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Variations in quality constituents of green tea leaves in response to drought stress under south Indian condition

Munivenkatappa Nalina[a], Sarikonda Saroja[b], Rajagopal Rajkumar[a], Balakrishnan Radhakrishnan[a], Krishnappa Nagarathana Chandrashekara[a,*]

[a] Division of Plant Physiology and Biotechnology, UPASI Tea Research Foundation Tea Research Institute, Nirar Dam P.O., Valparai 642 127, Coimbatore, Tamil Nadu, India

[b] Division of Entomology and Nematology, Indian Institute of Horticultural Research, Hessarghatta Lake P.O., Bangalore 560 089, Karnataka, India

A R T I C L E I N F O

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A B S T R A C T

Soil moisture stress seriously limits the growth and development of tea plants (*Camellia sinensis*), thus affecting crop yield and quality. Soil moisture reduction generally affects accumulation of tea secondary metabolites. To elucidate the variations in plant genotype response to abiotic stress during depleting soil moisture stress and changes in biochemical constituents of green leaf which determines the quality of black tea was investigated. An experiment was conducted at UPASI Tea Research Foundation Tea Research Institute, Distinctiveness-Uniformity-Stability center with drought tolerant clones namely UPASI-2, UPASI-6, UPASI-9, ATK-1, TRI-2025 and drought susceptible clones UPASI-3, UPASI-8, UPASI-17, TRF-1 of same age. Green leaves were collected from each plot was subjected to HPLC analysis for dihydroxyflavan-3-ols, trihydroxyflavon-3-ols and caffeine, and the results were statistically analyzed. The study indicated that the levels of total polyphenols, individual catechins, reducing sugars, amino acids and total lipids were significantly different at $P \leq .05$ among the treatments. Caffeine level increased ($P \leq .05$) with decrease in soil moisture for all the clones. Principal component analysis and hierarchical cluster analysis revealed differences between tea cultivars with respect to their quality and tolerance. Our study further indicated that the tolerant clones were able to accumulate higher levels of bio constituents during soil moisture stress than the susceptible ones. Quality of green tea leaf precursors is directly influenced by the soil moisture availability and is varietal dependent. This could be an important tool for the assessment of clonal performance and drought tolerance in tea cultivars.

1. Introduction

Tea (*Camellia sinensis* (L) O. Kuntze) belongs to the family Theaceae, is used to process the most popular beverage worldwide (Wheeler and Wheeler, 2004). It is estimated that of all the tea consumed worldwide, about 76–78 % is black tea (Cabrera et al., 2003). Tea flush (young tea shoots) consists of the terminal bud and two adjacent leaves are used for manufacturing. India being world's largest producer of black tea and consumer, black tea quality is affected by an array of factors namely; plant genetics, season, climate, growth stage, cultural practices and leaf quality. In nature, plants are continuously exposed to several biotic and abiotic stresses, productivity is decreasing due to its detrimental effect and therefore minimizing the losses is a major area of concern to maintain the plant growth and quality under changing climate (Anjum et al., 2011; Ahmed and Stepp, 2016). Drought stress is one of the most important environmental stresses which adversely modify the plant growth and productivity which is considered as severe threat for sustainable tea crop production in ever changing climatic conditions. Drought triggers a wide variety of plant responses, ranging from cellular metabolism to changes in its growth rates and crop yield, which limits the quality and the performance of tea plants more than any other environmental factor (Anjum et al., 2011; Cheruiyot et al., 2010; Shao et al., 2009). Plant adaptive strategies to stress are coordinated and fine-tuned by regulating physiological, biochemical and molecular activities (Upadhyaya et al., 2008; Das et al., 2012; Zhou et al., 2014). Soon after the perception and recognition of external changes, different signaling pathways are activated in order to convert an external stress into a biochemical response, each of them promoting the expression of a set of stress responsive genes. The full activation of signal cascades induced by a water stress event promotes acclimation of bio constituents and leads to stress tolerance (Mastrangelo et al., 2008).

Understanding the biochemical response of tea plants to drought is essential for a complete cognizance of plant resistance mechanisms to water stress conditions and quality of tea. There are contrasting

* Corresponding author.
E-mail address: knchandu1@gmail.com (C. Krishnappa Nagarathana).

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chemical compositions in different parts of tea crop shoots. The important biochemical parameters influencing tea quality includes green leaf polyphenols, catechins, free amino acids, proteins, total sugars, caffeine and especially the flavanols and their oxidative products. Flavanols are responsible for the formation of the black tea thearubigins and theaflavins (Hilton and Palmer 1973). The major catechins, namely (-) - epigallocatechin-3-gallate (EGCG), (-) - epigallocatechin (EGC), (-) - epicatechin-3-gallate (ECG), and (-) - epicatechin (EC), constitute around 90% of the total catechin fraction; and (+) catechin (C) and (+) - gallocatechin (GC) constitute about 6% of the fractions. The simple catechins (dihydroxyflavan-3-ols) undergo oxidative dimerization with the gallocatechins (trihydroxyflavan-3-ols) to produce theaflavins (Bowa et al., 2016). Being water-soluble and colorless, catechins contributes to astringency and bitterness to green tea (Kruidhof et al., 2012; Ahmed et al., 2013a,b). Total catechins content could be used to indicate the quality potential of tea, with high content being related to high quality (Gulati et al., 2009; Magoma et al., 2000). Fractions of catechins and its individual proportions could be important in the determination of tea quality and genetic diversity (Owuor and McDowell, 1994).

