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MINERAL NUTRIENTS AND SOME ENDOGENOUS METABOLITES IN ERECT AND INCLINED SHOOTS OF TEA (*CAMELLIA SINENSIS* (*L.*) O. KUNTZE)

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Erect shoots had more mineral nutrients except Ca and more organic acids than inclined shoots, while the latter had more sugars and amino acids. The xylem sap of erect shoots had more gibberellin-like activity whilst that of inclined shoots had more cytokinin-like activity. Differences in the rates of growth of erect and inclined shoots are discussed in relation to the levels of mineral nutrients and endogenous metabolites in these shoots.

INTRODUCTION

Tea is propagated by cuttings obtained from shoots 6-8 months after pruning the mature bushes. At this stage it is observed that shoots arising from the centre of the frame of the bush are erect in growth and more vigorous than peripheral shoots. and have produced about 20 to 25 leaves before becoming dormant, whilst peripheral shoots are inclined to varying degrees and less vigorous, with considerably fewer leaves, and usually go dormant more than once during the same period. Cuttings for propagation are taken from both types of shoots.

Variation in the growth of rooted cuttings has been noted in tea nurseries and may be due to several factors (Kathiravetpillai and Kulasegaram, 1980). Some of it may be related to the vigour of the shoots used, which in turn may be affected by mineral nutrients as well as various endogenous metabolites. In this study an attempt was made to see if erect and inclined shoots differed in their mineral nutrient status and in some important endogenous metabolites which could explain differences in the growth rates of such shoots.

MATERIALS AND METHODS

Mineral nutrients and sugars, starch, amino acids and organic acids were determined in stem tissue at three regions of 6-month-old erect (central) and inclined (peripheral) shoots. New shoots were designated as erect or central if they made an angle of 60-90° to the horizontal and as inclined or peripheral if this angle was less than 45°. The two clones used, TRI 2025 and DT1 were pruned at a height of 40 cm above ground level. The regions into which the shoots were divided after regrowth were (i) apical—from the terminal bud to the 4th node, (ii) middle—from the 5th to the 9th node, and (iii) basal—from the 15th node downwards. The mineral nutrient content of leaves was also determined at these three regions.

Quantitative analysis

Mineral nutrients.—The nutrients determined by standard methods of chemical analysis were N, P, K, Ca and Mg.

Sugars and starch.—For the quantitative analysis of sugars a 4g oven-dry sample was extracted in boiling hot water and clarified with Polyclar AT (Sanderson and Perera, 1966) concentrated and analysed for reducing power by the Shaffer-Somogyi method (1933). Reducing sugars were estimated by this method before hydrolysis and non-reducing sugars obtained by difference. Starch was estimated by the method used by Priestley (1962), employing 1g oven-dry stem tissue and expressing the starch content in glucose equivalents.

Total free amino acids.—Total free amino acids were determined by the ninhydrin method of Yemm and Cocking (1955). The optical density was determined on a Unicam Spectrophotometer SP 500 at 570 nm.

Qualitative analysis

Amino acids.—Amino acids were separated using Whatman No. 3mm paper with butanol : acetic acid : water (12 : 3 : 5) in the first direction (25 h) and phenol: water (500 : 125, w/v) in the second (16 h), and spraying the papers with 0.2% ninhydrin in butanol. The colour was developed by heating in an oven at 60°C for 15 min.

Organic acids.—Organic acids were separated using Whatman No. 3mm paper with ethanol : ammonia : water, 8 : 1 : 1 in the first direction (16 h) and butanol : formic acid : water, 75 : 15 : 10 in the second (7 h) and identified by spraying the papers with bromocresol green solution.

The developed spots were scored by visual observations of the relative intensity of their colour.

Analysis of xylem sap from erect and inclined shoots

Xylem sap was collected from comparable erect and inclined shoots (diam. 0.8 cm) in each of four randomly chosen bushes of clones TRI 2025 and DT1 by the method of Selvendran and Sabaratnam (1971) (Table 1). This was done five months after pruning and the samples of sap were immediately stored in a deep-freeze until required. The 700 ml of sap was concentrated *in vacuo* at 35°C to 150 ml and 10 ml of this was used for quantitative analysis. An aliquot of 0.1 ml was used for amino acid determination. The remaining sap (about 140 ml) was extracted for endogenous metabolites by a method essentially similar to that used by Pegg (1966) : it was partitioned with ethyl acetate and the acidic and neutral fractions were assayed for gibberellins (Frankland and Wareing, 1960); the basic fraction was assayed for cytokinins by the radish cotyledon test (Letham, 1971) and the chlorophyll preservation test (Loeffler and van Overbeek, 1964).

