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Principal Component Analysis based Clustering of UPASI Tea Cultivars for their Diversity on Free Radical Scavenging Activity

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ABSTRACT

Tea is one of the popular beverages which immensely have therapeutic activity against various diseases. The biochemical and therapeutic properties of tea was observed to be varying because of its seasonal and varietal difference which will reflect in the quality of the end product. Yield and quality are the major factors determining the profit. So, research is still going on to search high quality tea plant among the huge tea seedling population. This process is basically a time involving (in years) or cost of selection will be high. The present study, grouping of tea plant was made by using total catechins and polyphenols activity against free radicals such as DPPH and Hydroxyl radicals. UPASI-17 registered the highest polyphenol, catechin content as well as its scavenging activity against free radicals than that of other tea cultivars. When considering the catechin and polyphenols scavenging activity, catechin influence higher than that of polyphenols against DPPH and Hydroxyl radicals. IV distinct groups were observed through Principal Compound Analysis (PCA) and dendrogram analysis based on individual clones free radical scavenging activity. By this analysis all clones have high free radical scavenging property was grouped separately. By adapting this method of analysis, it will reduce the time and cost involvement in the screening of new tea varieties from the seedling population and help in the production of high free radical scavenging black tea.

Key words: Antioxidants, 1,1-diphenyl-2-picrydrazyl, free radicals, principle compound analysis, dendrogram

INTRODUCTION

Tea is one of the major economic crops of India which provides good foreign exchange to the nation. South India is playing a vital role in the production and exporting of tea in the world market, particularly Tamil Nadu. Planters are facing various problems to achieve this essential process for the country. Yield and quality of tea plants needs to improve every time to stand in the market. For this important process, screening of tea plants among the huge seedling population was continued to get good accessions in terms of quality and yield. Infilling, inter planting, inter row planting and replanting were based on these selected cultivars to improve the required yield and quality (Muraleedharan, 2005). To identify such high quality plants found to be a big task because of the huge seedling population of South India (90%). Quality of tea is one of the major criteria for the high price which immensely contains high antioxidant capability

(Owuor and Obanda, 2007; Nshimiyimana and He, 2010). A careful screening might be required for the selection in terms of quality.

It is well known that the crop shoots of tea contained various biochemicals such as flavonoids, alkaloids and enzymes besides the higher amount of polyphenols (John *et al.*, 2006a, b). Therapeutic functions of polyphenols can be listed more like antioxidant, anticancer and cholesterol content (Yang and Landau, 2000; Mukhtar and Ahmad, 2000; Davies *et al.*, 2003). One of the major properties of polyphenol was its scavenging activity against free radicals in humans (Mudgal *et al.*, 2010; Gupta *et al.*, 2008; Arnao *et al.*, 1999; Robards *et al.*, 1999). Because of the potential damaging nature of free radicals, oxidative stress may cause physiological stress when antioxidant defences are unable to cope with them (Gill *et al.*, 2011a; Frankel, 1993).

So, the individual catechin based tea characterization was made by Saravanan *et al.* (2005). But in that diversity study, we concentrated only on individual catechin molecules and not with total polyphenols and its antioxidant potential. The total polyphenol content in tea was found to be more than 30% among them catechin which contributes around 20%. Role of other phenolics compounds in free radical scavenging activity also to be consider when going for characterization. It will give a clear idea as to which group of plants will have high amount of free radical scavenging property. To develop such selection criteria an attempt was made to characterize tea clones on the basis of its free radical scavenging activity.

MATERIALS AND METHODS

Tea shoots comprising two leaves and a bud were harvested every month from UPASI Tea Research Experimental Farm, Valparai was used for the study. To avoid variations, uniformly aged and pruned plants were selected for this study.

Tea shoots collected were subjected to analyse its total catechins and polyphenol content using spectrometer (Model: Genesys 10 UV) adopting the method reported by Swain and Hillis (1959) and Dev Choudhary Goswami (1983).

