ANALYSES OF POLYPHENOLS, AMINO-ACIDS AND CHLOROPHYLL LEVELS IN TEA FLUSH AT DIFFERENT SEASONS

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The correlation between rainfall and levels of polyphenols, amino-acids and chlorophyll in flush from clonal tea has been studied over a period of one year. It was found that total polyphenols, the vanillin-reacting group of polyphenols, amino-acids and chlorophyll increased with rainfall, while the leucoanthocyanin group of polyphenols decreased with rainfall. These trends were uniform in all of the clones studied, but were not statistically significant in every case. It is suggested that the conflicting results of previous workers may have been partly due to the relative non-specificity of the methods used by them.

The polyphenols and amino-acids of tea are of importance in view of the part these compounds play in determining quality (Roberts 1962), and flavour (Wickremasinghe & Swain 1965), respectively. Studies of the effect of season on the quantitative changes in these compounds, however, have yielded conflicting results.

Evans (1930) concluded from his results that there were seasonal variations in the polyphenol and amino-acid contents of fresh tea flush, and Ramaswamy (1964), working with made tea, found an increase in soluble solids and nitrogenous constituents (which included amino-acids and caffeine), during the dry season compared to the wet season. The number of samples analysed in these investigations, however, was too small to allow definite conclusions to be drawn — a point which was emphasized by the latter author. The studies of Sanderson & Kanapathipillai (1964), indicated that season had no significant influence on levels of total nitrogen (amino-acids, caffeine and protein), caffeine, protein or total flavanols. Their results were based on analyses of flush from two clones, TRI 777 and TRI 740, plucked on ten-day rounds over a period of eight months. Finally, Wood, Bhatia, Chakraborty, Choudhury, Deb, Roberts & Ullah (1964), working with Assam tea flush, concluded that seasonal variations in amino-acids appeared to be too irregular and too small to have any real significance, but that there were wide variations in total polpyhenols.

The effect of dry and wet weather on polyphenols and amino-acids is, therefore, still obscure, and the present paper gives the results of analyses carried out at fortnightly intervals over a period of one year. Quantitative determinations were made of amino-acids and total as well as different groups of polyphenols, and a statistical study made of the relationship of the quantities of these compounds to rainfall. Five clones, (DT 1, TRI 2024, PA 22, CV5B1 and TRI 26), were followed in this investigation and a statistical analysis was also made of inter-clonal differences.

The results showed that rainfall did have some effect on the chemical entities studied, although the magnitude of the seasonal differences was not always statistically significant. Inter-clonal differences were also detected but these did not follow a uniform pattern which could be related to the classification of the clone.

The variations in chlorophyll content with season were studied in seven clones (DT 1, TRI 2024, PA 22, CV 5 B 1, TRI 26, CH 13 and TRI 777). In this case there were indications that chlorophyll content decreases during dry as compared with wet weather, although here too the trend was not statistically significant in all of the clones analysed.

Methods and materials

Flush samples used in this investigation were obtained from the clonal plots maintained by the Division of Plant Propagation at St Coombs Estate (elevation 4500 ft above mean sea level).

Polyphenols — Total and different groupings of polyphenols were estimated by the methods described by Swain & Hillis (1959).

Amino-acids — Amino-acids were estimated according to the method of Moore & Stein (1954).

Chlorophylls — Chlorophylls were extracted and determined as described previously (Wickremasinghe, Kirtisinghe, Perera & Perera 1965).

Rainfall figures were obtained from the records kept by the Division of Agricultural Chemistry.

Results and discussion

Table 1 shows the results of the statistical evaluation of the relationship between rainfall on the one hand and total amino-acids, total polyphenols, leucoanthocyanins and vanillin-reacting polyphenols on the other.

