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CORRELATION OF MAJOR FLAVANOLS OF SOME TEA CLONES WITH QUALITY OF TEA

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A trial was carried out to investigate whether a correlation exists between the levels of flavanols and the quality of some selected tea clones. The levels of flavanols were assayed by HPLC whilst the quality of the clones was determined by sending samples of tea to tasters. The results did not reveal the existence of a reliable correlation between the levels of flavanols and the quality of the clones studied.

INTRODUCTION

Analysis of fresh tea flush for soluble solids have indicated the presence of several groups of compounds including amino acids, carbohydrates, purines, organic acids and polyphenols (Millin and Rustige, 1967). Among these different groups of compounds, polyphenols comprise the highest percentage. Analysis of the polyphenol fraction of the tea flush has indicated that it is made of several sub groups, including flavanols, flavonol glycosides, leucoanthocyanins, phenolic acids and depsides. Among these sub groups, flavanols are highest in fresh tea flush (Millin and Rustige, 1967). Flavanols are made up of six catechins including (+) Catechin, (–) – Epicatechin, (+) – Gallocatechin, (–) Epigallocatechin, Epicatechin gallate and (–) – Epigallacotechin gallate. Of the six catechins, the highest amount seen in both clonal and seedling tea is (–) – Epigallocatechin gallate (Roberts, 1962).

During black tea manufacture these catechins interact with one another in the presence of atmospheric oxygen and an endogenous enzyme called polyphenol oxidase (PPO) to produce two groups of compounds namely theaflavins (TF) and thearubigins (TR) which contribute to the colour, quality and character as is detected in the tea cup (Sanderson, 1972). The quality of tea produced varies according to the type of leaf being used for manufacture. In addition, quality also varies according to the cultivar from which leaf is collected. The present mode of evaluating quality of different tea clones is by organoleptic means which is tedious and time consuming considering the fact that the leaf has to be manufactured prior to organoleptic assessment. Several attempts have been made in the past employing total flavanols, nitrogen content and PPO activity to evolve a chemical/biochemical procedure that could be correlated to quality but with little success (Sanderson, 1964; Sanderson and Kanapathipillai, 1964).

Subsequently Wickremasinghe (1965) found a correlation between the levels of TF and the valuation of some tea samples. However, no study was made to investigate whether a correlation existed between the level of TF and the quality of a selected number of clones.

In the present study an attempt has been made to find whether a correlation exists between the level of individual flavanols found in fresh tea flush of different clones and the quality of made tea. In this study the separation and quantification of flavanols was carried out using high performance liquid chromatography (HPLC) (Hoeffler and Coggon, 1976).

MATERIALS AND METHODS

The clones used in this study were from those planted at St. Coombs Estate, Talawakele: TRI 2025, TRI 2023, TRI 2142, TRI 2024, KEN 16/3, DT 1, N 2, CY 9, K 145, TRI 777, DG, DG 7, DG 39 and DN.

Extraction of flavanols

Fresh tea flush (10g) was plunged into boiling absolute ethanol (50 ml) and boiled for 10 min. The cooled extract was homogenized for 5 min and the homogenate centrifuged (4000 rpm) for 5 min. The supernatant was decanted into a 100 ml volumetric flask and the residue re-extracted with 50% aqueous ethanol (40 ml) and centrifuged as earlier. The pooled volume was made up to 100 ml with 50% aqueous ethanol.

Purification of the sample

Sep-pak C_{18} Cartridge (Waters Associates, Milford, MA, USA) was activated with acetone (10 ml) and washed with distilled water (10 ml). The sample (1 ml) was loaded into the cartridge and eluted with N, N – dimethyl formamide:methanol:acetic acid:water (200:10:5:785) – (5 ml x 3) into a 25 ml volumetric flask and the volume made up with distilled water.

The purified sample was filtered through a 0.45 μm filter (10 mm Rainin) and 20 μl was injected to the following high performance liquid chromatographic system.

High–performance liquid chromatographic conditions

A C_{18} (ODS)μ Bondapak (3.9 mm x 150 mm) column (Waters Associates, Milford, MA, U.S.A.) of 10 μm particle size was used. The mobile phase was N, N–dimethyl formamide:Methanol:acetic acid:water–200:10:5:785, at a flow rate of 2ml per min. Detector wavelength was set at 280 nm and sensitivity at 0.02 AUFS. A Waters HPLC system was used in the present study.

HPLC calibration curves were constructed using authentic flavanols (Mitsui Norin & Co. Japan). All flavanols were eluted at ca 25 min.

