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Screening of High Yielding Tea (Camellia Sinensis) Clones using Enzymes and Canonical Discriminate Analysis with Yield

Dr. D. Shalini
Assistant Professor, KG College of Arts and Science, Coimbatore, Tamil Nadu, India.

Abstract: ***In the present study, clonal and time of sampling (seasons) were significantly recorded variation in terms of productivity related enzymes and their participatory role in dry matter productivity. Rubisco (ribulose-bisphosphate caoxylarbse) played a prime role followed by MDH and PEPC. This was clearly established by the correlation studies where the Rubisco had higher correlation coefficient value followed by MDH and PEPC. Studies were conducted to analyze productivity related enzymes in UPASI tea clones and integrate it with yield data to develop a model to predict the yield of an unknown tea accession. Results showed that the Rubisco activity significantly differed among the UPASI tea accessions. It ranged from 0.058 (UPASI-11) to 0.122 (TRF-1). All UPASI clones were classified as moderate to good yielders. TRF-1 emerged as a high yielding clone with higher Rubisco activity. Linear regression analysis showed that there was a positive correlation between the Rubisco activity and yield.***

Keywords: RUBISCO, Phosphoenol pyruvate carboxylase, Malate dehydrogenase

I. INTRODUCTION

Important characteristic feature of green plants is photosynthetic carbon dioxide assimilation where the plants percept the radiant energy to fix the atmospheric carbon dioxide into simple sugars and then more complex organic molecule. This process provides the major input of energy into the biosphere and it is an important initiative reaction in terrestrial food chain and balancing the oxygen level in the atmosphere. The biochemical process supporting the life on the earth depends, in terms of energy, on oxidative reactions. In order to complete the carbon cycle it is necessary to release the carbon dioxide back to the food chain. Final product of metabolic pathways based on carbon where carbon dioxide is released into the atmosphere.

The only versatile enzyme capable of fixing the carbon dioxide in the presence of radiant energy is Rubisco (Ribulose-1-5-biphosphate carboxylase/oxygenase).

Several studies have reported that ribulose 1,5 bisphosphate carboxylase/oxygenase (Rubisco) activity and photosynthetic capacity are the possible limiting factors for plant growth and development .(Rogers et al., 1996). The rate of photosynthesis and biomass accumulation depends largely on the quantity and activity of Rubisco. Rubisco is the first and key enzyme in the Calvin cycle of photosynthetic carbon dioxide assimilation in C3 plants. It catalyzes the fixation of atmospheric CO_2 to ribulosa-1,5-bisphosphate (RuBP) to form two molecules of 3-phosphoglycerate (3PGA) which is subsequently used to build organic molecules. The enzyme is extremely inefficient and its carboxylation activity is compromised by competing side–reactions, the most notable being with another atmospheric gas, O_2. Both CO_2 and O_2 are mutually competitive at the same large subunit active site. Whereas carboxylation accounts for net CO_2 fixation, oxygenation leads to the loss of CO_2 in the photo respiratory pathway. In order to catalyze photosynthetic CO_2 fixation at high rates, large amounts of Rubisco are required to compensate the slow catalytic rate of the enzyme. It has been estimated that Rubisco accounts for a quarter of leaf nitrogen and up to half of the soluble protein in leaves of C3 plants and it is probably the most abundant protein of the world (Portis 1992).

Aoki (1990) reported that changes in the amount of proteins and RuBPC activity had no correlation with the changes in photosynthesis in tea. Significant positive correlation between RuBPC/RuBPO and photosynthesis was reported earlier in tea (Raj Kumar, 2005). There was a negative relationship existed between RuBPC/ RuBPO and photorespiration. These relationships indicated the importance of the specificity of between RuBPCO to CO_2 and O_2. When the specificity of RuBPCO to CO_2 increases, there would be an increase in photosynthesis besides an increase in RuPC/RuBPO. Increased catalase activity could also have contributed to an increase in photosynthesis by declining the photorespiration. Although the pathway of photorespiration is well understood, its regulation towards increasing biomass productivity is less known. Crop productivity influenced by a number of variables which can inhibit, stimulate, alter or modify the biomass productivity. Cultural operations like plucking (Spurgeon Cox and Raj Kumar, 2004), pruning (Spurgeon Cox and Raj Kumar, 2006; Siby Mathew, 2010), nutrient management (Verma et al., 2001), irrigation (Radhakrishnan and Venkateswaran, 2006), incidence of pests and diseases (Baby 2001), man made stress like mechanization (Marimuthu et al., 2001; Raj Kumar et al., 2010) and ecological variables (Raj Kumar and Mohan Kumar, 2009)

were found to influence the crop productivity significantly. These variables not only influence the crop productivity, they also affect the quality of made tea produce (Ilango et al., 1990; Venkatesan and Ganapathy, 2004; Baby 2002; Jeyaramraja et al., 2003).

