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Blending of Clonal Tea Leaves with Leaves from Seedlings in Order to Improve the Quality of Made Tea

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Abstract: The clonal tea is known for its quality, nutrionally and medicinally valuable biochemical components. But the availability of the clonal tea is limited. Tea plantations all over south India is maximum engaged by the seedlings. Thus in order to enhace the quality of the made tea from seedlings a blending attempt was made and the biochemical components were quantified using the available protocols. The results from the study shows that the quality of the made from seedlings was improved when blended with clonal leaves.

Key words:

INTRODUCTION

The heterozygous tea accessions possessed a number of biomolecules. Among them, polyphenols constitute greater proportions than any other constituents and catechins constitute two-third of total Polyphenols. Polyphenols, particularly, catechins gained much importance due to their therapeutic values such as anticancerous, antiallergic and antibacterial properties [1].

Recent studies have demonstrated that tea is more than a mere stimulant and may owe its special medicinal properties to the high levels of polyphenols present in the consumed beverage. Polyphenols account for about 25-35% of the total dry weight of freshly plucked tea leaves.

According to Wanyoko [2] black tea quality depends on the level of catechins. However, since the catechins in black tea infusions are colourless and present at levels below their flavour threshold, it is difficult to envisage how 'black tea quality depends on the level of catechins'.

Nutritional and therapeutic importance of tea arise from its unique combination of a large number of constituents such as proteins, carbohydrates, amino acids, lipids, vitamins, minerals, alkaloids and polyphenols [3]. These biochemical constituents determine the final commercial tea quality [4]. In the present study, edaphic and environmental conditions, genotypic variation, agronomic practices in relation to total polyphenols synthesis has been paid much attention besides certain quality parameters of made tea in relation to above said parameters.

Significance of Biomolecules Present in Tea: Plants are primarily having two kinds of metabolites, *i.e.* primary and secondary metabolites. Primary metabolites were involved in the metabolic architecture of the plants and the secondary metabolites, in the self defense mechanism against pests and diseases. All the plants are having their own secondary components among them, particularly polyphenols are very common. The phenolic components mainly involve in the protecttion of plants against pest or diseases attacking the plants. Many crops like asvakantha are having polyphenols groups. Some of the plants, for example tea, contain catechins as a group of polyphenols.

Polyphenols are extensively utilized in the pharmaceutical line because of its anticancer, antiviral, antibacterial and anticancer nature. The inhibitory effects of tea against carcinogenesis have been attributed to the biological activities of the polyphenols fraction in tea. Many laboratory studies have demonstrated the inhibitory effects of green tea polyphenols, especially (-) epigallocatechin-3-gallate (EGCG), on carcinogenesis in animals' models. Majority of these studies have been conducted in mouse skin tumor models, where tea polyphenolss were used either as oral feeding in drinking water or in direct local application. Black tea was also found to be effective, although the activity is weaker than that of green tea in some experiments. Other studies showed that black tea polyphenols-theaflavins exhibit stronger anticarcinogenic activity than did EGCG. Caffeine in tea is also important to prevent tumorigenesis. The molecular mechanisms of the cancer chemopreventive

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effects of tea polyphenols are not completely understood. They are most likely related to the mechanisms of biochemical actions of tea polyphenols, which include antioxidative activities, modulation of xenobiotic metabolite enzymes and inhibition of tumor promotion. In addition, tea polyphenols function as cancer chemopreventive agents through modulation of mitotic signal transduction. Similar antioxidant properties are found in cocoa which is beneficial for cardiovascular health befits [5].

Tea leaves contain high phenolic components, which account for 25-35% on dry weight basis [6]. As far as tea is concerned, plant synthesizes abundant polyphenols, proteins and lipids, as well as hydrolytic and oxidative enzymes. Tea polyphenols are having specific activities against free radical damaging of human cells. In tea polyphenols, Catechins are the major group and found to be two-third of the total polyphenols content.

From the study carried out by Leung *et al.*, [7] TF present in black tea possess at least the same antioxidant potency as catechins present in green tea and that the conversion of catechins to TF during fermentation in making black tea does not alter significantly their free radical-scavenging activity.

