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Influence of Some Bioregulators on Quality Traits of Pruned Tea (*Camellia sinensis* (L) O Kuntze)

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Abstract: During investigation, it was observed that all the applied bioregulators, namely, jibika, IAA, cycocel, thiourea, methanol, succinic acid and sucrose, have a significant effect on quality parameters such as polyphenol oxidase (PPO) activity, caffeine, crude protein, starch, nitrogen, carotenoid and ascorbic acid (vitamin C) content over the control. However, maximum PPO activity was observed with cycocel followed by succinic acid, jibika, thiourea and sucrose during the first phase of spray while at the second phase PPO was maximal in sucrose-treated bushes followed by cycocel, jibika, thiourea and succinic acid. The caffeine content was found to be maximal in methanol followed by cycocel, IAA, thiourea and jibika as compared to the control during the first phase of spray. Similarly, nitrogen content increased due to methanol application at both the phases. Starch and carotenoid content were significantly influenced by jibika treatment. Likewise, ascorbic acid content was highest in sucrose-treated bushes as compared to other treatments. © 1998 SCI.

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Key words: *Camellia sinensis*; quality traits; bioregulators; PPO; caffeine; ascorbic acid; IAA

INTRODUCTION

The tea came from plants belonging to a relatively large group of cultivated species of *Camellia sinensis* (L) O Kuntze. Only the tender apical shoots of the tea bushes are used for processing. Since tea contains many useful components in the leaves, including tannins (mainly catechins), caffeine, amino acids and vitamins, etc, it has long been utilised for beverages (Takeda 1994). These components are mainly responsible for the characters of tea. Moreover, the shoots are a rich source of polyphenol oxidase (PPO) a key enzyme in tea processing (Sanderson 1972) and tea quality is positively correlated with its content in the shoots (Biswas *et al* 1971). PPO plays an important role in the manufacture of black tea, because it is responsible for the oxidation of polyphenols and the formation of theaflavins (TF) and thearubigins (TR) (Graham 1983), compounds which are responsible for the brightness and much of the quality of tea liquors.

The popularity of tea as a refreshing drink and mild stimulant is principally due to the caffeine content of the tea plant. Caffeine (1,3,5-trimethyl-xanthine), along with the two isomeric dimethylanthines, theobromine and theophylline, which though present in minor amounts, are well known central nervous system stimulants (Stagg and Millin 1975). Caffeine also contributes to the characteristic taste and briskness of a tea infusion, forming a physicochemical complex with polyphenols (Collier *et al* 1972). This complex contributes to the formation of the coloured insoluble precipitate known as 'cream' when a tea liquor is allowed to cool (Roberts 1962; Bhuyan *et al* 1991). The degree of cream formation is largely dependent upon the caffeine content (Smith 1968) and is used by professional tea tasters as an indication of quality and hence for the evaluation of a tea. Further, the caffeine content in tea has been shown to be influenced by a number of factors,

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such as, varietal or clonal, genetics, seasonal, agronomic and cultural factors (Cloughley 1982). Owuor (1987), from Kenya, reported a linear increase in caffeine content in tea shoots along with the increased nitrogenous fertilisation.

Moreover, carotenoid, a pigment present in the tea leaves were also reported to influence the quality of tea (Venkatakrishna *et al* 1977). Good quality tea is usually made from the bud, the first and the second leaf which contains a lesser amount of carotenoid as compared to the third and fourth and mature (seventh and eight) leaves. However, Tirimanna and Wickrema-Singhe (1966, 1971) have studied the carotenoid pattern in tea flush, but so far a systematic qualitative and quantitative carotenoid analysis of tea leaves has not been carried out. Other biochemical parameters like crude protein starch content of leaf and vitamin C (ascorbic acid) content of the tea shoots also influence the quality of made tea, but there is a lack of detailed information.

The quality of black tea, which is of no less importance, is influenced by the tea-making potential of green leaf shoots as well as by manufacturing techniques (Yand and Thseng 1991; Kalita 1992; Mahanta and Baruah 1992). Therefore, it is the chemical composition of fresh tea flush which is of prime importance in determining its potential to make quality tea (Sanderson 1964). Those factors which influence the chemical composition of leaf also has a bearing on tea quality. However, there are reports on varietal and seasonal variations on chemical constituents of tea, studies on the effect of bioregulators on chemical composition of fresh tea flush is lacking. The aim of the present investigation is, therefore, to study the influence of bioregulators on certain chemical constituents of fresh tea flush which in turn govern the quality of made tea.