TABLE 1 — *Data on sap collection*

Amount of sap (ml)	*Clone TRI 2025* *Erect shoot*	*Clone TRI 2025* *Inclined shoot*	*Clone DT 1* *Erect shoot*	*Clone DT 1* *Inclined shoot*
Bush 1	170	195	160	155
Bush 2	135	150	210	190
Bush 3	245	180	190	220
Bush 4	150	190	145	145
Total sap	700	715	705	710
Time (h) taken for collection	18	48	26	56
Rate of collection per bush (ml/h)	9.72	3.72	6.78	3.17

RESULTS

Quantitative analysis

Mineral nutrients — The N, P, K and Mg content of stem tissue was almost always greater in erect shoots, while Ca content was greater in inclined shoots (Table 2). The nutrient content of leaves followed a very similar pattern. In general the apical region had the highest nutrient content, followed by the middle region.

TABLE 2 — *Mineral nutrient content (% dry matter) of stems and leaves in different regions of erect and inclined shoots*

Region on shoot	*Erect shoot* *N*	*P*	*K*	*Ca*	*Mg*	*Inclined shoot* *N*	*P*	*K*	*Ca*	*Mg*
Clone TRI 2025										
Stems										
Apical	3.96	0.26	2.15	0.38	0.215	3.47	0.16	1.70	0.56	0.180
Middle	2.03	0.26	1.65	0.50	0.145	1.86	0.19	1.10	0.63	0.135
Basal	1.40	0.23	1.23	0.44	0.130	1.47	0.16	0.70	0.75	0.100
Leaves										
Apical	3.85	0.25	1.35	0.56	0.130	3.50	0.21	1.25	0.88	0.095
Middle	3.15	0.20	1.38	0.94	0.095	3.57	0.20	1.15	1.19	0.115
Basal	3.15	0.17	1.38	1.29	0.105	3.08	0.16	0.85	1.94	0·105
Clone DT 1										
Stems										
Apical	3.93	0.25	2.10	0.38	0.180	3.12	0.25	1.95	0.38	0.185
Middle	1.65	0.27	1.75	0.38	0.180	1.68	0.26	1.25	0.38	0.135
Basal	1.19	0.19	1.15	0.31	0.105	1.26	0.17	0.70	0.35	0.080
Leaves										
Apical	3.85	0.26	1.75	0.48	0.095	3.43	0.25	1.60	0.60	0.100
Middle	2.87	0.21	1.95	0.75	0.097	3.08	0.18	1.65	1.00	0.110
Basal	2.87	0.16	1.80	0.81	0.095	3.08	0.16	1.50	1.00	0.135

Sugars and starch — Quantitative analysis confirmed that inclined shoots of both clones contained more sugars in all regions (Table 3). In both types of shoots the sugar content was greater in the apical region and least in the basal region. The starch content did not show much variation.

TABLE 3 — *Sugar and starch content (mg glucose/g dry weight) in different regions of erect and inclined shoots*

	Clone TRI 2025			
	Sugar content			
Region on shoot	*Total*	*Reducing*	*Non reducing*	*Starch content*
Apical				
Erect shoot	14.63	5.93	8.70	0.75
Inclined shoot	17.63	6.20	11.43	0.75
Middle				
Erect shoot	9.50	5.60	3.90	0.75
Inclined shoot	14.63	6.20	8.43	0.63
Basal				
Erect shoot	8.50	5.45	3.05	0.88
Inclined shoot	11.50	6.15	5.35	0.50
	Clone DT 1			
Apical				
Erect shoot	9.88	4.52	5.36	0.69
Inclined shoot	16.13	4.86	11.27	0.75
Middle				
Erect shoot	8.50	5.93	2.57	0.75
Inclined shoot	10.75	6.20	4.55	0.75
Basal				
Erect shoot	6.50	3.47	3.03	0.75
Inclined shoot	9.13	4.81	4.32	0.88

Total free amino acids — In both clones, the inclined shoots had more total free amino acids in all regions than the erect shoots (Table 4). Analysis of xylem sap also showed a greater amino acid content in inclined shoots of both clones (Table 5).