RESULTS

The separation of flavanols by high performance liquid chromatography is shown in Fig. 1. The levels of flavanols found in the different clones are presented in Figures 2 – 6. From the results it is evident that the highest level in all clones under investigation was that of EGCG (Fig. 2). Among the clones the highest level of EGCG was found in clones DN and TRI 2025, followed by K 145, N 2, TRI 2024, KEN 16/3, DG 7 while clones CY 9, DT 1, TRI 777 and DG 39 had the least amount of EGCG.

The next highest level of flavanol in all the clones except TRI 777 was EGC (Fig. 3) with peak levels in clone CY 9 followed by DG 39. Clones TRI 2025, TRI 2023, DG and DG 7 showed lesser amounts with the least in clone TRI 777.

The next highest level of flavanol in all the clones was that of ECG which peaked in clone TRI 777 (Fig. 4). The levels were lower in clones TRI 2023, CY 9, K 145, DG 7 and DG 39.

Clone CY 9 had the highest level of EC followed by clones DT 1 and N 2 (Fig. 5) while clones TRI 2023 and DG 7 had the lowest levels.

The highest level of catechin was found in clones TRI 2024 and N 2 (Fig. 6) while moderate levels were seen in clones, TRI 2025, TRI 2142, KEN 16/3, DT 1, CY 9 and K 145.

On the basis of organoleptic assessments of tea samples made by tea tasters the clones were grouped into 3 categories; viz. good, average and poor quality clones. DT 1, K 145, TRI 777 and N 2 were classified as good, KEN 16/3, DN, TRI 2025, DG 39 and TRI 2023 as average while clones TRI 2142, DG and CY 9 were classified as poor.

DISCUSSION

The results of this study does not indicate any definite trend in relation to the level of flavanols and quality of made tea of the individual clones. The level of the flavanol EGCG was low in the quality clones DT 1 and TRI 777 as well as in the poor quality clone CY 9. On the other hand its level was high in two other quality clones K 145 and N 2 as well as in the poor quality clone DG.

The flavanol EGC was high in one poor quality clone CY 9 whereas its level was low in another poor quality clone, DG. On the other hand it occurs only in moderate levels in the quality clone TRI 777. This indicates a lack of uniformity in the level of this compound in relation to quality in the respective clones.

While ECG was high in the two quality clones DT 1 and TRI 777 it was low in the quality clone K 145. Two other poor quality clones had moderate levels of this flavanol (DG and CY 9).

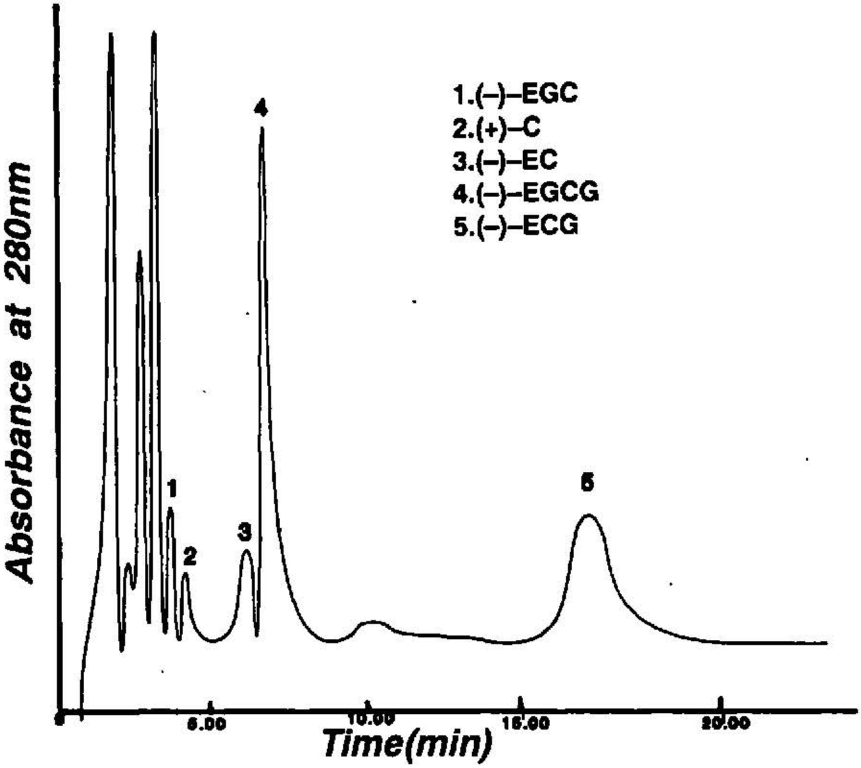

Fig. 1–A *typical high performance liquid chromatogram of five flavanols found in fresh tea flush.*