In acute feeding studies it is observed that flavanol-rich cocoa and chocolate increase plasma antioxidant capacity and reduce platelet reactivity. Based on the data, it was concluded that approximately 150 mg of flavonoids is needed to trigger a rapid antioxidant effect and changes in prostacyclin. Some dose-response evidences demonstrate an antioxidant effect with approximately 500 mg flavonoids brewed tea typically contains approximately 172 mg total flavonoids per 235 mL (brewed for 2 min); hence, consumption of one and three cups of tea would be expected to elicit acute and chronic physiologic effects, respectively. Collectively, the antioxidant effects of flavonoid-rich foods may reduce cardiovascular disease risk [8]. Recent research has shown that the polyphenolic antioxidants in green tea possess cancer chemopreventive effects and the molecular mechanisms that underlie the broad anticarcinogenic effect of polyphenolss in green tea [9].

Polyphenols are synthesized from in different biosynthetic pathways. Erythrose-4-phosphate and phosphoenolpyruvate are the two components, which plays an important role in polyphenol (catechin) biosynthesis besides other intermediates. From erythrose-4-phosphate and phosphoenolpyruvate, quinic acid and shikimic acid are formed. From quinic acid, chlorogenic acid is derived. Three pathways have been proposed for the biosynthesis of gallic acid. I) ß-oxidation of the side chain of 3,4,5-trihydroxy cinnamic acid, ii) dehydrogenation of shikimic acid, probably with 3-dehydro shikimic acid as an intermediate and iii) hydroxylation of protocatechuic acid in to shikimic acid [10]. Epicatechin, epicatechin gallate and epigallo catechin gallate were formed from gallic acid.. Shikimic acid leads to the formation of phenyl pyruvate (Scheme 1).

According to Wanyoko [2], black tea quality depends on the level of catechins. Since the catechins in black tea infusions are colorless and present at different levels and it is difficult to envisage how black tea quality depends on the level of catechin. It is true that the polyphenols present in the green leaves converted into theaflavins and thearubigins of black tea by the enzymatic oxidation and therefore their concentration in fresh green tea may influence black tea quality. Several factors influence synthesis of biological constituents of tea, particularly, tea polyphenols invariably; climate, genetic make of the accession, cultural operations, *etc*. [4]. There are many reports with regard to the pathway leading to polyphenol biosynthesis. Literature available on polyphenol/catechin biosynthesis and the factors influencing their production are plenty in tea and hence, details are not dealt in this chapter.

Very wide variation is observed in phenolic content in tea leaves. Distribution of polyphenols varied significantly among the different tea shoot component. Among the components, the bud exhibits the highest content of total polyphenols and catechins [11]. As the physiological maturity increases, the contents of polyphenols and catechins decreased [12]. There is a strong negative correlation exists between polyphenols and physiological maturity of tea leaves. It is interesting to note that higher the polyphenols higher the catechin content which, in turn, improves the quality of black tea [11].

Content of total catechin could be used as a tool to identify potential tea accession. High content of catechins relates to high quality [13]. Individual proportions of the catechins could be important in the determination of tea quality and genetic diversity [14]. Saravanan *et al*. [15] classified the UPASI clones into five different groups based on the catechins content. However, none of the clone segregated on the basis of parental /cultivar types because of their long term cross hybridization.

Magoma *et al*., [16] reported that catechin content in tea plants can be used as a reliable parameter to identify quality clones. Quality of black tea is correlated to polyphenols content, catechins and enzyme activity (PPO) in tea shoots [17,18].

Instead of taking allopathic tablets like aspirin, a cup of hot black tea with a few drops of limejuice in it will cure headache. It has been found that the decoction of tea acts as bronco and cardiovascular dilators. Hence tea-drinking is very useful to reduce bronchial problems like difficult breathing and asthma. Polyphenols like flavonols, flavonol gallates, flavonol glycosides, theaflavins, thearubigins bisflavonols, epitheaflavic acid and gallic acid, which constitute 48.5% of the total solids in a cup of tea, have a variety of pharmacological activities.