It may be noted that all the variables that influenced the productivity, affects the metabolic activities of the plants and thereby the crop productivity indirectly. Using the physiological parameters as independent variables, bush yield as dependent variable, multiple regression models was developed to predict the yield of unknown seedlings, necessitating the importance of metabolic functions in plant improvement programme (Jeyaramraja *et al*., 2003). Above cited literature indicated that the studies were carried out to emphasize the physiological attributes in crop productivity and the importance of physiological markers to identify the desirable traits. However, no pertinent literature are available with particular reference to enzymes involved in photosynthetic carbon dioxide assimilation in tea and crop productivity, except a scanty information on Rubisco (Jeyaramraja et al., 2002; 2003; Shalini and Rajkumar 2011). Jeyaramraja et al. (2003) reported the photoassimilatory and photorespiratory behaviour of certain drought tolerant and susceptible clones. It has been reported that concentrations of RuBP carboxylase or oxygenase had no relationship with the drought tolerant nature of tea clones but their ratio correlated with the same. However, Jeyaramraja et al. (2002) applied Rubisco, peroxidase and polyphenol oxidase as markers to identify the productive and drought tolerant tea clones. Even though utility of physiological markers in evaluation of tea germplasm was reported in early 1990's (Raj Kumar et al., 1993 a, b), until the turn of the millennium physiological and biochemical features were not considered in clonal selection programme. In 2001, UPASI released a clone, TRF-1 was first described on the basis of morphological, physiological and biochemical attributes (Balasubramanian *et al*., 2001). In this present study specific enzymes involved in the metabolic functions of green plants (Rubisco, PEPC and MDH) were considered as biochemical markers in the present study. Rubisco activity was correlated with yield and a regression model was developed to predict the yield of UPASI clones and using. Except a few preliminary works on Rubisco in tea, no other productivity related enzymes were not assayed/reported in tea.

II. MATERIALS AND METHOD

A. *Experimental Design*

Experiments were carried out at UPASI (United Planters' Association of Southern India) Tea Research Foundation, Tea Research Institute, Valparai 642 127, Tamil Nadu located in the Anamallais of Western Ghats. The decennial mean maximum temperature ranges from 24 to 27^{o}C, the minimum hovers around 14-17^{o}C.

B. *Plant Material*

Irrespective of the taxonomic classification, Crop shoots collected from UPASI clones and estate selections (total 30 in numbers, detailed in the results) were subjected to quantification of enzyme assays. Crop shoots with uniform physiological maturity were collected from the field grown tea bushes, labeled and brought to the laboratory. Fresh samples were used for enzyme assay.

C. *Enzymes and Biochemical Constituents*

Enzyme assays (detailed in the results) was replicated two times for each clone and for each sampling. The results were pooled together according to the sampling month and analyzed statistically adopting factorial design, where months considered as main treatments and clones as sub treatments (Gomez and Gomez, 1984).

D. *Productivity Related Enzymes*

Freshly harvested two leaves and bud was ground with a pre-chilled pestle and mortar while grinding the buffer (50 mM Tris HCl (pH 8.0), 50 mM MgCl2, 5 mM 2-mercaptoethanol and 1 mM EDTA) was added at 1.0 mL/g tissue. Homogenate was passed through four layered cheese cloth and the filtrate was centrifuged at 3000 rpm for 20 min at 5ºC. The supernatant was used as crude enzyme source. Malate dehydrogenase (MDH) was assayed following the method reported by Sadasivam and Manickam (1996) and computed (web mnstate edu/provost/MDH/Assayprotocol.pdf) the reaction velocity determined by monitoring the decrease in absorbance at every 30 sec for 3 minutes under room temperature. MDH is expressed as µmoles NADH oxidised /min. Phosphoenol pyruvate carboxylase (PEPC) The oxaloacetic acid formed by the action of PEPC is coupled with MDH and followed by NADH oxidation. Set the spectrometer to zero absorbance at 340 nm without adding NADH in the test against the blank in the reference cuvette. Add NADH as quickly as possible into the test, mix well and record the initial absorbance. Record the absorbance every 30 sec for 3 min. Ribulose biphosphate carboxylase (Rubisco) Extraction and enzyme assay was followed as described by Chantal et al.(1997). The NADH oxidation was initiated by adding 0.5 mM RUBP and the reaction was followed for 1 min. Rubisco activity was expressed as U/mg protein.