TABLE 4 — *Total free amino acids (mg alanine equivalent/g dry weight) at different regions of erect and inclined shoots*

	Clone TRI 2025	*Clone DT 1*
Apical		
Erect shoot	3.15	3.30
Inclined shoot	6.48	7.88
Middle		
Erect shoot	1.43	1.12
Inclined shoot	7.95	2.49
Basal		
Erect shoot	2.02	0.98
Inclined shoot	4.12	1.54

TABLE 5 — *Total free amino acids (μg/ml) in xylem sap of erect and inclined shoots*

Type of shoot	*Clone TRI 2025*	*Clone DT 1*
Erect	93.86	38.30
Inclined	117.90	102.46

Qualitative analysis

Amino acids — In both clones, amino acids were present in greater amount in the inclined shoots (Table 6). In TRI 2025 glutamic acid was most intense at all 3 regions of inclined shoots. In both clones glutamine was most intense at the 3 regions of inclined shoots. In TRI 2025 theanine was high in the apical region of inclined shoots while in DT 1 its density was relatively high in both types of shoots at the apical region.

TABLE 6 — *Amounts of amino acids in erect and inclined shoots based on visual scoring of chromatogram (scores 1 — 15 denote increasing density of spots)*

	Region on shoot					
	Apical		*Middle*		*Basal*	
Amino acid	*Erect shoot*	*Inclined shoot*	*Erect shoot*	*Inclined shoot*	*Erect shoot*	*Inclined shoot*
Clone TRI 2025						
Aspartic acid	Trace	1	1	3	Trace	1
Serine	Trace	1	Trace	—	Trace	Trace
Glutamic acid	2	6	5	7	2	3
γ-methylene glutamic acid	5	5	Trace	—	—	—
Threonine	Trace	2	Trace	—	—	—
Basic amino acid	—	—	2	5	Trace	—
Glutamine	8	12	4	15	3	6
Alanine	7	7	3	3	2	1
γ-amino butyric acid	6	8	5	7	4	3
Theanine	6	11	3	8	4	5
Total	34	53	23	48	15	19
Clone DT 1						
Aspartic acid	1	Trace	Trace	Trace	Trace	1
Serine	Trace	Trace	Trace	—	Trace	—
Glutamic acid	3	2	2	4	Trace	1
γ-methylene glutamic acid	5	5	—	5	—	—
Threonine	4	3	—	Trace	—	—
Basic amino acid	—	—	—	—	Trace	—
Glutamine	4	9	—	10	Trace	4
Alanine	7	4	2	4	Trace	Trace
γ-amino butyric acid	9	7	2	5	2	3
Theanine	11	10	4	6	1	4
Total	44	40	10	34	3	13

In TRI 2025 the amount of amino acids in the middle region of inclined shoots was more than twice that in the erect shoots while the same position of the inclined shoots of DT 1 showed a three-fold increase. Among the clones, TRI 2025 showed a greater amount of amino acids than DT 1.

Organic acids — In both clones, organic acids were present in greater amount in the erect shoots and most intense in the apical and basal regions (Table 7). In the erect shoots of clones TRI 2025 and DT 1 oxalic acid was present in greater amount in the basal and middle regions respectively. In both clones citric and quinic acids were denser at the apical region while in clone DT 1 an unknown organic acid (Unknown 1) was more intense at the basal region.

TABLE 7 — *Amounts of organic acids in erect and inclined shoots based on visual scoring of chromatogram (scores 1-8 denote increasing density of spots)*

	Region on shoot					
	Apical		*Middle*		*Basal*	
Organic acid	*Erect shoot*	*Inclined shoot*	*Erect shoot*	*Inclined shoot*	*Erect shoot*	*Inclined shoot*
Clone TRI 2025						
Oxalic	5	3	6	6	8	3
Citric	8	4	5	2	6	1
Succinic	2	—	—	—	2	—
Quinic	7	3	6	1	4	2
Unknown 1	—	—	6	6	4	4
Unknown 2	3	4	3	5	2	—
Unknown 3	2	1	1	2	Trace	Trace
Unknown 4	4	—	—	—	—	—
Total	27	15	27	22	26	10
Clone DT 1						
Oxalic	4	1	8	3	4	4
Citric	8	3	2	4	3	1
Succinic	1	—	—	—	—	—
Quinic	8	3	3	2	1	—
Unknown 1	3	—	7	4	8	3
Unknown 2	3	2	3	—	—	—
Unknown 3	3	1	2	2	—	Trace
Unknown 4	4	—	—	—	—	—
Total	34	10	25	15	16	8

