speed	—	0.25 cm/min

Sample preparation

The clones used in this study were TRI 2023, TRI 2024, TRI 2025, TRI 2026, DN, DTI, CY 9, N 2, KEN 16/3 and TK 48. Fifty grams of either the first or second leaf was taken from each clone and the total surface area was measured. The wax from the adaxial and abaxial surfaces were extracted separately using chloroform delivered from a burette (Hunt, Holloway and Baker, 1976). The chloroform extract was evaporated to dryness *in vacuo*. The residue was weighed, dissolved in 1 ml chloroform and examined by Gas Chromatography. The experiment was done in triplicate for each clone and mean values taken.

RESULTS AND DISCUSSION

The wax content per unit area (cm^2) from the adaxial and abaxial surfaces of tea shoots obtained by extraction with chloroform are presented in Figs 1 and 2 respectively. It will be seen that among the clones selected for this study the drought resistant clone CY 9 contained the most amount of wax. The wax content in the adaxial

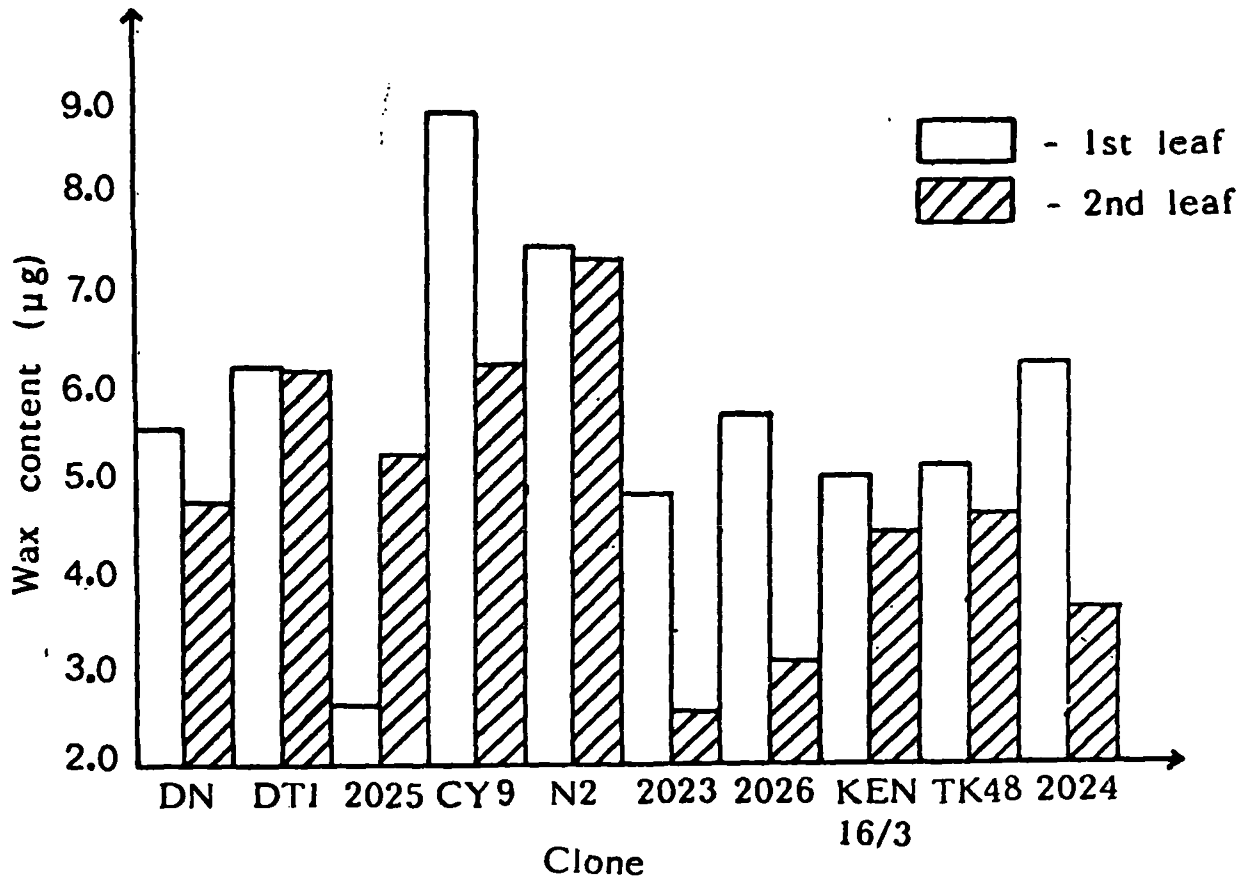

Fig. 1—Wax content per unit area (cm²) in the adaxial tea leaf surface of different clones.