E. Yield and Yield Potential

Over and above the productivity related enzyme assay, yield monitoring was carried out between September 2008 and August 2010 corresponding to the biochemical estimation/enzyme assay period. Each clonal block consisted of 10 bushes and harvesting of crop shoots was done at periodical intervals and recorded. Green leaf harvested (kg/plot) was computed as yield kg made tea per ha on the basis of 22.5% out turn and 10,000 bushes/ha. Since the yield recorded in a non replicated manner, statistical analysis was not performed to ascertain the clonal variation with critical difference. Two years mean data was considered for correlation analysis. Yield potential was calculated using the formula, Yield potential = (yield of respective clone per unit area)/(mean field yield per unit area) as reported by Raj Kumar (2005). Relationship existed between productivity related enzymes and yield was established through regression analysis (SPSS Ver. 10.0). Canonical discriminate analysis was performed particularly with the values of Rubisco, malate dehydrigebase and yield (SPSS Ver. 10.0) and profiling of tea clones was executed.

III. RESULT AND DISCUSSION

A. Malate Dehydrogenase

Irrespective of the cultivar studied, sampling time significantly influenced the activity of malate dedhydrogenase (Table 1). During moisture stress period, its activity was significantly lower irrespective of the clones studied. Under favourable conditions, malate dehydrogenase activity was more pronounced, especially between June and December. However, June and December samplings were showed significant variation in malate dehydrogenase activity, irrespective of the clones studied. There was a significant variation exhibited by the clones in the native levels of malate dehydragenase, irrespective of the seasons. Malate dehydrogenase activity was ranged between 0.034 **(**UPASI-5**)** and 0.089 µ moles NADH oxidised min-1 0.2 ml enzyme-1 (UPASI-17). According to the class interval analysis, most of the UPASI clones grouped in to moderate group which possess 0.053 to 0.070 µ moles NADH oxidised min-1 0.2 ml enzyme-1**.** Only four clones (UPASI-9, UPASI-17, UPASI-18 and CR-6017) recorded relatively higher enzyme activity.

B. Phosphoenol Phyruvate Carboxylase

Both "Jat" specific cultivars and time of sampling (seasons) were significantly different in terms of phosphoenol phyruvate carboxylase (µ moles NADH oxidised min-1 0.05 ml enzyme-1) activity (Table 2). Unlike the malate dehydrogenase activity, phosphoenol phyruvate carboxylase activity was significantly higher during soil moisture stress period.

Irrespective of the jat specific cultivars, phosphoenol phyruvate carboxylase activity was significantly higher during March followed by September sampling.

Under drought conditions, phosphoenol phyruvate carboxylase activity was more pronounced and it was expressed less especially between June and December. However, June and December samplings were showed significant variation irrespective of the clones studied. Irrespective of the seasons, there was a significant variation exhibited by the clones in the native levels of phosphoenol phyruvate carboxylase activity.

Phosphoenol phyruvate carboxylase activity was ranged between 0.004 (in most of the clones) and 0.011 µmoles NADH oxidised min-1 0.05 ml enzyme-1 (UPASI-3). According to the class interval analysis, most of the UPASI clones grouped in to moderate group which possess 0.0063 µmoles NADH oxidised min-1 0.05 ml enzyme-1 of phosphoenol phyruvate carboxylase activity**.**

C. Rubilose Bisphosphate Carboxylase (RUBISCO)

Clonal and time of sampling (seasons) were significantly different in terms of RUBISCO (U mg/protein) activity (Table 3). RUBISCO activity was assayed during different seasons as it was documented in other productivity related enzymes.

Activity of RUBISCO varied among the UPASI clones significantly (Table 3) and it ranges from 0.058 (UPASI-11) to 0.122 (TRF-1). About two third UPASI clones registered lower values of RUBISCO activity wherein all three varieties were grouped together. However out of 19 clones grouped in to lower RUBISCO category, most of them found to be broad leaved "Assam/Cambod" cultivars.

Similar trend was observed with the moderate RUBISCO activity group; but most of the tea clones belongs to "China" category or "China" hybrids. TRF-1 which registered higher RUBISCO activity represents the "Cambod" cultivar. Among the seasons, the second crop season registered higher values of RUBISCO activity followed by June and December sampling. Both December and June sampling were found to be on par with each other. Significant fall in RUBISCO activity was noticed during soil moisture stress.

D. Productivity and Yield Potential of Tea Clones

When considering the national mean productivity (~2,000 kg made tea per hectare), in general, all the UPASI clones classified into moderate to good yielding category (Table 4). Class interval analysis revealed that most of the UPASI clones fall under category of moderate yielder (>3100 to 4800 kg/ha) and hardy in nature when compared to TRF-1. Certain clones, UPASI-2, UPASI-10, UPASI-12 and UPASI-8 known for their yield potential, their tolerance towards soil moisture stress and moderate RUBISCO activity categorically push them to moderate yielder. UPASI-3, UPASI-9, UPASI-17 are regarded as high yielding clones which registered relatively higher RUBISCO activity. Exceptionally, TRF-1 emerged as a high yielding clone with higher RUBISCO activity. Earlier, it has been anticipated that TRF-1 will record >10,000 kg of made tea per hectare. However, in the present study, under prevailing ecological conditions and agronomic practices TRF-1 could registered ~7,100 kg made tea per ha.