Analysis of xylem sap for endogenous hormones

Gibberellins — The acidic fraction of the xylem sap of erect shoots of clones TRI 2025 and DT 1 showed evidence of gibberellin activity, with peak values of 123% and 188% respectively (Fig. 1). The inclined shoots showed strong zones of inhibition. In both clones the neutral extracts of erect and inclined shoots were characterized by the absence of any promotional activity and are not presented here.

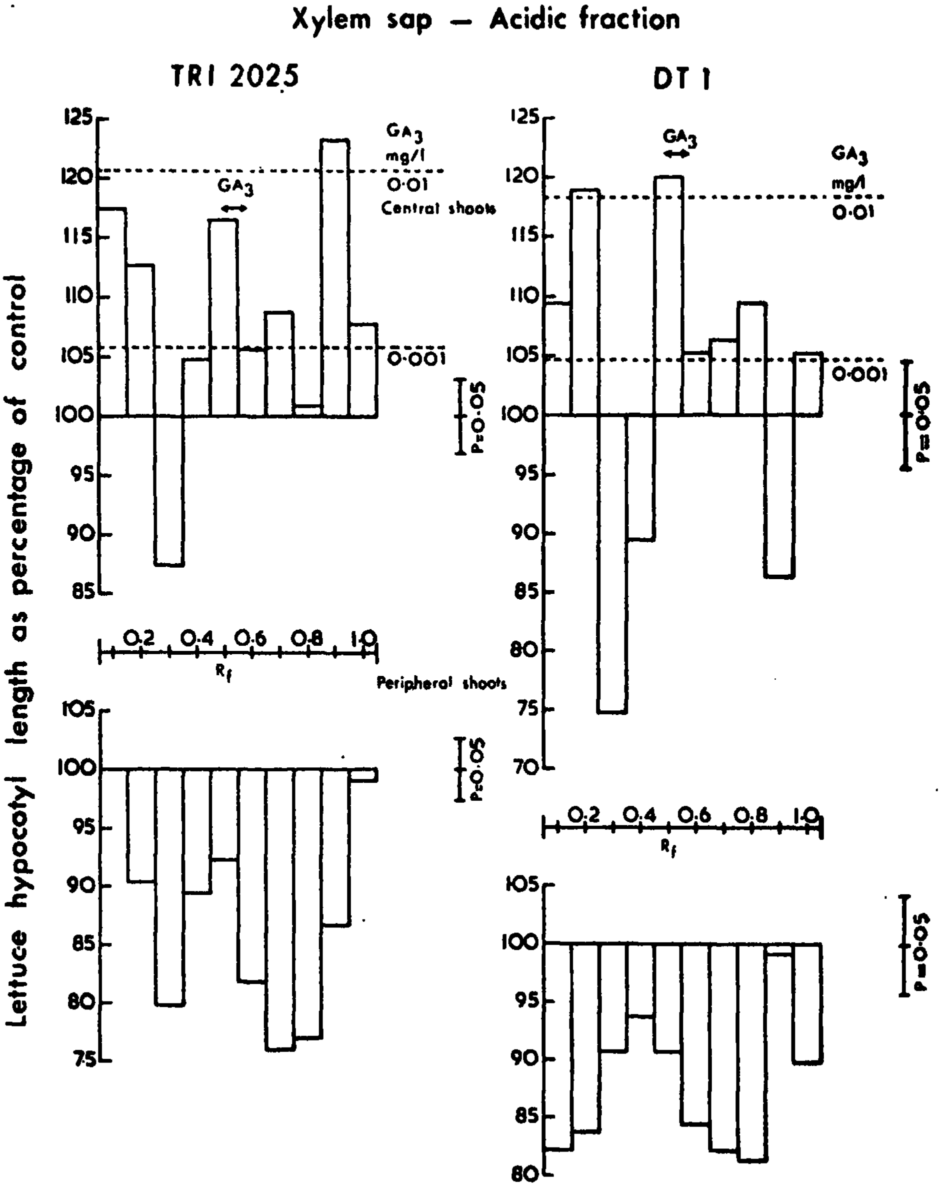

Fig. 1.—Gibberellin-like activity (lettuce hypocotyl extension test) in acidic fraction of approximately 175 ml of xylem sap from erect and inclined shoots of clones TRI 2025 and DT 1 chromatographed in isopropanol : ammonia : water, 10 : 1 : 1 V/V. Vertical bars represent LSD for P=0.05. Short horizontal lines indicate the position of authentic GA_3. Levels of response to GA_3 are given by dotted horizontal lines.

Cytokinins — In clone TRI 2025 the basic fraction of the xylem sap of inclined shoots in the radish cotyledon test showed greater cytokinin activity, with a peak of 126% at Rf 0.9 (Fig. 2). The same fraction of erect shoots showed smaller peaks. In clone DT 1 the basic fraction from erect and inclined shoots also showed evidence of stimulatory activity at Rf 0.1 - 1.0, and this was again more pronounced in the inclined shoots.