MATERIALS AND METHODS

The experiment was carried out on light pruned mature tea bushed of clone TV_{18} in 1994. A randomised block design with three replications for each treatment was followed. A total of seven treatments and a control (Waterspray) were given to the bushes: jibika (0·5%), IAA (0·02%), cycocel (0·05%), thiourea (1·5%). methanol (30%), succinic acid (0·01%) and sucrose (2·0%). Spraying was done twice: the first spray was done after light pruning followed by a second spray in mid-May. Similarly, the chemical analysis of various quality parameters were done in two phases, that is, one month after each spray.

Biochemical analysis

Fresh tea shoots were collected and analysed for their chemical composition. However, the leaves were not manufactured to get black tea.

Polyphenol oxidase (PPO) activity

PPO activity was assayed by measuring dioxygen (O_2) consumption with a Clark electrode at 30°C by following the method of Matheis *et al* (1987). Five grams of fresh leaf sample was homogenised with 25 ml of 0·1 M acetate buffer (pH 5·6) for 2 min. The homogenate was filtered and 0·3 ml of suspension was added to the cell of an Oxygraph (Gilson Model 5/6) containing 1·5 ml of air saturated 0·1 M acetate buffer. The oxygen consumption was followed at 30°C for 3 min. Oxidation rates were expressed as the amount of green leaf (dry weight) which catalyses the consumption of 1 μmol of O_2 min^{-1}.

Caffeine

It was estimated by the method of Ullah *et al* (1987). One gram of dried sample was soaked in 6 ml ammonia solution in an RB flask. Twenty millilitres of chloroform was added and refluxed for 30 min. Caffeine was extracted by washing the residue with chloroform and the concentration was determined spectrophotometrically. The optical density was measured at 276 nm and the caffeine content was obtained from a calibration graph derived from measurements on standard caffeine solutions and expressed on a dry mass basis.

Total nitrogen

The N content in the plucked shoots were estimated by micro-kjeldhal method (AOAC 1960) taking 0·2 g of dried leaf sample.

Crude protein

Crude protein: was estimated by multiplying the value of nitrogen with the factor 6·25 after estimating nitrogen content by micro-Kjeldhal method.

Starch

Fresh tea shoots collected for analysis were steamed and dried. The starch content was determined by the method of McCready *et al* (1950).

Carotenoid

Total carotenoid in the harvested shoots were estimated following the method as suggested by Taylor (1993). For analysis, 0·5 g of fresh sample was ground in methanol and filtered through a sintered glass funnel. After dilution with methanol the absorbancy was read at 470 nm, 653 nm and 666 nm. Using the following formula, total carotenoid was estimated:

$$T_c\ (\mu g\, g^{-1} \text{ fresh tissue}) = \frac{(1000 \times A_{470}) - (2{\cdot}86 \times Ca) - (129{\cdot}2 \times Cb)}{245}$$

where C*a* is chlorophyll *a* and C*b* is chlorophyll *b*.

Ascorbic acid (vitamin C)

The method of Sadasivam and Balasubramaniam (1987) was followed to estimate the ascorbic acid (AA) content in freshly harvested shoots. Four grams of leaf sample was ground with 2% oxalic acid and filtered by adding charcoal. Charcoal is added to remove any polyphenols present in the tea, which imparts colour and interferes with the colorometric estimation of AA. The concentration was measured spectrophotometrically at 518 nm immediately after adding 2,6-dichlorophenol indophenol dye. The amount of AA was estimated from a standard curve and expressed as mg per 100 g fresh leaf.

RESULTS AND DISCUSSION

Response of bioregulators on PPO activity in tea leaves

Significant variation among the bioregulators treatments on the PPO activity at both the phases, ie after first and second spray of bioregulators was observed. However, during the first phase maximum PPO activity was observed with cycocel treatment followed by succinic acid, jibika, thiourea and sucrose as compared to the control. In the second phase, sucrose treatment showed maximal PPO activity followed by cycocel, jibika, thiourea and succinic acid. Methanol recorded the lowest PPO activity in both the phases as shown in Fig 1. This is in accordance with the findings of Sangeeta and Varshney (1991) who also found a positive association of PPO with cycocel. According to them, GA_3 suppressed the activity of PPO probably by exerting its effects through oxidative metabolism. Further, Poljakoff-Mayber and Mayer (1960) found that thiourea strongly inhibited *in vitro* PPO in lettuce seeds at comparatively low concentrations. Very few reports are available on the effect of growth regulators on PPO activity though some work has been done with chemicals like methanol, succinic acid and sucrose on enzyme activity. Furthermore, PPO activity was found to be higher in the second phase with the same treatments. According the Sanderson and Kanapathipillai (1964), PPO activity varied during the wet and dry seasons. They found a sharp drop in the level of PPO during dry season. In the present study, also during the first phase of analysis, the amount of rainfall received was less than the second phase, ie the first phase was comparatively drier than the second phase which accounts for less PPO activity in first phase.