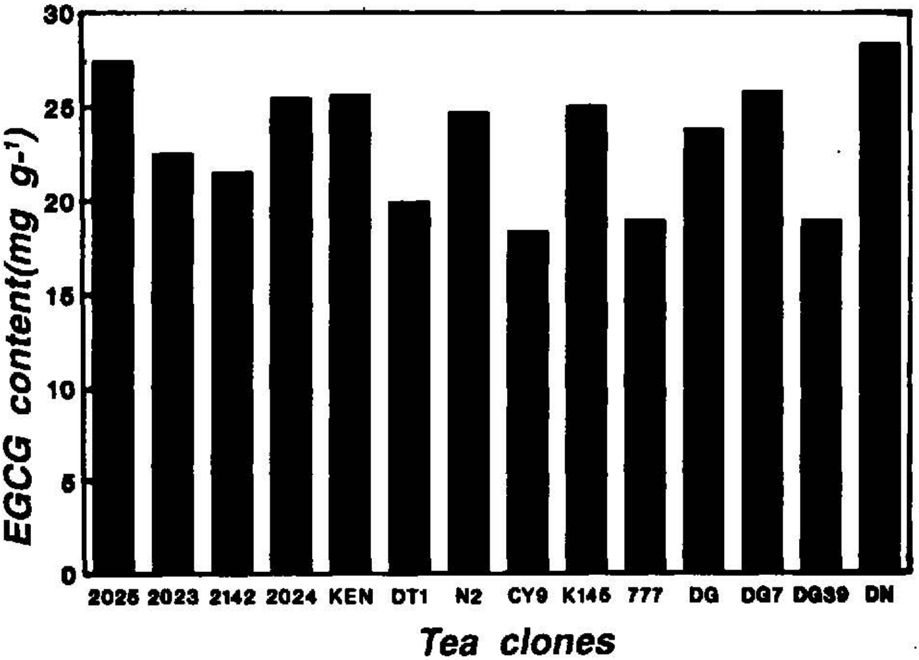

Fig. 2–*The level of (–)-Epigallocatechin gallate (EGCG) in flush of tea clones.*

