Activities of Certain Enzymes in the Shoots of Different Tea Clones

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A number of chemical and biochemical changes take place in the tea flush shoots, resulting in the development of aroma and other black tea characteristics during processing. These biochemical changes are mediated by certain macromolecular and proteinaceous substances called enzymes, which are required only in small quantities to effect changes in large amount of substrates. Fermenting ability and quality of made tea mainly depend on endogenous levels of certain enzymes like polyphenol oxidase (PPO), peroxidase (PO), phenylalanine ammonia lyase (PAL) and cellulase and their specific substrates. PO, PPO, PAL are involved in the phenol metabolism while cellulase acts upon glucose polymer.

Polyphenol oxidase

Tea leaf polyphenol oxidase, a copper containing protein, plays a vital role in black tea processing. It has been already established that the level of PPO activity has a direct relationship with the fermenting time, colour of the infusion and aroma in tea.

Peroxidase

Although the presence of peroxidase, a hemoprotein, reported as early as 1938 in tea flush, the exact role in tea processing is not understood. Unlike PPO, PO has a wide range of substrate specificity but only in presence of H_2O_2. Earlier reports showed that the deterioration of fresh tea shoots at 25 to 30°C after plucking could have been caused, as the activity of peroxidase increased. However, recent reports on the molecular characterization of both PO and PPO indicated that the oxidative role of PO may be useful in assisting the PPO in oxidizing polyphenols and ultimately contributing the colour and flavour of tea.

Phenylalanine ammonia lyase

Since the polyphenolic compounds are quantitatively major components of tea shoots, the biosynthesis of polyphenols is of particular interest to evolve potential clones. The enzyme, phenylalanine ammonia lyase, transforms the phenylalanine into cinnamic acid (Comm and Towers, 1973) (Fig. 1). Cinnamic acid is the precursor for the synthesis of catechins which is the substrate for formation of TF and TR.

Cellulase

Cellulose is one of the main constituents of plant cell wall (a linear polymer of glucose units with 1,4 linkages). The glucose polymer can be degraded by the plant enzyme, cellulase. It has been reported that exogenously added cellulase along with macerozyme in made tea increased the extraction rate of solubles by 50 per cent. There appears to be no report on the endogenous level of cellulase activity in fresh tea shoots.

Considering the importance of these enzyme, it is felt essential to generate information on the endogenous level of enzyme activity, characterization and the changing pattern during tea processing.

A preliminary investigation was undertaken to determine the activities of PO, PPO, PAL and cellulase in fresh shoots (three leaves and a bud) of UPASI-1, UPASI-3, UPASI-9, UPASI-15, UPASI-22, TRI-2024, CR-6017 and SA-6. Enzyme extracts were prepared, allowed to react with specific substrates; the end product was assayed spectrophotometrically.

The endogenous levels of PO, PPO, PAL and cellulase activity are presented in Table 1. It could be observed from the data that these enzymes in tea shoots showed clonal variation. The present results indicate that in the clones known for quality, such as UPASI-22, UPASI-3, and CR-6017, peroxidase activity was found to be higher while in the poor fermenting clone, SA-6, peroxidase activity is very low. However, no such distinct variation was obtained in PPO activity except in CR-6017. Hence, the sum of PO and PPO activity may give a better index to identify the potential quality clones, since, the two enzymes share certain common molecular and functional properties.

PAL activity was found to be higher in clones UPASI-9, UPASI-3 and UPASI-22, in that order, than in any other clone studied. Lesser activity in clone SA-6 may be attributable to poor biosynthesis of polyphenols via sugars, as reported earlier.

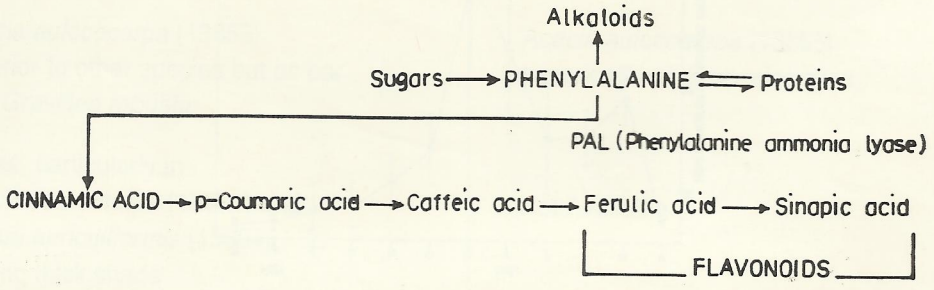

Fig. 1. Pathway of flavonoids formation

The cellulase activity was found to be almost similar in all clones except in UPASI-9, which exhibited higher amount of cellulase activity.

The present results, thus give a native level of enzyme activity in various tea clones which could be used to screen the potential clones as a source of enzymes for specific purpose. Such selected clones having higher enzyme activity may be blended with poor quality clones to improve the fermentation and other black tea characteristics.

Table 1. Enzyme activities in fresh clonal tea shoots.

Clone	Peroxidase*	Polyphenol oxidase *	Sum of PO and PPO	PAL**	Cellulase$
UPASI-1	15.20	37.55	52.75	13.13	2.67
UPASI-3	64.19	39.44	103.63	17.96	2.51
UPASI-9	36.47	48.22	84.69	18.43	6.03
UPASI-15	16.76	35.54	52.30	10.76	3.06
UPASI-22	63.66	31.30	94.96	17.95	2.67
TRI-2024	39.12	74.23	113.35	10.56	3.26
CR-6017	44.69	137.96	182.65	13.53	2.82
SA-6	3.65	28.04	31.69	6.16	2.80

Values represent mean of three different observations

* Units per gram dry weight

** μ mole cinnamic acid formed per hour per gram acetone powder

$ mg glucose liberated per minute per gram dry weight

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