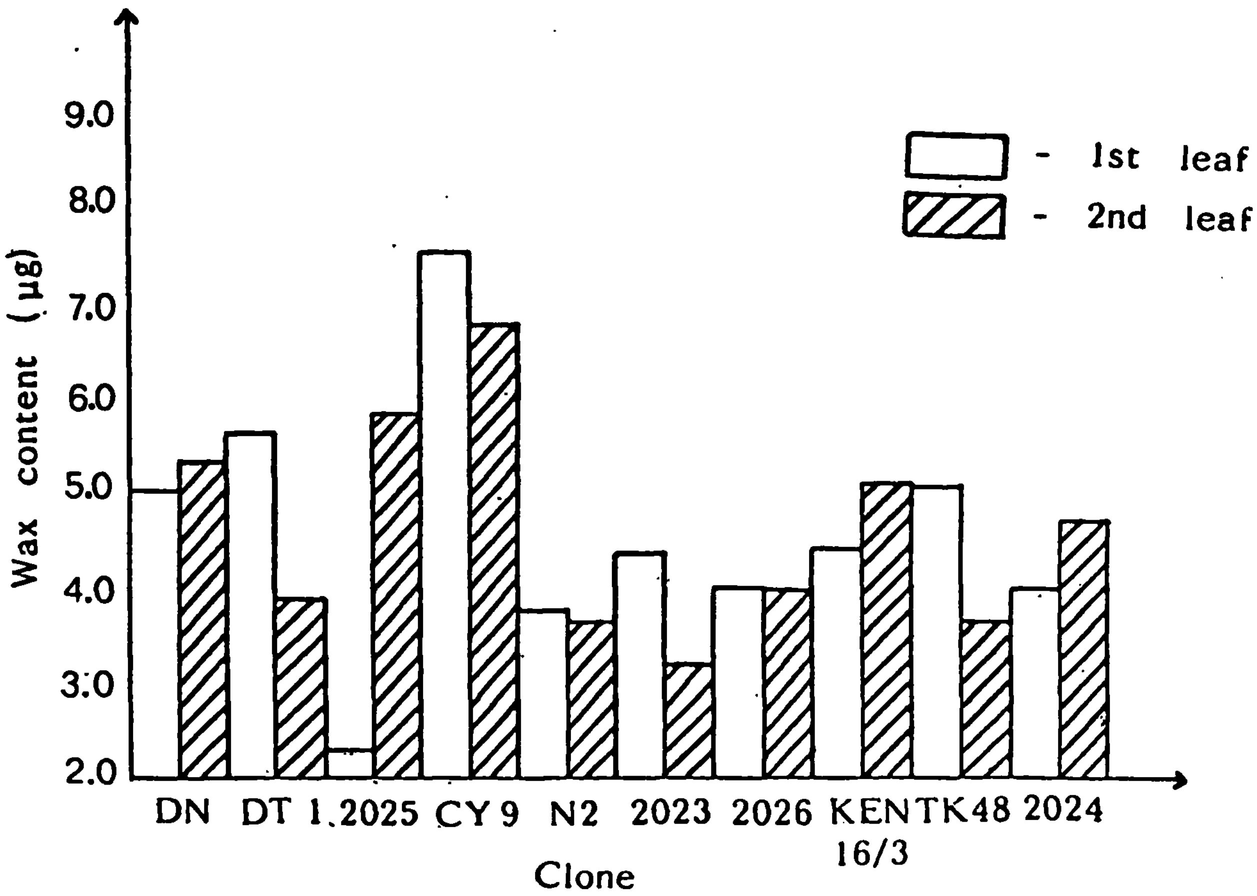

Fig. 2—Wax content per unit area (cm²) in the abaxial tea leaf surface of different clones.

surface was higher in drought resistant clones like DN, DT 1, CY 9 and N 2 than in drought suseptible clones like TRI 2023, TRI 2026, KEN 16/3, TK 48 and TRI 2024. Nagarajah (1979 a) has shown that leaves of drought resistant clones have a comparatively higher cuticular resistance compared to leaves of drought susceptible clones and it is possible that this is associated with the high epicuticular wax content. From Fig. 1, it can also be seen that the adaxial wax content in the first leaf is generally more than in the second leaf. It is known that the vigorously growing fresh leaves are capable of producing more wax than the mature leaves.