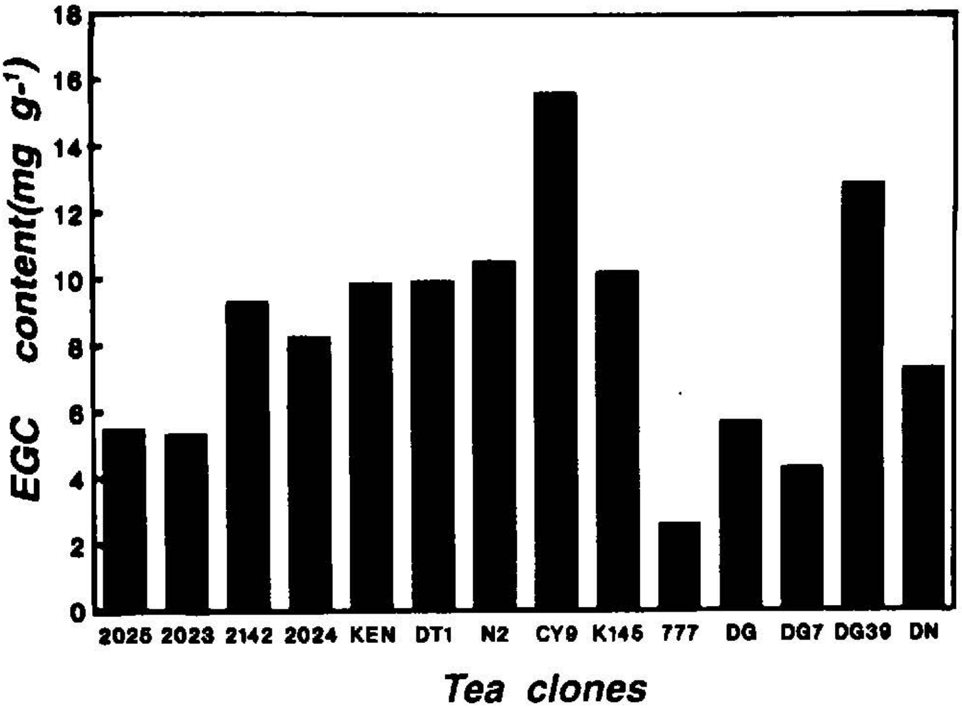

Fig. 3–*The level of (–)-Epigallocatechin (EGC) in flush of tea clones.*

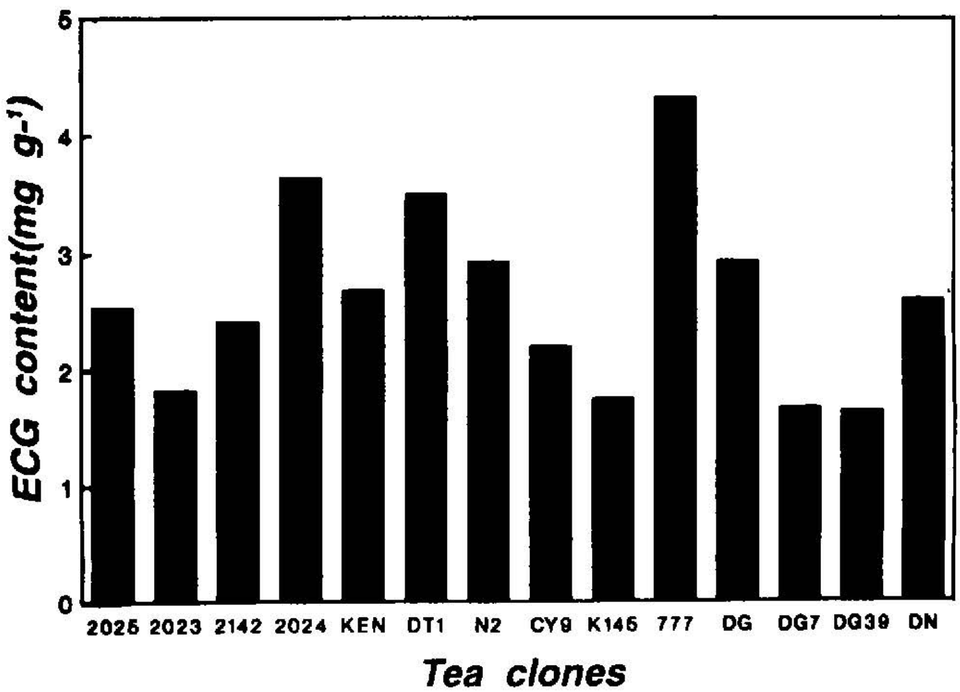

Fig. 4–*The level of (–)-Epicatechin gallate (ECG) in flush of tea clones.*

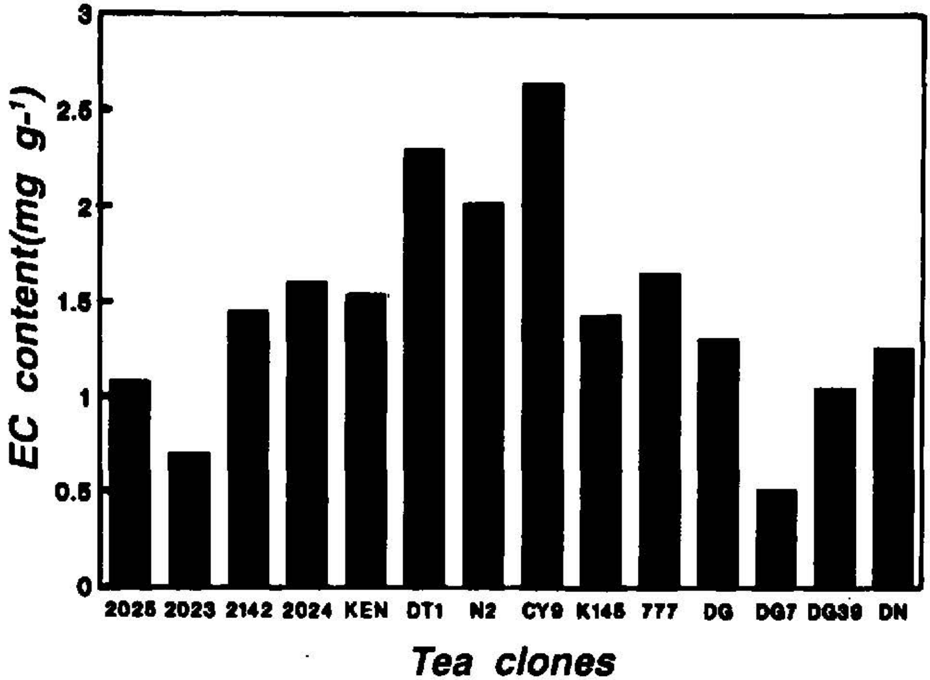

Fig. 5–*The level of (–)–Epicatechin (EC) in flush of tea clones.*

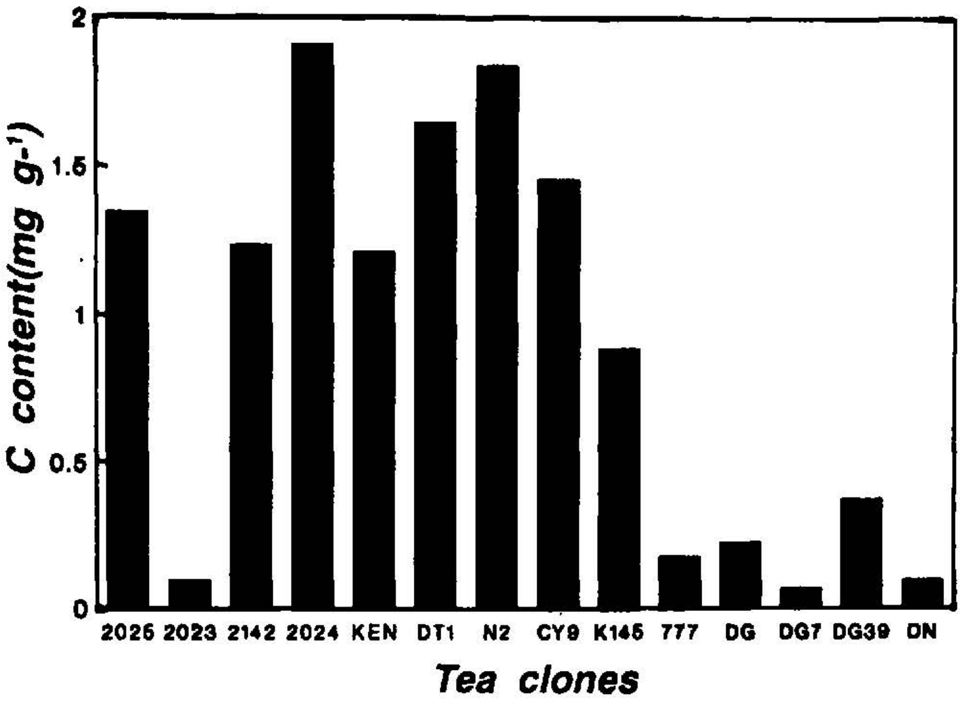

Fig. 6–*The level of (+)–catechin (C) in flush of tea clones.*

The quality clone DT 1 as well as the poor quality clone CY 9 had high levels of EC but this was low in the other quality clone K 145.

The level of catechin was high in the quality clone N 2 as well as in the average quality clone TRI 2024. The quality clone TRI 777 and the poor quality clone DG had low levels of catechin.

These results indicate that none of the major flavanols of tea could be used as suitable indices to classify clones according to their quality potential.

CONCLUSION

On the basis of the above observations it is apparent that none of the flavanols could be used as an index in classifying the clones for their quality potential. It appears that there are other factors which contribute