Scavenging activity: Free radical scavenging activity of OH was carried out following the procedure of Halliwell and Gutteridge (1981), whereas DPPH free radical was assayed following the method of Choi *et al.* (2002) and Tagashira and Ohtake (1998).

DPPH free radical scavenging activity was analysed by using 0.5 mL of substrate, 1.0 mL of 0.1 mm DPPH in ethanol and 0.5 mL of 50 mm Tris-HCl bufler (pH 7.4), after 30 min of incubation the OD was taken at 517 nm.

OH radical scavenging activity was analysed by adding 0.5 mL substrate with 1.0 mL of EDTA solution and mixed with 1.0 mL of DMSO (0.85%) in 0.1 m phosphate bufler (pH 7.4) to initiate the reaction followed by the addition of 0.5 mL of 0.22% ascorbic acid. After incubation and colour development the absorbance was observed at 412 nm. The difference between control and treatment OD was taken for scavenging activity calculation.

PCA analyses: Based on the data obtained, values were subjected to linear regression analysis and principal component analysis using the Special Software SIMCA P for clustering the tea clones. For PCA analysis total catechins and polyphenols scavenging activity against free radical were considered.

RESULTS AND DISCUSSION

The polyphenol and catechin level in all the UPASI tea clones were examined and presented in Fig. 1. DPPH and hydroxyl free radicals scavenging property of UPASI clones as well as its corresponding catechin and polyphenol values were also analysed and presented. Total polyphenol content was found to be high with UPASI 17 clone followed by UPASI 8 (29.03 and 28.18%) and lowest amount was registered with UPASI 14 followed by UPASI 4 clones respectively (21.55 and 22%). From the same extract the total catechin content was also analysed and the results revealed that UPASI 17 (19.83) registered high catechin content followed by UPASI 3 (18.07), the lowest catechin content was registered with UPASI-16 (14.57) followed by UPASI 25 (14.95). The DPPH and hydroxyl free radical scavenging activity of all the UPASI tea clones were studied. The scavenging activity was found to be high with UPASI-17 followed by UPASI 19 (32.52, 30.39) in case of DPPH radicals. The lowest amount of DPPH scavenger was UPASI-26 and UPASI-27, respectively (27.05 and 27.09). UPASI-17 followed by UPASI-3 (26.4 and 24.71) registered the highest hydroxyl radical scavenging activity. The lowest activity was noticed with UPASI- 16 and UPASI-5 (21.29 and 21.32).

With the available data a PCA and dendrogram analysis was made by using SIMCA P software. The catechin and polyphenol content of the clones were plotted in X axis and the free radicals scavenging percentage of the individual clones were taken in Y axis. While analysed with this conditions IV different groups had been segregated from the data. The free radical scavenging property of catechin and polyphenols against DPPH and hydroxyl freeradicals are analysed with PCA and presented in Fig. 2. The dendrogram of the UPASI clones polyphenol and catechin content against DPPH and Hydroxyl radicals were presented in Fig. 3. Based on the PCA clustering analysis and dendrogram of the clones, four distinct clustering was observed based on the clones polyphenol and catechin content against DPPH and hydroxyl radical scavenging activity. Group 1 showed the lowest scavenging activity against free radicals. UP 16, UP 25, UP 5, UP 4 and UP 14 falls under this group and were found to be average quality clones released by UPASI Tea Research institute. UP 26, UP 27, UP 22, UP 13, UP 7, UP 11, UP 18, UP 6 and UP 9 falls under group 2. All the medium quality clones were clustered under group 3