Response of bioregulators on caffeine, total nitrogen and crude protein content of tea leaves

Data mentioned in Fig 2, revealed that the caffeine content of tea leaves varied significantly due to bioregulator treatments at both the phases. In the first phase, ie early season, methanol had the highest (4·87%) caffeine followed by cycocel, IAA, thiourea and jibika as compared to the control. Similarly in the second phase the same was highest (4·27%) with methanol treatment followed by IAA, succinic acid, thiourea, jibika and sucrose. Nandi *et al* (1995) reported that NAA resulted in increased amino acid and protein content while nitrogen remained unaffected. Also, GA_3 increased caffeine content in addition to protein and amino acid content. The higher caffeine content with applied treatments was mainly due to increased nitrogen content in leaf tissues as was observed in the present experiment. These findings are in close confirmation with the result of Owuor (1987) who was also of the opinion that higher nitrogen content in leaf tissues increases caffeine content in tea. However, there is no such report on the effect of these chemicals on caffeine content in tea.

Further, the highest nitrogen content of leaves was observed (7·21%) with succinic acid in the first phase followed by sucrose methanol, thiourea and cycocel while in the second phase it was highest (6·39%) with thiourea treatment followed by methanol, cycocel and IAA (Table 1). Crude protein was also found to be increased in the similar manner in both the phases (Fig 3). Succinic acid and thiourea obtained maximum crude protein in the first and second phase, respectively. Dzhamankulov (1967) found a slight increase in N content in soybean due to succinic acid treatment. Ramanathan *et al* (1980) reported that triacontanol spray registered the highest NPK uptake in both grain

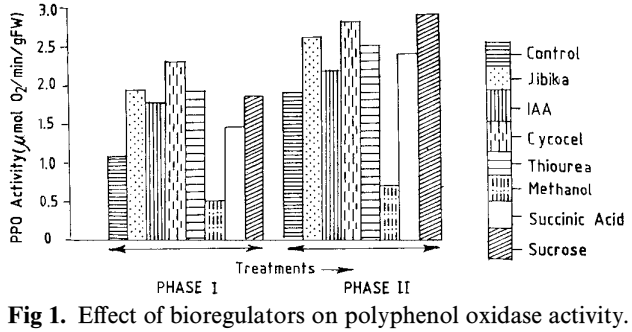

Fig 1. Effect of bioregulators on polyphenol oxidase activity.

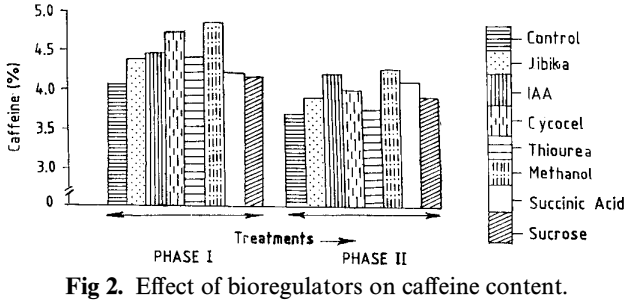

Fig 2. Effect of bioregulators on caffeine content.

TABLE 1
Response of bioregulators on starch, total carotenoids, ascorbic acid and total nitrogen content

Treatment	*Starch (%) DW*		*Carotenoid (μg g^{-1} FW)*		*Ascorbic acid (mg 100 g FW)*		*Nitrogen (% DW basis)*	
	Phase I	*Phase II*	*Phase I*	*Phase II*	*Phase I*	*Phase II*	*Phase I*	*Phase II*
Control	8·04	7·82	88·04	70·55	75·31	54·81	5·32	5·11
Jibika	9·59	7·92	108·92	119·26	129·67	109·17	5·60	4·83
IAA	8·41	6·93	76·08	86·16	139·17	118·67	5·32	5·32
Cycecel	7·01	6·49	101·05	108·55	102·81	82·31	5·71	5·39
Thiourea	7·23	5·75	73·55	93·43	58·81	43·61	5·76	6·39
Methanol	5·39	5·53	95·98	100·73	93·44	72·94	5·85	6·34
Succinic acid	7·01	5·75	88·93	86·54	125·94	105·44	7·21	5·08
Sucrose	4·28	6·28	101·17	112·76	155·00	134·50	6·85	4·48
SE (d)	±0·012	±0·008	±2·190	±2·424	±0·539	±0·745	±0·029	±0·152
CD (5%)	0·026	0·017	4·698	5·199	1·134	1·598	0·062	0·326
CV (%)	23·48	11·33	14·31	17·079	28·17	29·72	10·42	12·32

and straw of rice. An increased N content in tea leaves due to triacontanol have been reported by Vijayaraghavan and Balakrishnan (1985). Further, it is reported by Kathiravetpillai and Kulasegaram (1981) that cycocel increased N content of leaves of tea clones. Poljakoff-Mayber and Mayer (1960) suggested that thiourea has a positive effect on nucleic acid metabolism by increasing the RNA/DNA ratio. This might be the reason for higher N content of the plucked shoots as envisaged in the present investigation.