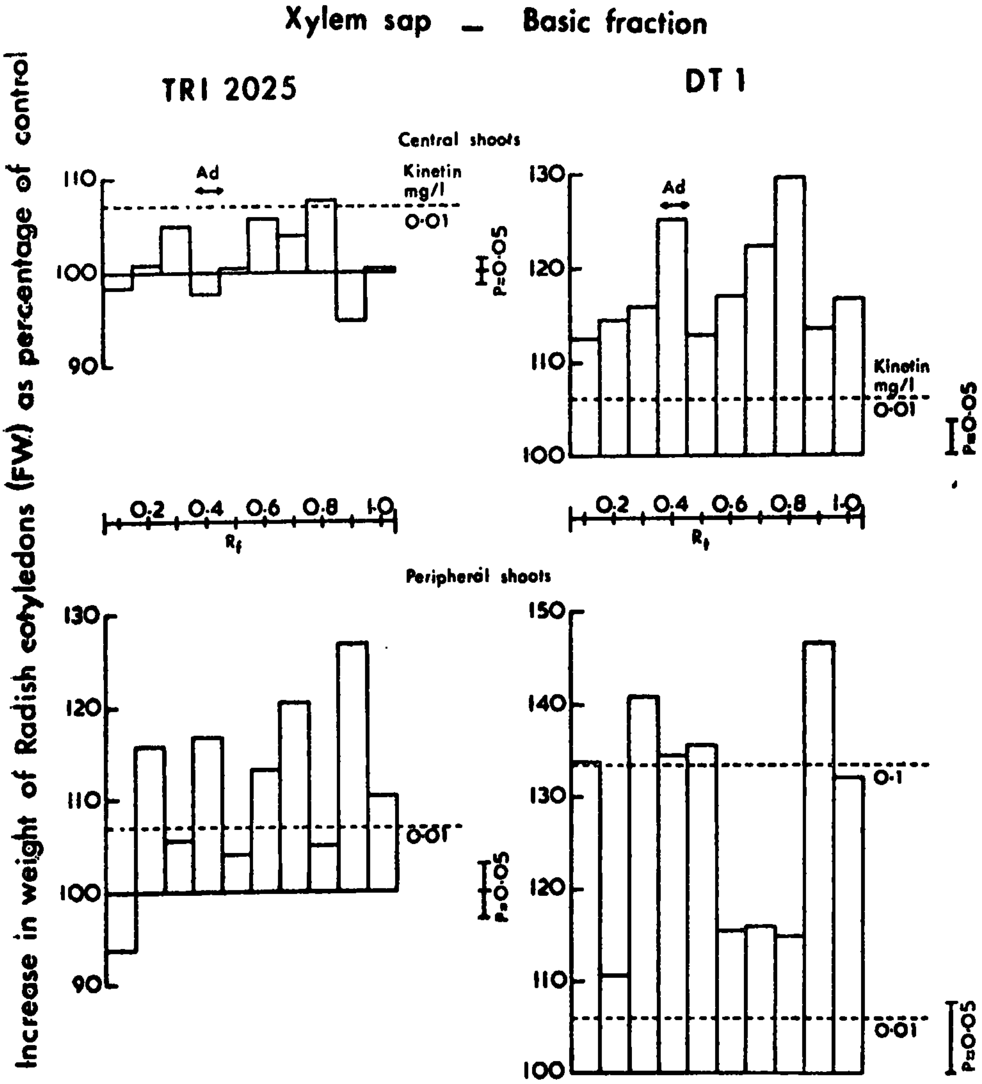

Fig. 2.—Cytokinin-like activity (radish cotyledon test) in basic fraction of approximately 87.5 ml of xylem sap from erect and inclined shoots of clones TRI 2025 and DT 1 chromatographed in n-butanol : ammonia : water, 4 : 1 : 1 V/V. Vertical bars represent LSD for $P=0.05$. Short horizontal lines indicate the position of authentic adenine (Ad). Levels of response to kinetin are given by dotted horizontal lines.

In the chlorophyll preservation test, the inclined shoots of both clones showed evidence