Yield potential is the ratio between the actual yields of the tea clone towards the mean yield level of the field. The values >1.00 is on par with the mean field yield level. Mean yield level of the field is not the same as the potential clones were released for adoption under south Indian tea plantations. Since the clones selected for the study is of different tea fields planted in different years, the mean yield level 3800 kg is considered for computation (Table 4). Furthermore, clones planted in the field were of various age from pruning cycle. According to the present computation and the class interval analysis, five different classes were segregated (Table 4). Yield potential of the tea clones varied between 0.83 (UPASI-16) to 1.79 (TRF-1). As high as 13 clones were registered very low yield potential of 0.83 to 1.05 while another nine clones recorded yield potential of 1.06 to 1.23. Only four clones registered moderate to high yield potential where its values ranges from 1.29 to 1.46. UPASI-9, CR 6017 and TRF-1 formed individual category with significant variation in yield potential among them and with other groups.

E. Relation Between Productivity Related Enzymes And Crop Yield Of Different Clones

Linear regression analysis revealed that there is a positive correlation existed between malate dehydrogenase activity and yield. Correlation coefficient value (r = 0.405) was not significant at five per cent probability (Table 4). Linear regression analysis revealed that there was a very week positive correlation existed between phosphoenol phyruvate carboxylase activity and yield. Correlation coefficient value (r = 0.398) was not significant at five per cent probability. Linear regression analysis revealed that there is a positive correlation existed between Rubisco activity and the yield fitting the regression formula, Y = a + bx (where Y is made tea yield in kg, x is the Rubisco activity, a (-127.31) & b (58303) are regression constants). The correlation coefficient value (r = 0.821) was highly significant at one per cent probability.

Certain tea clones which registered higher RUBISCO activities were not matched with the yield. However, among the three enzymes, role played by RUBISCO is predominant followed by malate dehydrogenase and PEPC. Yield to malate dehydrogenous activity, yield to rubisco activity showed significant correlation at five per cent probability against yield followed by yield to PEPC and yield. Thus the interrelationship among the productivity related enzymes on yield of the tea clones was established.

F. Principal Component Analysis

Principal component analysis of the data related to productivity related enzymes revealed that RUBISCO alone contributed 58.771% contribution towards yield while other two enzymes contributed another 35% towards the yield. All the three enzymes cumulatively contributed 92.9% towards yield. Since other enzymes involved in productivity are not documented in the present study, the results presented herein are not conclusive. However, the data presented in this study revealed the role of different roductivity related enzymes and their role in dry matter accumulation.

G. Canonical Discriminate Analysis Among three Different Components on Productivity

Canonical discriminate analysis with three different components particularly Rubisco, malate dehydroxigenase and yield segregated the tea clones in three different groups (Fig. 1). Since the relationship between PEPC and yield was very low, PEPC was not included in the analyses. Only two clones, UPASI-17 and UPASI-25 segregated in group I in accordance with canonical discriminate analysis. Remaining 28 UPASI clones and estate selections were segregated in other two groups. Thirteen tea clones were separated in group II (TRF-1, UPASI-18, UPASI-9, UPASI-5, TRF-2, UPASI-3, UPASI-12, UPASI-2, UPASI-10, UPASI-27, UPASI-7, UPASI-8 and CR-6017). On the other hand about 50% of the tea clones segregated in group III (UPASI-24, UPASI-14, UPASI-20, UPASI-13, UPASI-28, UPASI-16, UPASI-11, SA-6, UPASI-22, UPASI-1, UPASI-15, UPASI-4, UPASI-6, UPASI-26 and TRI-2043). Except UPASI-17, most of the productive tea clones segregated in Group II and most of them are drought hardy. Majority of Group III clones represents “China” hybrids however, group II cultivars belonging from all the three jats representing, “Assam”, “China” and “Cambod” cultivars. Canonical descriminate analysis not only represents the predominant functional

IV. DISCUSSION

In the present study, clonal and time of sampling (seasons) were significantly recorded variation in terms of productivity related enzymes and their participatory role in dry matter productivity. Rubisco played a prime role followed by MDH and PEPC. This was clearly established by the correlation studies where the Rubisco had higher correlation coefficient value followed by MDH and PEPC. Over and above, Rubisco is one of the 11 enzymes involved in the carboxylation cycle and gained importance because of its catalyzing action. Earlier Shalini and Raj Kumar (2011) established the linear relationship existed between the Rubisco activity and UPASI clones and certain estate selections. TRF-1 recorded significantly higher Rubisco activity in the present study. Contrarily, Jeyaramraja *et al*. (2002) reported lower Rubisco activity in TRF-1 and concluded that rather than the quantitative levels of carboxylase or oxygenase, its ratio determines the productivity.