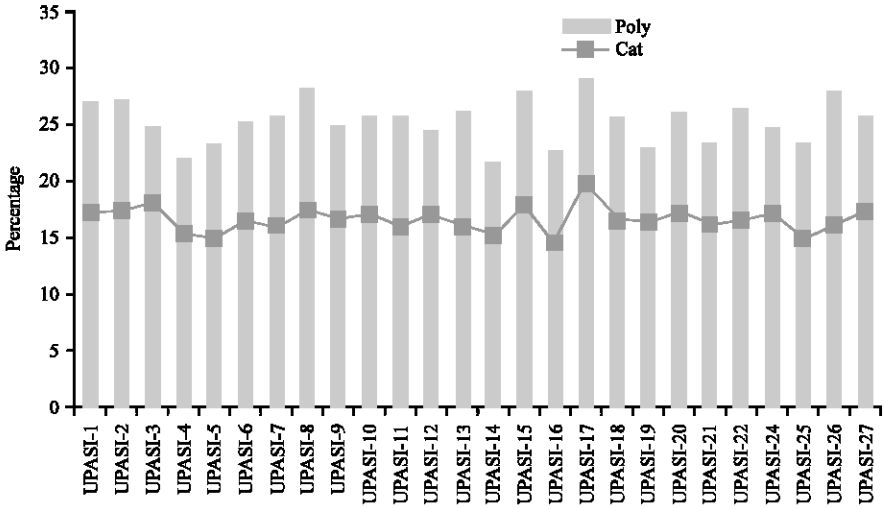

Fig. 1: Total polyphenol and catechin content of UPASI tea clones

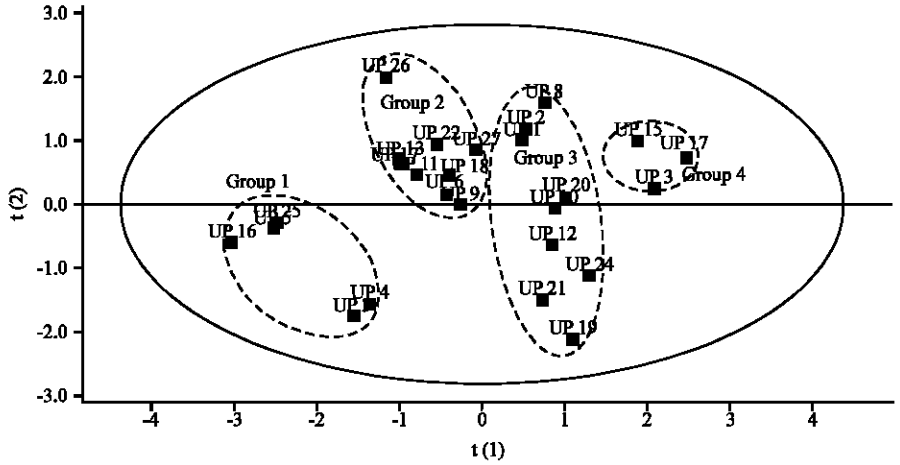

Fig. 2: Principle compound analysis (PCA) of UPASI tea clones against DPPH and Hydroxyl scavenging potential

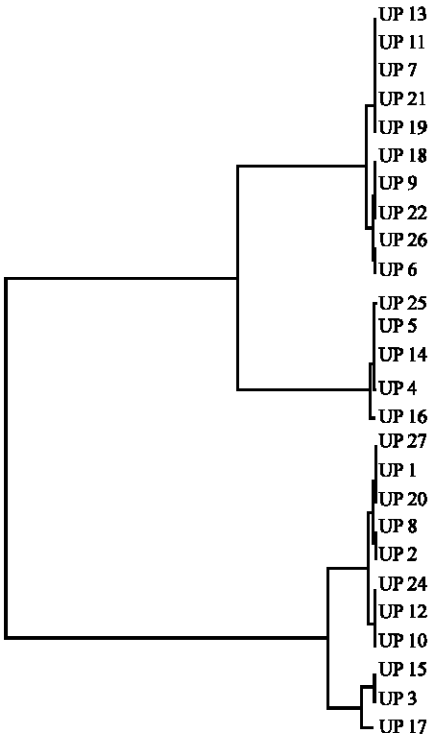

Fig. 3: Dendrogram of UPASI tea clones based on free radical scavenging potential against DPPH and Hydroxyl Radicals

(UP 1, UP 2, UP 8, UP 10, UP 12, UP 19, UP 20, UP 21 and UP 24). There is no trend on the basis of jats were observed with the clustering analysis.