In all the clones the adaxial wax content was always higher than the abaxial wax content in the first leaf. This is probably because of the plant's adaptation to reduce epicuticular evaporation and due to the fact that the cuticular transpiration is less than the stomatal transpiration. Furthermore tea being a dicotyledonous plant stomata are distributed only in the lower surface (abaxial) of the leaf and the remaining portion is covered by a cuticle. Since the adaxial surface is completely covered by a cuticle without any stomata, higher transpiration from the abaxial surface compared to the adaxial surface is expected.

On investigating the nature of the wax constituents, it was found that one of the compounds was caffeine. It was identified by comparing the retention time and mass fragmentation pattern by Gas Chromatography-Mass Spectrography. Since the presence of an alkaloid in the leaf surface is very unusual (Hubert, Kwasny and Turner, 1977) the extraction procedure was re-checked using cotton wool swabs which gave the same result.

The caffeine content per unit area (cm^2) from the adaxial and abaxial surfaces of tea shoots are given in Figs 3 and 4 respectively. It can be seen that clone CY 9 has the highest caffeine content. It can also be seen that the caffeine content of the abaxial surface is higher than that of the adaxial surface in the first and second leaves of almost all the clones. This could be due to the diffusion of caffeine from the inner cells to the leaf surface either through the cell walls or through the stomatal pores. It is probable that greater diffusion may have taken place through the stomatal pores than through the cell walls as diffusion is quicker through the former.

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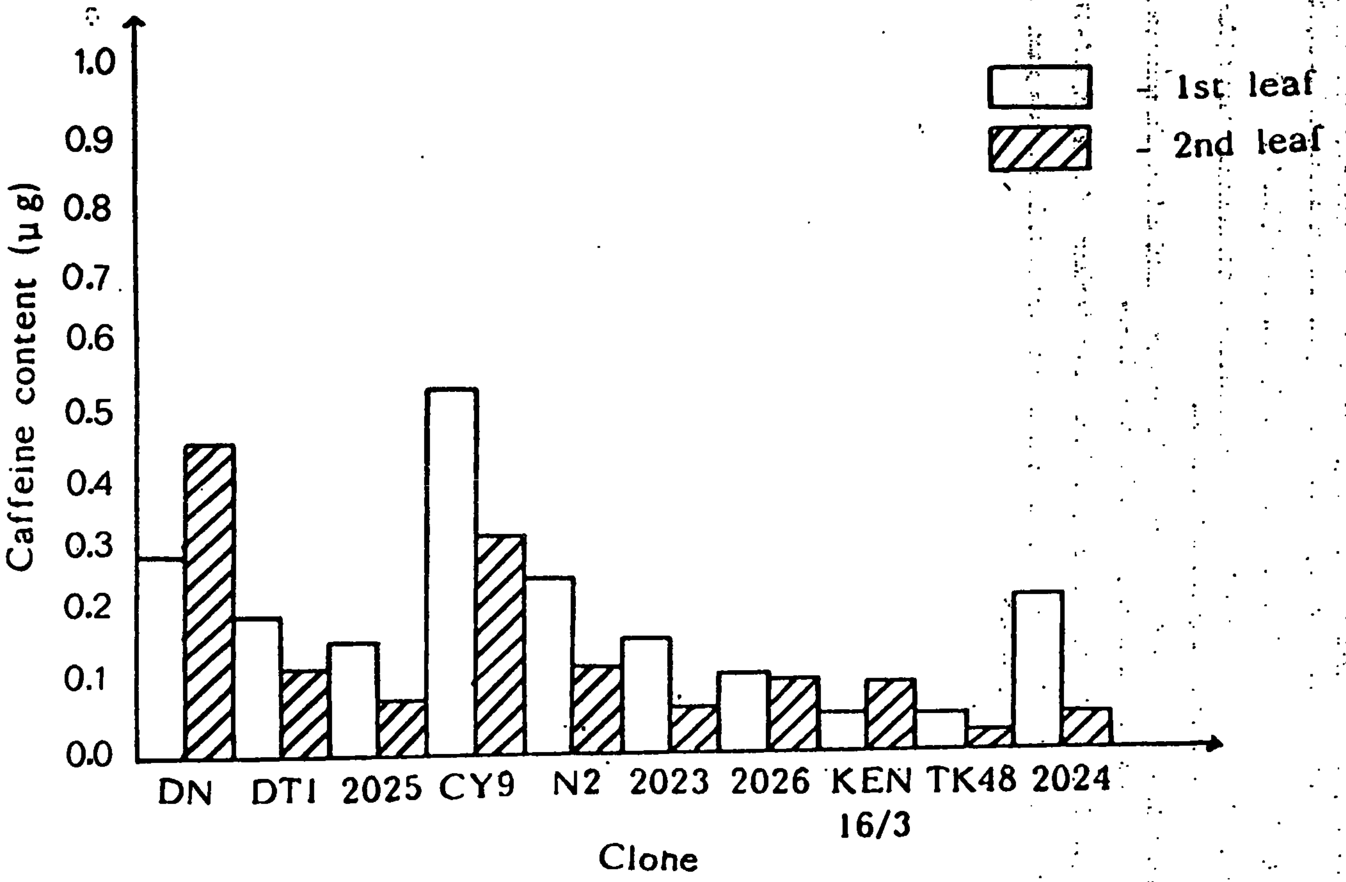