Out of 11 enzymes involved in photosynthetic carbon assimilation cycle, Rubisco is a versatile enzyme which has been reported earlier in tea with particular reference to productivity (Jeyaramraja *et al*., 2002; Raj Kumar, 2005; Shalini and Raj Kumar, 2011). However, there was no report available on MDH and PEPC in tea and this is the first report on MDH and PEPC in UPASI clones and certain estate selections. Though the methodologies adopted to assay the MDH and PEPC are well established, further studies are required to authenticate the results. Relationship existed between yield and MDH/PEPC was established, in the present study. It may be noted that correlation coefficient value between Rubisco and yield was higher followed by MDH and PEPC which signifies the participatory role of these yield related enzymes in the hierarchical order. Still there is a huge lacuna in carbon cycle enzymes studies in tea and hence further research on these aspects are required which will through more light on carbon metabolism in tea and serve as a baseline data in future plant improvement programmes.

V. CONCLUSION

When considering the national mean productivity, all UPASI clones classified into moderate to good yielding category. Certain clones, UPASI-2, UPASI-10, UPASI-12 and UPASI-8 known for their yield potential, their tolerance towards soil moisture stress and moderate Rubisco activity categorically pushed them to moderate yielder. UPASI-3, UPASI-9, UPASI-17 are regarded as high yielding clones which registered relatively higher Rubisco activity. Exceptionally TRF-1 emerged as a high yielding clone with higher Rubisco activity. It may be noted that the excess sugars may be diverted towards defense and other biosynthetic pathways rather than structural growth and development which in turn class interval analysis designate them as moderate clones rather than high yielder's (Shalini and Raj Kumar, 2011).

Table 1.Clonal variation in malate dehydrogenase activity (µ moles NADH oxidised min^{-1} 0.2 ml $enzyme^{-1}$)

Clone	March	June	September	December	Mean (Clone)
UPASI-1	0.034	0.043	0.060	0.043	0.045
UPASI-2	0.045	0.058	0.057	0.040	0.050
UPASI-3	0.058	0.065	0.072	0.065	0.065
UPASI-4	0.030	0.037	0.050	0.037	0.039
UPASI-5	0.024	0.034	0.045	0.034	0.034
UPASI-6	0.030	0.039	0.035	0.039	0.036
UPASI-7	0.028	0.044	0.054	0.044	0.043
UPASI-8	0.065	0.069	0.066	0.069	0.067
UPASI-9	0.069	0.084	0.053	0.084	0.072
UPASI-10	0.047	0.050	0.066	0.050	0.053
UPASI-11	0.045	0.063	0.072	0.063	0.061
UPASI-12	0.043	0.069	0.069	0.069	0.062
UPASI-13	0.047	0.061	0.068	0.061	0.059
UPASI-14	0.041	0.054	0.060	0.054	0.052
UPASI-15	0.055	0.064	0.069	0.064	0.063
UPASI-16	0.039	0.063	0.068	0.063	0.058
UPASI-17	0.076	0.095	0.092	0.095	0.089
UPASI-18	0.069	0.075	0.079	0.075	0.074
UPASI-20	0.038	0.068	0.077	0.068	0.062
UPASI-22	0.036	0.054	0.065	0.054	0.052
UPASI-24	0.041	0.052	0.070	0.052	0.054
UPASI-25	0.056	0.063	0.078	0.075	0.068

UPASI-26	0.038	0.055	0.065	0.055	0.053
UPASI-27	0.043	0.062	0.070	0.062	0.059
UPASI-28	0.045	0.074	0.079	0.074	0.068
TRI-2043	0.044	0.070	0.067	0.059	0.060
TRF-1	0.040	0.053	0.065	0.053	0.053
TRF-2	0.049	0.060	0.064	0.060	0.059
SA-6	0.036	0.054	0.061	0.054	0.051
CR-6017	0.048	0.085	0.073	0.085	0.073
Seasonal mean	0.046	0.062	0.067	0.062	

Statistical significance at P = 0.05:	S.E.	C.D.	C.V. (%)
MT (Clone)	0.002	0.003	6.69
ST (Season)	0.001	0.001	
MT x ST	0.003	0.007	

*S.E.: standard error; C.D.:critical difference; C.V.(%): co-efficient of variance

Table 2. Clonal and seasonal variation in activity of phosphoenol phyruvate carboxylase (µ moles NADH oxidised min^{-1} 0.05 ml $enzyme^{-1}$)