A linear regression was plotted against the radical scavenging activity against polyphenols andcatechins by means of predicted and observed values. Only few predicted results coincide with the observed results. In DPPH against polyphenols and catechins only 4 clones such as

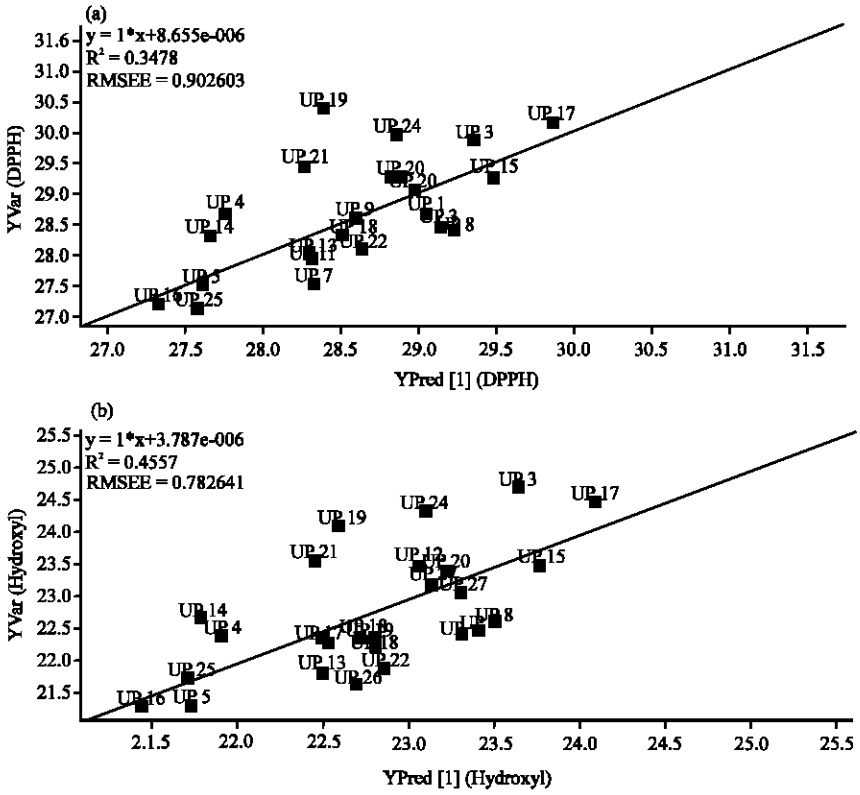

Fig. 4(a-b): Linearity between the catechin and polyphenols content against free radicals

UPASI 5, 9, 15 and 16 fall under the predicted line (Fig. 4a). Thirteen clones show lower values than the predicted values. Nine of them were found to have higher value than the observed values. In the case of hydroxyl radicals, only 3 clones such as UPASI 10, 16 and 25 were plotted in the predicted line. 14 clones show lower values than the predicted values and 9 of them were found to have higher value than the observed values (Fig. 4b). In UP 17, the level of catechin and polyphenols was high resulted with high free radical scavenging activity was observed. The influence of total catechins and total polyphenols against DPPH and Hydroxyl radicals were compared and presented in Fig. 5. Results revealed that the catechin influences more scavenging activity against free radicalsthan total polyphenol content of the plant.

Free radical scavenging activity of plants is found to be very important for human's day to day life to overcome certain illness (Choi *et al*., 2002; Semalty *et al*., 2009; Gill *et al*., 2011b; Arora *et al*., 2011). Tea is one of the major crops which produce various phenolics components which served as free radical scavengers. Bravo *et al*. (2007) studied the antioxiadant activity of tea and compared with commercially available drinks. Wide variation in terms of such antioxidant compounds present in tea was observed. Grouping of tea was found to be one of the best tools to characterise tea plant from seedlings and to develop into good quality or high yielding accession. Based on our earlier study, we found that grouping of tea can be done not only with morphological, physiological and molecular but also can be done with biochemical parameters such as EGCG, EC, EGC and ECG (Saravanan *et al*., 2005). The clustering of tea clones based on free radical scavenging potential was found to be an important aspect to select a cultivar. Thomas *et al*. (2008) studied the diversity among various forms of flavanols in selected UPASI tea germplasm. While