Amino acids constitute around 3–4% in tea flush, Theanine (5-*N*-ethylglutamine) is the most abundant amino acid which is unique to tea and it is found at a level of 2% dry weight (Juneja et al., 1999). The production of secondary metabolites contributes to rich flavors, clean taste, and nutrient content of tea. These metabolites are known to be beneficial to human health (Li et al., 2015). Tea harvested during the dry spring is rated with the highest quality and best sensory preference. High quality deteriorates and sensory preference is ranked low during monsoon season, due to increase in soil moisture (Ahmed et al., 2014). The impact of soil moisture is expected to have a notable effect on tea production and the quality of black tea under south Indian conditions. The present study aims to document the quality variations prevailing due to depleting soil moisture levels and its implication on quality deterioration in elite clones, which are categorized as tolerant and susceptible varieties belonging to Assam, China and Combod jats in the Annamalais region.

2. Materials and methods

2.1. Experimental site and experimental design

The field experiment was conducted at UPASI Distinctiveness-Uniformity-Stability (DUS) center in the year 2015–2016, located at Valparai (11°59′ 0.69″ N, 76°57′2.32″ E and 1050 m above MSL). The soil in the experimental field is of sandy clay loam with sand 54.23%, clay 32.07%, and 12.13% silt. The 9 elite tea clones were selected for the study, based on their contrasting traits and field performance, they were categorized in to two groups namely drought tolerant (UPASI-2, UPASI-6, UPASI-9, ATK-1, TRI-2025) and drought susceptible (UPASI-3, UPASI-8, UPASI-17, TRF-1) (Durairaj et al., 2015). The experiment was performed with four-year-old tea plants, and were managed under standard cultural practices recommended by UPASI (Durairaj et al., 2015). The study was conducted in same experimental plot to minimize soil spatial variability, spacing between the plants were 75 cm apart and between the plots is 105 cm. The experiment was carried out in a complete randomized block design; each block was sampled with three replicates for analysis. The experiment was analyzed as 2 factorial design (with the genotype and soil moisture as the treatments).

2.2. Weather characteristics of experimental site

The field experiment was carried out under natural climatic variables. The climate is semi-warm, sub-humid with frequent day-to-day and year-to-year variability in the weather patterns. The comprehensive weather data was obtained during study period from weather station located at UPASI experimental farm. The average annual temperature ranged from 15.5 to 22.5 °C and rainfall ranging from 175.0 to 300 cm per year. During drought period (December-2015–March-2016) day temperature mean max was 32.1 °C and night temperature mean max was 18.5 °C. There was no precipitation during study period and relative humidity (RH %) ranged from 40 to 49%.

2.3. Leaf sampling

Fresh tea crop shoots consisting of one apical bud and two adjoining leaves, were collected from the experimental plots. The samples were hand plucked just before each commercial harvest at regular intervals (3–4 weeks) apart and soil samples were also collected to estimate soil moisture level during each sampling day. Sampling was done across two seasons (wet season: during September 2015 and dry season: during December 2015–March 2016). At each harvest, 200 g of fresh tea crop shoots were collected, wrapped with aluminum foil and carried to lab in cryocan. All samples were stored in −80 °C freezer until analysis.

2.4. Soil moisture analysis

The composite soil samples were collected adjacent to the root zone at the experimental site at depths of 0.00–22.5 cm top soil and from 22.6 to 45.0 cm bottom soil as per the UPASI guidelines (Durairaj et al., 2015), using a stainless-steel auger (5 cm diameter). Three samples were collected from each location and then combined to form one composite sample. The fresh soil samples were put in to a moisture free plastic valve bag and taken to the laboratory and immediately used to determine soil moisture content gravimetrically by drying at 105 °C.

2.5. Physicochemical characteristics of soil from experimental site

The fresh (un-dried) sub samples were subjected to soil pH analysis using potentiometric method with soil: solution suspensions (1:2.5 w/v) by using Elico LI 120 pH meter. Portion of the sub soil was air-dried at room temperature and passed through a 1-mm sieve and subjected to texture and chemical analysis. The chemical properties of the soil were assessed in triplicates following the standard methods. The total nitrogen (N) was determined using Kjeldahl method (Bremner, 1996) by using Kelplus-Elite EX instrument. Soil organic carbon content was determined using the dichromate oxidation method (Walkley and Black, 1934). In the diluted digests, phosphorous (P) was measured spectrophotometrically at 882 nm (UV–Vis Pro 1100, Amersham Biosciences) by the molybdenium blue method (Bray and Kurtz, 1945) after its reaction with ascorbic acid. The 1N ammonium acetate extracts was used for potassium (K), calcium (Ca), magnesium (Mg) analysis. Ca and Mg were determined by titrimetric methods and K was measured by flame photometer (Systronics-120) (Hanway and Heidal, 1952). The particle size analysis was done by the Universal pipette method.

2.6. Leaf moisture analysis

The moisture content of fresh tea crop shoots was determined using ISO1572:1980 procedure with the following changes to the oven conditions. Temperature was maintained at 80 °C to get steady state weight for 48 h and then cooled in desiccator to measure dry weight. Analysis was carried out on two separate determinations for the same leaf samples. All results reported in the present study are on dry weight basis.

2.7. Sample preparation for biochemical analysis

One gram of frozen plant material was homogenized by using precooled pestle and mortar using liquid nitrogen to obtain a fine powder. Then, the fine powder was extracted with 10 mL of double distilled ethanol, filtered through Whatman No. 1 filter paper. Filtrate was