Due to the climatic nature, tea leaves are harvested almost through out the year in some places of the tea grown areas, while tea plants experiences dormancy during winter season, where the harvesting is affected for certain periods of the year [19]. In such places, tea cultivation may be regarded as seasonal. There are many reports available on the seasonal yield of tea plants. But a very few reports are available on the seasonality of South Indian teas.

Recently, Senthil Kumar [20] reported that under mid elevation conditions green leaf bioconstituents are high when compared with high elevation. But the tea manufactured from the leaves at high altitude is better in quality than mid elevation. This is because during manufacturing process the clones studied took more time for fermentation. Similar type of observations was also reported by Owuor and Obanda [21] under Kenyan conditions.

Apart from the cultural practices, black tea parameters are influenced by manufacturing conditions as well. Particularly, handling of harvested crop, withering, fermentation and drying process of black tea manufacture influence the quality parameters to a considerable extent. Moreover teas made from seedling teas/poor raw materials yielded only moderate to poor quality black [11], while high grown raw material produced flovoury teas.

MATERIALS AND METHODS

All the experiments were carried out at TATA Tea Limited, Research and Development Centre, Munnar, Kerala, between the period 2000 and 2006 using UPASI released tea clones and certain popular estate selections. Samples (crop shoots comprising two to four leaves and a bud) were collected from the tea bushes at periodical intervals for biochemical analyses *viz*., polyphenols, catechins, catechin fractions (ISO/TC34/SC 8 N 488), amino acids and soluble sugars. Black tea samples (BOPF grade) was manufactured by crus, tear and curl (CTC) and were analysed for TF, TR and TLC by spectrophotometeric method [12], Caffeine and Total soluble solids.

RESULTS

In order to improve the quality of made tea, blending studies were carried out, where different proportions of seedling leaves were blended with known quantity of clonal leaves. When the seedling teas proportion increased 3:2) or equal (1:1) to that of clonal leaves, the values of polyphenols content of made tea was significantly lesser. On the other hand, when the clonal leaves proportion enhanced, the polyphenols content enhanced dramatically. The results of the present investigation are presented in Fig. 1 and Tables 1-3.

Table 1: Effect of blending seedling leaves with quality cultivar TTL-2 leaves in various proportions for one cycle

	Seed:TTL-2				
Treatments alone	1:1	2:1	3:2	2:3	Seed
Polyphenol (%)	31.10	32.87	32.73	30.58	27.17
CD	2.18	2.12	2.01	2.29	2.15
Catechin (%)	14.31	15.12	15.06	14.07	12.50
CD	2.14	2.11	2.06	2.19	2.12
Solubel Sugars (%)	8.09	8.55	8.51	7.95	6.95
CD	2.12	2.11	2.08	2.15	2.11
Amino Acid (%)	1.11	1.23	1.15	1.10	1.05
CD	0.06	0.08	0.07	0.07	0.08
Theaflavin (%)	1.15	1.50	1.48	1.23	0.81
CD	0.02	0.10	0.11	0.04	0.05
Thearubigin (%)	11.64	12.36	12.29	10.79	9.68
CD	1.65	1.63	1.71	1.69	1.86
Total Liquor Color	2.64	3.12	3.02	2.83	2.75
CD	0.39	0.36	0.38	0.31	0.34
Total Soluble Solids (%)	35.93	37.89	37.72	35.51	31.67
CD	0.26	0.52	0.26	0.20	0.35
Caffeine (%)	3.06	3.24	3.22	3.01	2.68
CD	0.22	0.21	0.20	0.23	0.22

Table 2: Effect of blending seedling leaves with quality cultivar TTSS-1 leaves in various proportions for one cycle