The increase in crude protein content is mainly due to increased nitrogen content in leaves indicating high amino acid synthesis and thereby improved crude protein content. Ghosh and Srivastava (1995) reported an increased level of total free amino acids and crude protein in oak leaves due to IAA treatment. Since caffeine contributes to tea quality (Wood and Roberts 1964; Millin *et al* 1969; Sanderson *et al* 1976), increase in its quantity in tea leaf improves the quality. Increase in N content due to bioregulator treatment results in higher crude protein and caffeine content. Further caffeine in the tea plant is formed by nucleic acid catabolism (Bhattacharyya and Ghosh 1968; Ogutunga and Northcote 1976). Thus, any increase in nucleic acid metabolism due to bioregulators treatment results in enhanced caffeine content. However, Sekiya *et al* (1984) studied the interaction of tea catechins with proteins and reported that during fermentation process the greater part of *O*-guinoues precipitate with proteins which gives a more intense coloured product known as thearubigins. This compound contributes colour to the tea liquor which is no doubt of considerable importance in the tea industry.

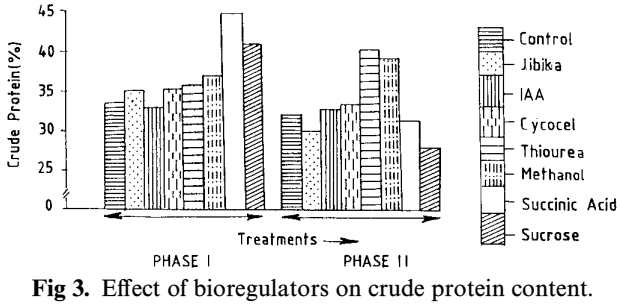

Fig 3. Effect of bioregulators on crude protein content.

Response of bioregulators on starch and carotenoids contents in tea leaves

The results presented in Table 1 showed that there was significant variation in the starch content of tea leaf tissues due to bioregulator treatments in both the phases. However, during the first phase, jibika produced highest (9.59%) starch followed by IAA while the lowest (4.28%) was recorded with sucrose treatment. Similarly in the second phase also, jibika produced the highest (7.92%) starch content as compared to other treatments and control. The rest of the treatment did not show significant effect in comparison to control. This result is in confirmation with the findings of Jain and Aggarwal (1987) who found increased starch content in *Trigonella* sp due to GA application. Further, the carbon assimilation by leaves is reported to be increased by gibberellic acid (Chatterjee *et al* 1976) which is brought about by an increase in the rates of cyclic and non-cyclic photophosphorylation (Yakushkina and Pushkina 1975), enhanced RuBP carboxylase activity and increased chlorophyll content (Briant 1974).

Total carotenoid was also found to differ significantly due to bioregulator treatments (Table 1). In both the phases, jibika treated leaves produced highest 109 $\mu g\ g^{-1}$ and 119 $\mu g\ g^{-1}$ total carotenoids. Other treatments like sucrose, cycocel and methanol also obtained increased carotenoid content over control. Suryanarayana (1981) supported that cycocel increased the level of carotenoid content in mango leaf. However, the reports on influence of starch and carotenoid content on tea quality are very scanty.

Response of bioregulators to ascorbic acid

Ascorbic acid is an important growth regulator which occurs naturally in plants. All the bioregulators tested showed significant variation with respect to ascorbic acid (AA) content in tea leaves (Table 1). The maximum of 155.0 mg and 134.50 mg AA was obtained in sucrose-treated plants and the lowest of 58.81 mg and 43.61 mg in thiourea at both the phase, respectively. Chinoy *et al* (1961) were also of the opinion that a higher concentration of sucrose or other sugar is essential for rapid biosynthesis of AA. Likewise, Arnon *et al* (1954) have ascribed the role of an electron carrier to AA in the oxidative chain of photosynthetic phosphorylation in illuminated chloroplast or as a factor stabilising the activity of the chloroplasts. In fact, Chinoy (1962) found that AA is an important constituent of the redox system influencing development of plants. However, there is no evidence of the influence of AA on tea quality. But, as the AA is vitamin C, it would improve the tea quality. Thus, the results of biochemical analyses are suggestive of a hormonal influence on the quality of made tea. It has been reported that epicatechin gallate, theaflavins and theogallin of black tea are the main 'quality constituents' desirable in the north east Indian Plains black tea (Biswas *et al* 1971). It would, therefore, be appropriate in future to determine the main quality constituents of Indian teas like TF, theogallin and epicatechingallate, following the application of jibika, sucrose and other growth substances which favourably affect growth.

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