Fig. 3 — Caffeine content per unit area (cm²) in the adaxial tea leaf surface.

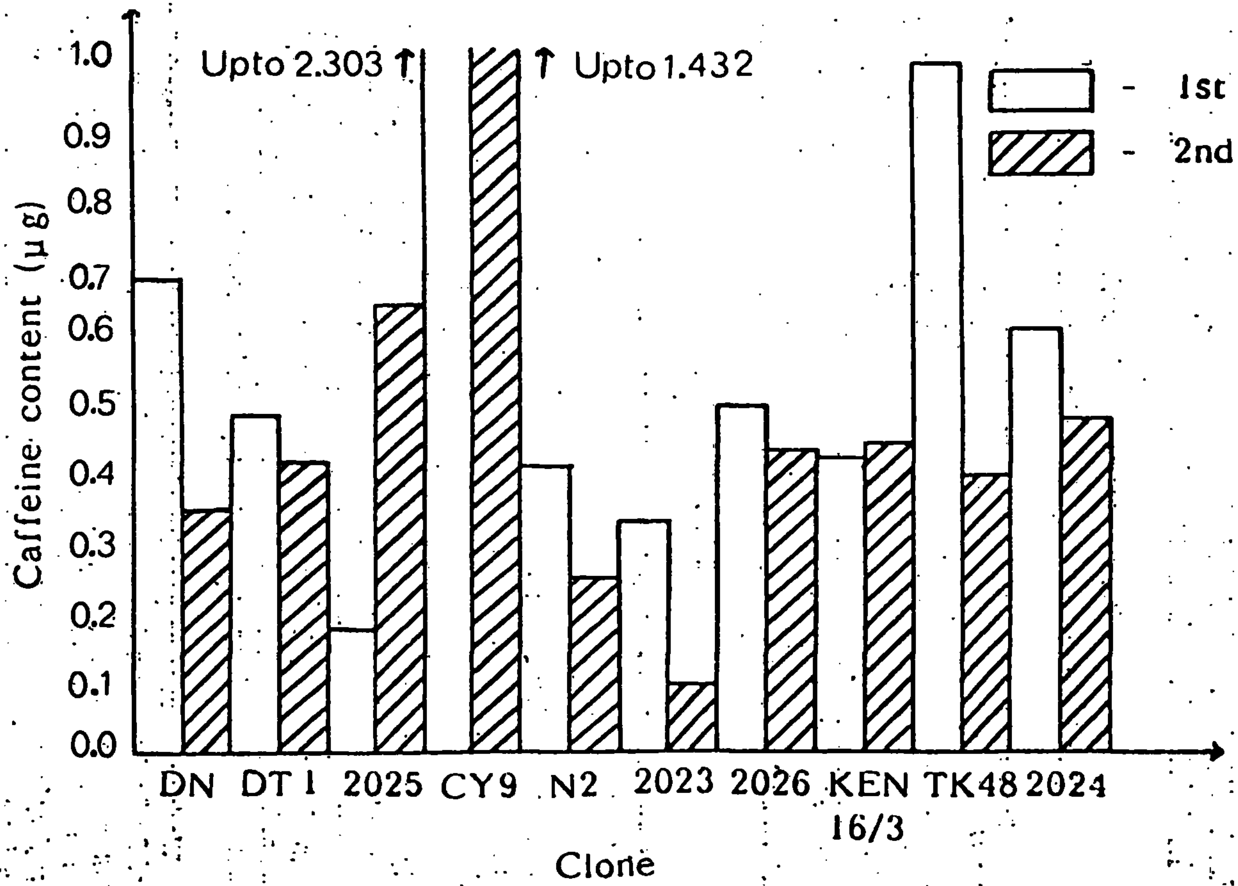

Fig. 4.—Caffeine content per unit area (cm²) in the abaxial tea leaf surface.