	Seed:TTSS-1				
Treatments alone	1:1	2:1	3:2	2:3	Seed
Polyphenol (%)	31.43	33.39	33.39	31.00	27.17
CD	2.24	2.16	2.21	2.02	2.15
Catechin (%)	14.46	15.36	15.36	14.26	12.50
CD	2.17	2.13	2.15	2.06	2.12
Solubel Sugars (%)	8.17	8.58	8.64	8.05	6.95
CD	2.14	2.12	2.13	2.08	2.11
Amino Acid (%)	1.20	1.30	1.46	1.07	1.05
CD	0.06	0.09	0.08	0.10	0.08
Theaflavin (%)	1.00	1.32	1.28	1.08	0.81
CD	0.02	0.14	0.09	0.18	0.05
Thearubigin (%)	10.61	11.56	11.81	10.31	9.68
CD	1.66	1.79	1.81	1.71	1.86
Total Liquor Color	3.06	3.37	3.27	2.55	2.75
CD	0.36	0.31	0.30	11.16	0.34
Total Soluble Solids (%)	34.45	36.34	36.25	33.80	31.67
CD	0.34	0.25	0.17	0.24	0.35
Caffeine (%)	3.10	3.29	3.29	3.05	2.68
CD	0.22	0.21	0.22	0.20	0.22

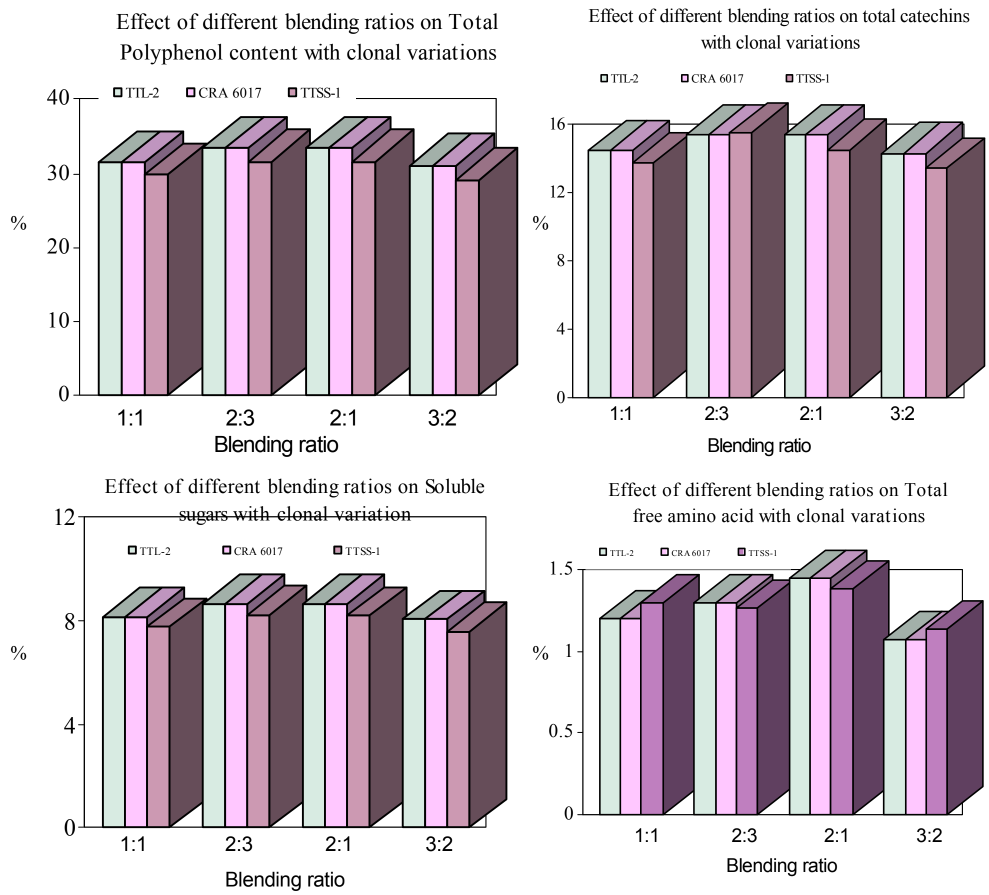

Fig. 1: Effect of different blending ratios on biochemical parameters of green leaves with clonal variation

Table 3: Effect of blending seedling leaves with quality cultivar CR-6017 leaves in various proportions for one cycle