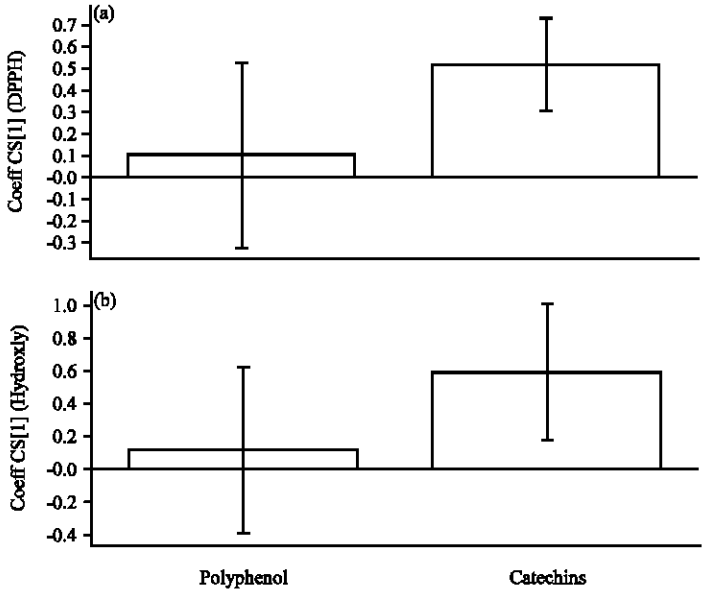

Fig. 5(a-b): Effect of total catechins and polyphenols on free radical scavenging activity

analysing the clusters, the group was not segregated based on the jats of the tea plants. It clearly indicates that the radical scavenging property of the clones will not depend upon the jats or its morphology. Based on our study it indicates that the amount of EGCG was high with UP 17, so the high free radical scavenging activity was obtained. Amount of polyphenols and catechins was found to be high in the case of group IV and the free radical scavenging property was also observed high. This result was coinciding with Muthiah *et al.* (2009) stating that the amount of free radicals scavenging found to be influenced by the catechins content. Borse *et al.* (2007) reported that marginal variations in the total catechins do not interfere with its radical scavenging activity. But, we observed that marginal variation in polyphenol content of the plant does not influences the free radical scavenging activity but the catechins influences the activity. Xu *et al.* (2004) stated that the epicatechin molecules are high free radical scavengers among the polyphenols. The UPASI clones contains high catechin content, had high free radical scavenging activity and the influence of catechins on free radicals scavenging property was higher than that of total polyphenols in the plants. The iron toxicity on polyphenol content in tea was reported by Hemalatha and Venkatesan (2011). According to John *et al.* (2006c), the higher DNA protection activity was observed with high concentration of catechin molecules. These results coincide with the present work as stated by Saravanan *et al.* (2005), the seasonal and clonal influence was observed in the case of catechin and polyphenols as well as its free radical scavenging activity. When analysed this data with PCA, it gave IV distinct groups but in our earlier study by using individual catechin molecules for characterization produces 5 distinct groups were observed.

In this study, we found that there is no relationship between grouping and jats. All the groups were segregated based on its free radical content. Gulati *et al.* (2009) studied tea catechins as biomarker to study the diversity of Indian tea clones. Same result was observed in the present study stating that no relationship between biochemical content and its varietal differences. Selecting a cultivar based on its antioxidant property might be one of the best tool because tea is concern for its anti inflammatory properties. So, the ratio of major secondary metabolites such as

polyphenols and catechins played a vital role in the quality potential of tea (Kottur *et al.*, 2010), not only for quality of black tea but also for its therapeutic aspects.

It is a simple and reliable technique to screen the tea plants from the huge seedling populations with high therapeutic potential. Tea is not only taken for its simulative activity as beverage but also for its immense therapeutic property. The cultivars screened by this method will provide good source of catechins in made tea leads to good therapeutic activity in the end product.

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