of stimulatory activity while the erect shoots showed zones of inhibition (Fig. 3).

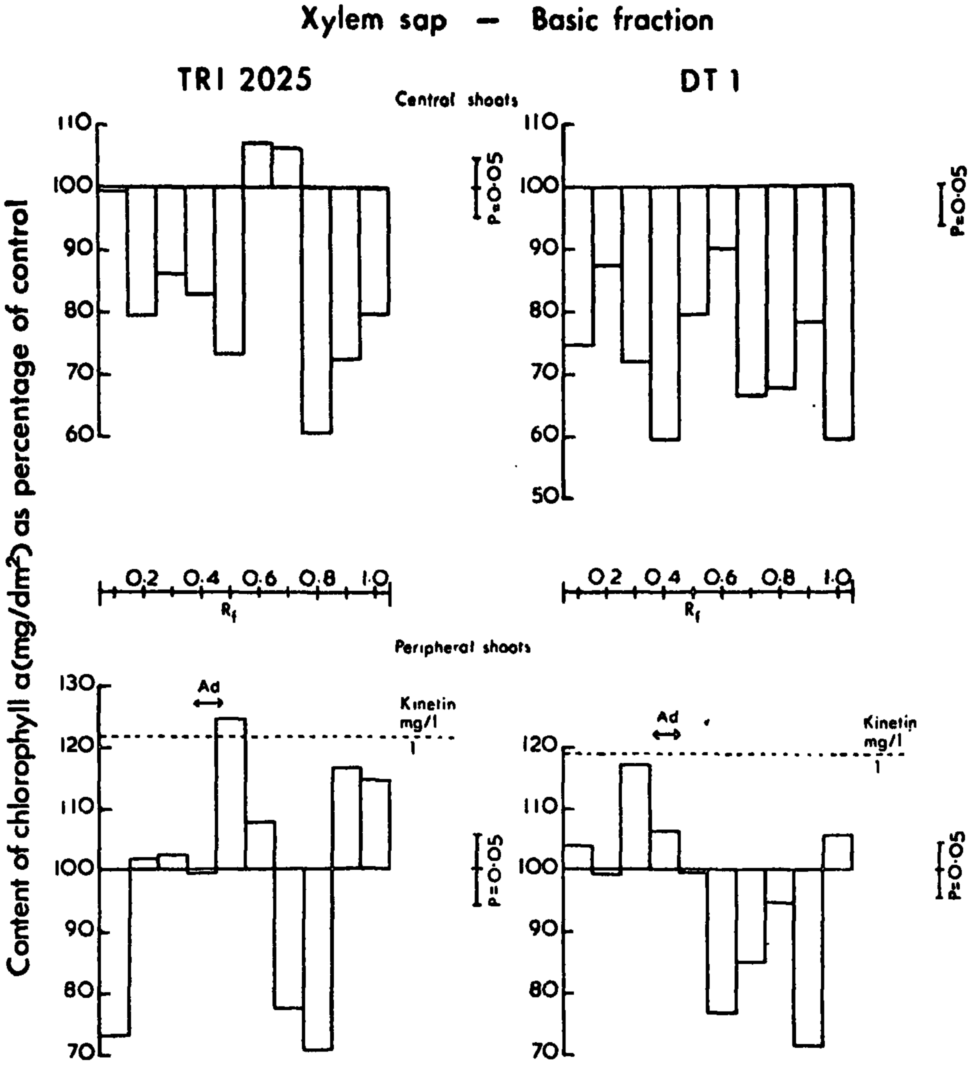

Fig. 3.— Cytokinin-like activity (chlorophyll preservation test) in basic fraction of approximately 87.5 ml of xylem sap from erect and inclined shoots of clones TRI 2025 and DT1 chromatographed in n-butanol : ammonia : water, 4 : 1 : 1 V/V. Vertical bars represent LSD for P=0.05. Short horizontal lines indicate the position of authentic adenine (Ad.). Levels of response to kinetin are given by dotted horizontal lines.