TABLE 1 — *Correlation coefficients between rainfall and amounts of polyphenols and amino-acids*

Clone	Total poyphenols	Vanillin-reacting polyphenols	Leuco-anthocyanins	Amino-acid
DT 1	0.349	0.070	-0.412	-0.175
TRI 2024	0.152	0.286	-0.440*	-0.083
PA 22	0.334	0.156	-0.472*	-0.376
CV5B1	0.512*	0.166	-0.304	-0.037
TRI 26	0.430	0.308	-0.138	-0.106

*Significant at $P < 0.05$

It is seen that increased rainfall causes a general increase in the level of total are vanillin-reacting polyphenols in the five clones tested. The rise in total polyphenols, however, was statistically significant only in the case of the clone CV5B1, whilst the rise in vanillin-reacting polyphenols was not significant in any of the clones tested. The leucoanthocyanin group of polyphenols showed the reverse trend in that there was a general decrease of leucoanthocyanins with rainfall, and this increase was significant for clones TRI 2024 and TRI 26. In the case of amino-acids there was a slight, non-significant but nevertheless uniform decrease with rainfall. It should be mentioned here that quantitative methods used in the present investigation differed from those used by previous workers, the majority of whom had estimated polyphenols by the non-specific Loewenthal procedure, which would react with all reducing substances. The combination of more specific methods used here indicated that the amounts of one group of polyphenols showed a trend which was opposite to that of other groups and this finding could also be partly responsible for the conflicting results reported previously. With respect to amino acids the uniform decrease with rainfall was so small that it may have escaped detection by the relatively non-specific Kjeldahl procedure employed in previous studies.

Inter-clonal correlations

Table 2 lists the correlation between the five clones.

TABLE 2 — *Mean values in different clones*

Clone	Total polyphenols	Vanillin-reacting polyphenols	Leuco-anthocyanins	Amino-Acids
DT 1	346.1	263.7	8.85	9.31
TRI 2024	361.5	274.4	8:71	15.58
PA 22	331.2	252.6	8.41	13.75
CV5B1	339.4	274.4	9.30	11.50
TRI 26	337.6	265.0	11.75	10.35
LSD at P=0.05	16.8	8.5	0.6	1.84

All values expressed as mg/g dry weight

The results did not follow a coherent pattern, although it would appear that a low level of free amino-acids together with a sufficient level of polyphenols as exemplified by the high quality clone, DT 1, are desirable for quality. High levels of amino-acids with high total polyphenol content (TRI 2024), or high amino-acids with low total polyphenols (PA 22), or low amino-acids with low total polyphenols (TRI 26) do not seem to be conducive to the production of teas of optimal quality.

Chlorophylls

Analysis of the correlation of chlorophyll content and rainfall are shown in Table 3.

TABLE 3 — *Correlation between rainfall and amounts of chlorophyll*

Clone	r value
DT 1	0.504
TRI 2024	0.398
PA 22	0.476
CV5B1	0.559
TRI 26	0.687*
TRI 777	0.657*
CH 13	0.462

*Significant at $P < 0.05$

There was a uniform increase of chlorophyll with rainfall, although this increase was of statistically significant proportions in only two (TRI 777 and TRI 26), of the seven clones studied.

The biosynthesis of chlorophyll in the tea leaf is governed partly by the amount and quality of the available light. If the amount and quality of sunlight is reduced by cloud, dust, *etc*, the plant responds by producing more chlorophyll to enable it to make maximal use of the light that reaches it. Hence, plants growing in dull, rainy weather or at low elevations, (where part of the radiant energy from the sun is cut off by the dust, *etc* of the atmosphere), would be expected to produce more chlorophyll than those growing in sunny weather at high elevations. These considerations could explain the increased chlorophyll content during rainy weather and also in tea growing at low elevations. In this connection a sample of flush growing at an elevation

of 7000 ft in dry, sunny weather was found to have an extremely low level of chlorophyll and also to produce a tea of very good quality and flavour. It is tempting to speculate that freedom from the necessity to manufacture chlorophyll allows the plant to produce and conserve compounds which are conducive to the production of quality and flavour.

Summary

1 — Total polyphenols, vanillin-reacting polyphenols, amino-acids and chlorophyll increase with rainfall.

2 — The Leucoanthocyanin group of polyphenols and amino-acids decrease with rainfall.

3 — The results on polyphenols are discussed in relation to previous findings.

4 — The results on chlorophylls are discussed in relation to quality and flavour.

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