Clone	March	June	September	December	Mean (Clone)
UPASI-1	0.005	0.005	0.005	0.005	0.005
UPASI-2	0.006	0.006	0.006	0.006	0.006
UPASI-3	0.012	0.010	0.011	0.011	0.011
UPASI-4	0.005	0.005	0.005	0.005	0.005
UPASI-5	0.004	0.004	0.004	0.004	0.004
UPASI-6	0.005	0.005	0.005	0.005	0.005
UPASI-7	0.004	0.004	0.004	0.004	0.004
UPASI-8	0.007	0.007	0.007	0.007	0.007
UPASI-9	0.010	0.008	0.009	0.009	0.009
UPASI-10	0.004	0.004	0.004	0.004	0.004
UPASI-11	0.004	0.004	0.004	0.004	0.004
UPASI-12	0.005	0.005	0.005	0.005	0.005
UPASI-13	0.005	0.005	0.005	0.005	0.005
UPASI-14	0.005	0.005	0.005	0.005	0.005
UPASI-15	0.005	0.005	0.005	0.005	0.005
UPASI-16	0.006	0.006	0.006	0.006	0.006
UPASI-17	0.010	0.008	0.009	0.009	0.009
UPASI-18	0.007	0.007	0.007	0.007	0.007
UPASI-20	0.005	0.005	0.005	0.005	0.005
UPASI-22	0.004	0.004	0.004	0.004	0.004
UPASI-24	0.005	0.005	0.005	0.005	0.005
UPASI-25	0.010	0.008	0.009	0.009	0.009
UPASI-26	0.005	0.005	0.005	0.005	0.005
UPASI-27	0.005	0.005	0.005	0.005	0.005
UPASI-28	0.005	0.005	0.005	0.005	0.005
TRI-2043	0.009	0.007	0.008	0.008	0.008
TRF-1	0.005	0.005	0.005	0.005	0.005
TRF-2	0.004	0.004	0.004	0.004	0.004
SA-6	0.004	0.004	0.004	0.004	0.004
CR-6017	0.007	0.007	0.007	0.007	0.007
Seasonal mean	0.006	0.005	0.006	0.006	

Statistical significance at P = 0.05:	S.E.	C.D.	C.V. (%)
MT (Clone)	0.0001	0.0004	7.64
ST (Season)	0.0001	0.0002	
MT x ST	0.0004	0.0008	

Table 3. Clonal and seasonal variation in RUBISCO activity (U mg/protein)

Clone	March	June	September	December	Mean (Clone)
UPASI-1	0.051	0.064	0.089	0.064	0.067
UPASI-2	0.070	0.090	0.089	0.062	0.078
UPASI-3	0.071	0.080	0.089	0.080	0.080
UPASI-4	0.045	0.056	0.075	0.056	0.058
UPASI-5	0.048	0.068	0.089	0.068	0.068
UPASI-6	0.051	0.067	0.060	0.067	0.061
UPASI-7	0.048	0.076	0.093	0.076	0.073
UPASI-8	0.074	0.079	0.076	0.079	0.077
UPASI-9	0.084	0.102	0.064	0.102	0.088
UPASI-10	0.064	0.069	0.090	0.069	0.073
UPASI-11	0.043	0.060	0.069	0.060	0.058
UPASI-12	0.054	0.087	0.087	0.087	0.079
UPASI-13	0.056	0.072	0.080	0.072	0.07
UPASI-14	0.056	0.073	0.082	0.073	0.071
UPASI-15	0.075	0.087	0.094	0.087	0.086
UPASI-16	0.042	0.067	0.072	0.067	0.062
UPASI-17	0.079	0.099	0.096	0.099	0.093
UPASI-18	0.091	0.099	0.104	0.099	0.098
UPASI-20	0.042	0.076	0.086	0.076	0.070
UPASI-22	0.042	0.063	0.076	0.063	0.061
UPASI-24	0.058	0.074	0.099	0.074	0.076
UPASI-25	0.063	0.071	0.088	0.085	0.077
UPASI-26	0.044	0.063	0.074	0.063	0.061
UPASI-27	0.056	0.081	0.091	0.081	0.077
UPASI-28	0.042	0.069	0.073	0.069	0.063
TRI-2043	0.051	0.082	0.078	0.069	0.070
TRF-1	0.093	0.123	0.150	0.123	0.122
TRF- 2	0.073	0.090	0.096	0.090	0.087
SA-6	0.044	0.066	0.075	0.066	0.063
CR – 6017	0.057	0.100	0.086	0.100	0.086
Mean (Season)	0.059	0.070	0.861	0.077	

