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Influence of Externally Added Substrates on Total Catechin Content in Tea Leaves (*Camellia* sp.)

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Abstract: Tea shoots treated with different substrates *viz*. tyrosine, gallic acid, glutamic acid, phenylalanine and shikimic acid at different concentrations was studied. Tea shoots were incubated under *in vitro* conditions at varying time periods to evaluate their efficacy in biosynthesis of catechins. Among the substrates, shikimic acid followed by phenylalanine synthesised 20.34 and 19.59% of catechins, respectively. Among the clonses used, UPASI-17 recorded higher values of catechins. Irrespective of the substrates, 20 mM concentration was found to be optimum level for catechin production. Incubation period of 5 h produced maximum amount of catechins, irrespective of clone, substrate type and concentration there of.

Key words: Catechins, substrate, incubation time, substrate concentration, clonal variety

INTRODUCTION

Tea (*Camellia* sp.) is an important plantation crop, cultivated in more than three million hectares in the world have many health benefits. Owing to the abundant occurrence of flavonoids, proteins and lipids, as well as hydrolytic and oxidative enzymes, tea plays an important role in the generation of many therapeutic attributes. Recently, catechin has received much attention because of its antioxidative, antibacterial and antiallergetic activities[1-3]. In catechin biosynthesis, different pathways are involved and erythrose-4-phosphate and phosphoenolpyruvate are the two components, which plays an important role in catechin biosynthesis besides other intermediates.

From erythrose-4-phosphate and phos phoenolpyruvate, quinic acid and shikimic acid are formed. From quinic acid chlorogenic acid was formed. Three pathways have been proposed for the biosynthesis of gallic acid. a) β-oxidation of the side chain of 3,4,5-trihydroxy cinnamic acid, b) dehydrogenation of shikimic acid, probably with 3-dehydro shikimic acid as an intermediate and c) hydroxylation of protocatechuic acid[4,5] in to shikimic acid. From gallic acid, epicatechin, epicatechin gallate and epigallo catechin gallate were formed. Shikimic acid leads to the formation of phenyl pyruvate. Phenylalanine, trans-cinnamic acid, coumaric acid and Phenylalanine Ammonia Lyase (PAL), chalcone synthase are the few enzymes involved in this pathway to form catechins and epicatechins. On the other hand, from prephenic acid the substrates like phenyl pyruvate, tyrosine, DOPA are involved to form catechins and epicatechins by the enzyme hydroxylases. Available literature revealed that shikimic acid pathway forms an important one towards the biosynthesis of catechins and its fractions in plant tissues. Involvement of other substrates like gallic acid, tyrosine and phenylalanine are also elucidated in catechin biosynthesis pathway[6,7]. However, only a few attempts were made on the influence of externally added substrates on catechin production in tea. In this context, exogenous addition of substrates at varying concentration and duration were studied with particular reference to the catechin biosynthesis under laboratory conditions.

MATERIALS AND METHODS

Tea shoots comprising of apical bud and two leaves harvested at 11.00 am from tea clones, UPASI-3, UPASI-9, and UPASI-17, representing Assam, China and Cambod cultivars, respectively, were used for this study. This study was conducted from the month of February to May 2003 at UPASI Tea Research Institute. Tea shoots were cleaned with running tap water and the moisture was removed with Whatman No. 1 filter paper. Three shoots of uniform physiological maturity were transferred to glass vials each containing two ml of different concentrations (1, 2, 5, 10, 20, 30 and 40 mM) of the substrates viz., gallic acid, glutamic acid, phenylalanine, tyrosine and shikimic acid. The pH of the substrates was adjusted to 7.0 and thereafter the vials were placed in a desiccator. Vacuum was applied for about five minutes so that the air trapped at the interface of the shoots and the liquid would be sucked away. By this method the entry of substrates into

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the leaves have been ensured[8]. Tea shoots were illuminated by a fluorescent light source and each vial was incubated for 2,5,10,15 and 20 h. After incubation, the shoots were washed thoroughly with distilled water and they were incubated for 24 h in the distilled water. Analysis of total catechin was carried out with the treated shoots, apart form the untreated control, by using the modified procedure of Swain and Hillis[9].