	Seed:CR-6017				
Treatments alone	1:1	2:1	3:2	2:3	Seed
Polyphenol (%)	29.84	31.66	31.52	29.13	27.17
CD	2.18	2.21	2.08	2.09	2.15
Catechin (%)	13.73	14.56	14.50	13.40	12.50
CD	2.14	2.15	2.09	2.09	2.12
Solubel Sugars (%)	7.79	8.24	8.15	7.55	6.95
CD	2.12	2.13	2.09	2.10	2.11
Amino Acid (%)	1.30	1.27	1.38	1.14	1.05
CD	0.04	0.05	0.04	0.05	0.08
Theaflavin (%)	1.02	1.36	1.36	1.10	0.81
CD	0.06	0.12	0.03	0.05	0.05
Thearubigin (%)	10.89	11.84	11.79	10.25	9.68
CD	1.82	1.70	1.63	1.74	1.86
Total Liquor Color	2.86	3.22	3.55	2.76	2.75
CD	0.31	0.31	0.39	0.30	0.34
Total Soluble Solids (%)	34.99	36.94	36.74	34.54	31.67
CD	0.29	0.14	0.27	0.43	0.35
Caffeine (%)	2.94	3.12	3.10	2.87	2.68
CD	0.22	0.22	0.20	0.21	0.22

As discussed earlier, seasons played a major role in quality parameter. Here again, polyphenols content during high cropping months, was marginally higher. Among the clones tested, TTL-2 resulted higher performance in blending in terms of black tea polyphenols followed by CR-6017 and TTSS-1.

Polyphenols content of green leaves with respect to seasons and different pruning cycles had been evaluated. Polyphenols content of green leaves blended with seedling leaves and TTL-2 at 3:2 or 1:1 proportions enhanced significantly. Seedling leaves registered invariably significantly lesser amount of polyphenolss throughout the seasons and pruning cycle. Among the clones tested, TTL-2 resulted higher performance in blending in terms of total polyphenols followed by CR-6017 and TTSS-1. Among the pruning cycle, there was a fluctuation in polyphenols content in third year.

Catechins of green leaves with respect to seasons and different pruning cycles had been evaluated. Catechins of green leaves blended with seedling leaves and TTL-2 at 3:2 or 1:1 proportions enhanced marginally.

Seedling leaves registered invariably significantly lesser amount of catechins throughout the seasons and pruning cycle. Among the clones tested, TTL-2 resulted higher performance in blending in terms of green leaves catechins followed by CR-6017 and TTSS-1. Among the pruning cycle, there was a fluctuation in catechins in third year.

Soluble sugars of green leaves with reference to seasons and different pruning cycles had been evaluated. Soluble sugars of green leaves blended with seedling leaves and TTL-2 at 3:2 or 1:1 proportions enhanced marginally. Seedling leaves registered invariably significantly lesser amount of soluble sugars throughout the seasons and pruning cycle. Among the clones tested, TTSS-1 resulted higher performance in blending in terms of green leaves soluble sugars followed by CR-6017 and TTL-2. Among the pruning cycle, there was a fluctuation in soluble sugars in third year. Same trend was followed in the amino acids.

Theaflavin content of the clone, TTL-2 blended with seedling was significantly higher than TTSS-1 and CR-6017 Invariably the seedling population showed, in all the seasons/pruning cycle, very low amount of TF value. Similar trend was noticed with other quality parameters like TR, TLC, TSS and caffeine.

DISCUSSION

During the course of processing of tea leaves, various biochemical changes occur resulting in the formation of compounds responsible for quality and aroma of tea. Thus, the quality of raw material and the processing conditions determine the ultimate quality of black tea. In the present study, the manufacturing conditions are maintained identical in all the experiments carried out.

It has been recommended to blend clonal leaves with seedling leaves to improve the quality of made tea produced from the later [11,22]. In this study, it is found that blending of clonal leaves with seedling populations improves the quality parameters of made tea.

The present study has helped to elucidate the controllable and independent factors like developmental changes, agronomic factors, genetic potential and environmental factors that singly or in combination influence the dry matter allocation in tea plants which is deliberately manifested on crop productivity to a great extent. The study also indicates the probable refinement in the conventional agronomic practices that need to be reviewed in future to enhance productivity index in tea.

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