Statistical significance at P = 0.05:	S.E.	C.D.	C.V %
MT (Season)	0.03	0.06	7.98
ST (Clone)	0.06	0.12	
MT x ST	0.11	0.22	

Table 4. Differential yield level of tea clones and their productivity index

Clone	Yield, kg ha^{-1}	Yield potential
UPASI-1	3803	1.00
UPASI-2	4439	1.17
UPASI-3	5067	1.33
UPASI-4	4066	1.07
UPASI-5	3557	0.94
UPASI-6	3301	0.87
UPASI-7	3876	1.02
UPASI-8	4893	1.29
UPASI-9	5666	1.49
UPASI-10	4675	1.23
UPASI-11	3248	0.85
UPASI-12	4670	1.23
UPASI-13	3167	0.83
UPASI-14	3787	1.00
UPASI-15	4321	1.14
UPASI-16	3160	0.83
UPASI-17	5119	1.35
UPASI-18	5451	1.43
UPASI-20	3353	0.88
UPASI-22	3864	1.02
UPASI-24	3617	0.95
UPASI-25	3507	0.92
UPASI-26	4123	1.09
UPASI-27	4398	1.16
UPASI-28	3649	0.96
TRI-2043	4346	1.14
TRF-1	7154	1.88
TRF- 2	3989	1.05
SA-6	3973	1.05
CR - 6017	6103	1.61

Yield kg/ha derived at an out turn of 22.5% with a mean population of 9000 plants/ha; yield potential is nothing but the ratio between actural yield calculated for the particular clone and mean yield of the field. Since the clones are grown at different field conditions the yield levels were not subjected to statistical analysis

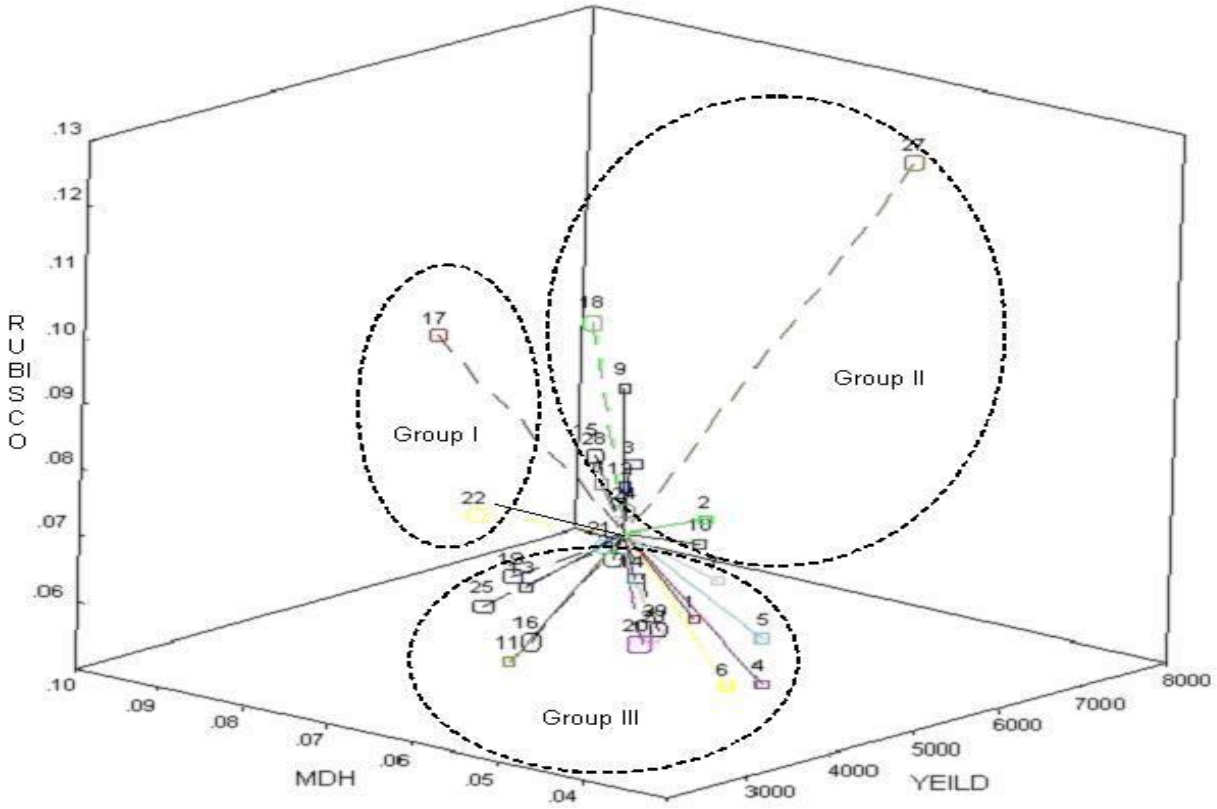

Fig1. Canonical discriminate analysis among three different components on productivity