to quality in differernt clones. These factors could be the activity of enzymes such as polyphenol oxidase and peroxidase, the organoleptic contribution made by other polyphenolic substances such as flavonols and their glycosides as well as the contribution made by flavour.

REFERENCES

HOEFFLER, A. C. and COGGON, P. (1976). Reverse phase high performance liquid chromatography of tea constituents. *J. Chromatogr.* **129**, 460.

MILLIN, D. J. and RUSTIGE, D. W. (1967). Tea Manufacture. *Process Biochemi* **2**, 9–13.

ROBERTS, E. A. H. (1962). Economic importance of flavanoid substances, pp. 468–512. In *Chemistry of Flavanoid compounds* ed. T. A. Giesmann, Pergamon Press, London.

SANDERSON, G. W. (1972). The chemistry of tea and tea manufacturing, pp 247-316. In *Recent advances in Phytochemistry,* eds. V. C. Runekless and T. C. Tso, Academic Press, London.

SANDERSON, G. W. and KANAPATHIPILLAI, P. (1964). Further studies on effect of climate on the chemical composition. *Tea Q.* **35**, 222-229.

WICKREMASINGHE, R. L. (1965). Studies on quality and flavour: 1 – The polyphenols and low boiling volatile compounds. *Tea Q.* **36**, 59-63.