DISCUSSION

In a pruned tea bush the erect shoots grow faster than the inclined shoots (Kathiravetpillai *et al.*, 1980). The present work showed that erect shoots have a greater mineral nutrient content except for Ca, which is higher in inclined shoots, whilst in either type of shoot the nutrient content is greater in the actively metabolizing apical region (Table 2). Hasselo (1965) found that the NPK content of tea leaves on bushes in plucking declined with age, while that of Ca and Mg increased. Mullins (1965) attributed the reduced terminal growth of horizontally-trained trees to some gravitationally-induced deficiency of essential growth factors.

The low level of sugars in erect shoots may be due to their rapid utilization for extension growth (Table 3). Other workers have shown the presence of these sugars in tea flush (Bhatia and Chanda, 1960). In evergreen trees generally, depletion of carbohydrates occurs at the time of most rapid shoot extension, apparently as a result of the demands of growth exceeding the immediate supply of photosynthates (Priestley, 1962). However, reserves accumulate when meristematic activity declines, and the accumulation of sugars in the inclined shoots of tea may therefore be associated with the restricted growth of these shoots. It may be noted that in both clones the variation in total sugars was largely due to differences in sucrose, the major translocatory form.

In both clones, the amino acids in both types of shoots at all three regions followed the same trend as the total sugars, with more amino acids being present in the apical region of inclined shoots (Tables 4 and 6). It will be seen that glutamine and theanine are the major amino acids present in the shoots. Glutamine and theanine are the major amino acids in the exudate of tea plants (Selvendran, 1970). Selvendran and Selvendran (1973) in a survey of nitrogenous constituents of the tea plant found theanine to be the most prominent followed by glutamic acid, aspartic acid and glutamine. The roots are the chief sites of the synthesis of theanine from which it is translocated to the aerial parts (Wickremasinghe and Perera, 1972). The universal distribution of theanine in the plant suggests an underlying function for it though at present nothing definite is known about its role. The relatively lower quantities of amino acids in the erect shoots suggests a rapid mobilization of this acid, resulting in better shoot growth (Tables 4, 5 and 6). It appears that in the inclined shoots there is very little enzymatic activity for the conversion of amino acids. However, erect shoots contained more organic acids, and this may be associated with the functioning of actively metabolizing tissues.

The analysis of xylem sap demonstrated the presence of more gibberellin-like activity in erect shoots and more cytokinin-like activity in inclined shoots (Figs. 1, 2 and 3). There is evidence that roots are also a site of gibberellin biosynthesis, some, if not all, of the root-synthesised gibberellins being exported to the shoot in the xylem sap (Sitton,Richmond and Vaadia, 1967). Extracts from the apical region of erect shoots of tea have shown the presence of more gibberellin-like and auxin-like activity, while those of inclined shoots have shown greater cytokinin-like and inhibitory activities (Kulasegaram and Kathiravetpillai, 1979). Again the greater nutrient content in the apical region of erect shoots may be due to preferential movements of nutrients into these tissues induced by auxin (Marschner and Ossenberg-Neuhaus, 1977).

Variation in the growth of tea plants is commonly seen in commercial tea nurseries. Standard nursery practices are more easily carried out with a uniform group of plants, whereas non-uniform plants present problems to the nurseryman. Several factors may be responsible for variation in the growth of young tea: node position of cuttings affect shoot-growth in the early stages (Kulasegaram and

Janakiram, 1970); cuttings are also taken from secondary and tertiary shoots after ermoval of the primary shoots, and plants from these successive batches grow with decreasing vigour; cuttings obtained from such different sources as rested mature bushes, bushes in plucking following a light prune, or young unpruned plants, also lead to variation in growth. Shoot inclination, the locality from which shoots are obtained for propagation, shoot age and cultural practices all augment variability. It is thus apparent that while variation in the growth of tea plants may be connected with several factors, differences in the levels of mineral nutrients and endogenous metabolites in the two types of shoot described here could have also contributed towards the different rates of growth of these shoots.