1: UPASI-1, 2: UPASI -2, 3: UPASI -3, 4: UPASI -4, 5: UPASI-5, 6: UPASI -6, 7: UPASI-7, 8: UPASI -8, 9: UPASI -9, 10: UPASI-10, 11: UPASI-11, 12: UPASI-12, 13: UPASI-13, 14: UPASI-14, 15: UPASI-15, 16: UPASI-16, 17: UPASI-17, 18: UPASI-18, 19: UPASI-20, 20: UPASI-22, 21: UPASI-24, 22: UPASI-25, 23: UPASI-26, 24: UPASI-27, 25: UPASI-28, 26: TRI 2043, 27: TRF-1, 28: TRF-2, 29: SA-6 and 30: CR-6017

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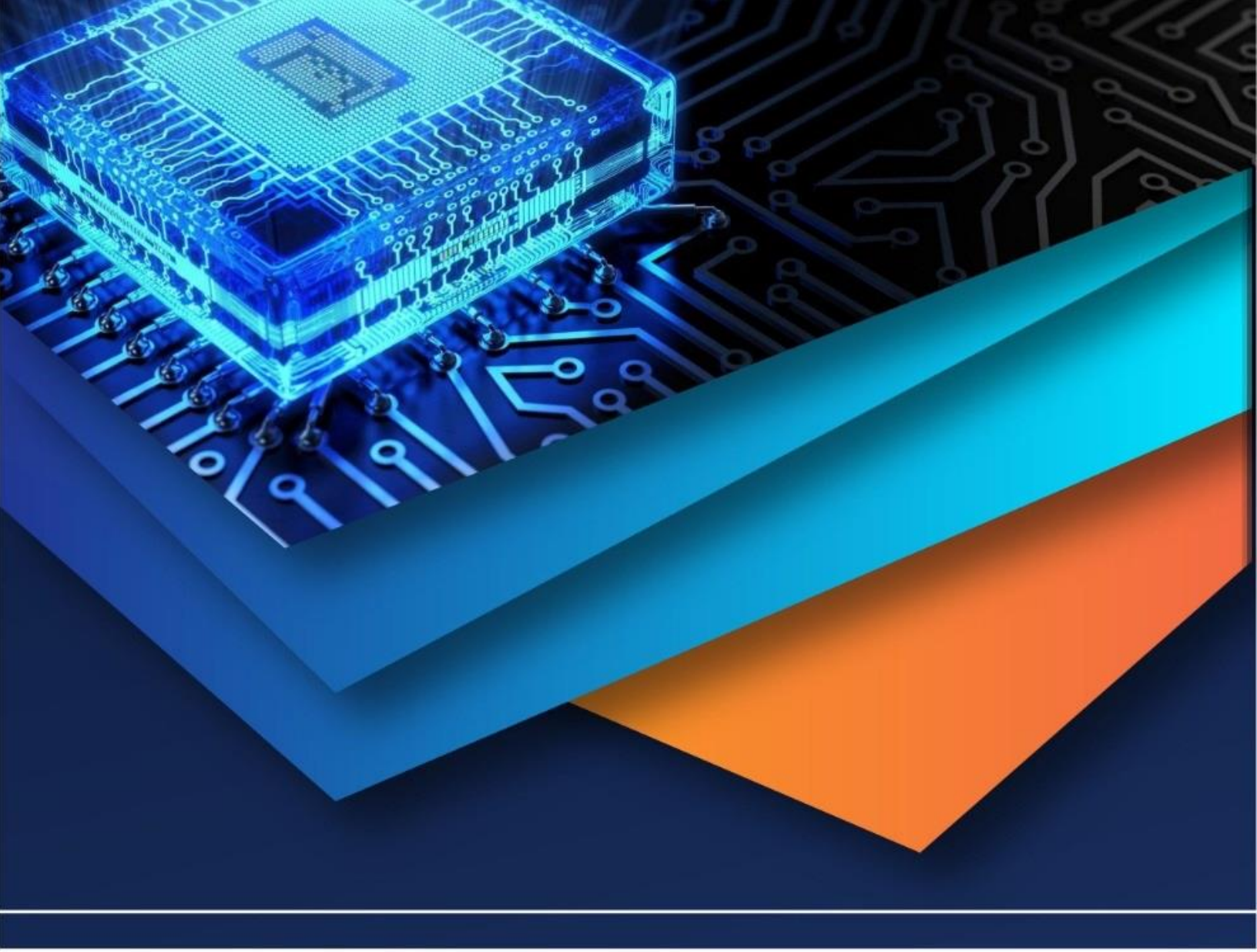

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