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REFERENCES

BHATIA, I. S., and CHANDA N. B. (1960). Report of the Senior Biochemist. *Rep. of the Tock. Exp. Stn for* **1959.** 255-270.

FRANKLAND B., and WAREING, P. F. (1960). Effect of gibberellic acid on hypocotyl growth of lettuce seedlings. *Nature,* Lond. **185,** 255-256.

HASSELO, H. N. (1965). The nitrogen, potassium, phosphorus, calcium, magnesium, sodium, manganese, iron, copper, boron, zinc, molybdenum and aluminium contents of tea leaves of increasing age. *Tea Q.* **36,** 122-136.

KATHIRAVETPILLAI, A., and KULASEGARAM, S. (1980). Variation in growth of young vegetatively propagated tea (*Camellia sinensis*). *Expl. Agric.* **16** (399-40).

KULASEGARAM, S., and JANAKIRAM, D. (1970). The influence of node position on the development of tea cuttings. *Tea Q.* **41,** 53-63.

KULASEGARAM, S. and KATHIRAVETPILLAI, A. (1979) Endogenous hormones in actively growing central and dormant peripheral shoots of tea (*Camellia sinensis*) (L.) O. Kuntze). *J. Plantation Crops,* **7,** 105-116.

LETHAM, D. S. (1971). Regulators of cell division in plant tissues. XII. A cytokinin bioassay using excised radish cotyledons. *Physiol. Plant.* **25,** 391-396.

LOEFFLER, J. E., and VAN OVERBEEK, J. (1964). Kinin activity in coconut milk. In *Regulateurs Naturels de la Croissance Vegetale.* Centre National de la Recherche Scientifique. Paris No. **123,** 77-82.

MARSCHNER, H., and OSSENBERG-NEUHAUS, H. (1977). Z. *Pflanzenphysiologie,* **85,** 29-44, cited in MENGEL & KIRBY, "Principles of Plant Nutrition", *Publ. Int. Potash. Inst.* **1978.**

MULLINS, M. G. (1965). The gravitational responses of young apple trees. *J. hort. Sci.* **40,** 237-247.

PEGG, G. F. (1966). Changes in levels of naturally occurring gibberellin-like substances during germination of seed of *Lycopersicon esculentum* Mill. *J. exp. Bot.* **17,** 214-230.

PRIESTLEY, C. A. (1962). Carbohydrate resources within the perennial plant. *Comm. Bur. Hort. and Plantation Crops. Tech. Comm.* **27,** pp. 116.

SANDERSON, G. W., and PERERA, B. P. M. (1966). Removal of polyphenolic compounds interfering with carbohydrate determinations in plant extracts with an insoluble polyphenol adsorbent. *The Analyst,* **91,** 335-336.

SELVENDRAN, R. R., (1970). Changes in the composition of the xylem exudate of tea plants (*Camellia sinensis* L.) during recovery from pruning. *Ann. Bot.* 34, 825-833.

SELVENDRAN, R. R., and SABARATNAM, S. (1971). Composition of the xylem sap of tea plants (*Camellia sinensis* L.) *Ann. Bot.* 35, 679-682.

SELVENDRAN, R. R. and SELVENDRAN, S. (1973). The distribution of some nitrogenous constituents in the tea plants. *J. Sci. Fd. Agric.* 24, 161-166.

SHAFFER, P. A., and SOMOGYI, M. (1933). Copper-iodometric reagents for sugar determination. *J. Biol. Chem.*, 100, 695-713.

SITTON, D., RICHMOND, A., and VAADIA, Y. (1967). On the synthesis of gibberellins in roots. *Phytochemistry* 6, 1101-1105.

WICKREMASINGHE, R. L., and PERERA, K. P. W. C. (1972). Site of biosynthesis and translocation of theanine in the tea plant. *Tea Q.* 43, 175-179.

YEMM, E. W., and COCKING, E. C. (1955). The determination of amino-acids with ninhydrin. *The Analyst*, 80, 209-213.