RESULTS AND DISCUSSION

Performance of all substrates was optimum in the production of catechin at the specific molar concentration of 20 mM. In all the substrates tried there was a steady increase in the synthesis of catechin up to 20 mM concentration and thereafter declined very sharply (Table 1- 3). Among the substrates tried, shikimic acid at 20 mM concentration was found to have synergistic activity in production of catechins where they recorded 17.12 -20.34%, irrespective

Table 1: Total catechin content in UPASI-3 shoots treated with different substrates

Treatments	2 h	5 h	10 h	15 h	20 h
Tyrosine (mM)					
Control	14.45	14.72	14.56	14.59	14.66
1	14.32	14.44	14.39	14.29	14.23
2	14.67	14.97	14.84	14.53	14.28
5	15.00	15.43	15.21	14.93	14.83
10	15.04	15.56	15.41	15.14	14.90
20	15.51	15.77	15.68	15.31	14.94
30	14.58	14.66	14.77	14.64	14.57
40	14.41	14.54	14.47	14.47	14.42
Gallic acid (mM)					
1	14.77	14.89	14.84	14.74	14.68
2	15.12	15.42	15.29	14.98	14.73
5	15.45	15.88	15.66	15.38	15.28
10	15.49	16.01	15.86	15.59	15.35
20	15.96	16.22	16.13	15.76	15.39
30	15.03	15.11	15.22	15.09	15.02
40	14.86	14.99	14.92	14.92	14.87
Glutamic acid (mM)					
1	14.81	15.20	15.14	14.80	14.62
2	15.22	15.82	15.52	14.99	14.78
5	15.58	16.11	15.74	15.38	14.91
10	15.62	16.43	15.98	15.44	15.13
20	16.02	16.68	16.30	15.89	15.59
30	15.54	16.49	16.16	15.32	15.22
40	15.13	16.21	15.78	14.86	14.61
Phenylalanine (mM)					
1	14.84	15.30	15.21	14.87	14.71
2	15.37	15.92	15.63	15.14	14.89
5	15.63	16.25	15.81	15.27	15.17
10	15.85	16.69	16.08	15.48	15.28
20	16.24	16.81	16.66	15.95	15.67
30	15.71	16.68	16.28	15.47	15.38
40	15.27	16.46	16.16	15.08	14.95
Shikimic acid (mM)					
1	15.04	15.90	15.68	14.96	14.55
2	15.48	16.19	15.99	15.39	14.63
5	15.82	16.40	16.12	15.71	14.84
10	16.08	16.62	16.41	15.78	15.39
20	16.42	17.12	16.73	16.15	15.86
30	15.82	16.27	16.39	15.74	15.45
40	15.47	16.15	15.56	15.32	15.06

Table 2: Total catechin content in UPASI-9 shoots treated with different substrates

Treatments	2 h	5 h	10 h	15 h	20 h
Tyrosine (mM)					
Control	15.19	15.22	15.31	15.13	15.09
1	14.77	15.21	15.00	14.74	14.66
2	14.90	15.27	15.11	15.07	14.68
5	15.22	15.46	15.26	15.12	14.92
10	15.36	15.68	15.52	15.32	15.13
20	15.58	15.99	15.68	15.39	15.16
30	15.00	15.73	15.41	14.87	14.79
40	14.83	15.29	15.14	14.69	14.67
Gallic acid (mM)					
1	15.22	15.66	15.45	15.19	15.11
2	15.35	15.72	15.56	15.52	15.13
5	15.67	15.91	15.71	15.57	15.37
10	15.81	16.13	15.97	15.77	15.58
20	16.03	16.44	16.13	15.84	15.61
30	15.45	16.18	15.86	15.32	15.24
40	15.28	15.74	15.59	15.14	15.12
Glutamic acid (mM)					
1	15.36	15.44	15.38	15.18	15.13
2	15.48	15.69	15.55	15.26	15.18
5	15.61	15.89	15.71	15.49	15.37
10	15.96	16.29	16.05	15.77	15.49
20	16.26	16.43	16.33	15.97	15.63
30	16.12	16.21	16.18	15.85	15.48
40	15.64	15.99	15.74	15.54	15.33
Phenylalanine (mM)					
1	15.51	15.77	15.64	15.44	15.34
2	15.65	15.99	15.82	15.57	15.48
5	15.87	16.27	16.09	15.62	15.52
10	16.11	16.45	16.33	15.82	15.63
20	16.37	16.81	16.62	16.10	15.86
30	16.18	16.46	16.38	15.87	15.79
40	15.85	16.29	16.10	15.69	15.58
Shikimic acid (mM)					
1	15.75	16.00	15.88	15.58	15.44
2	15.86	16.31	16.05	15.76	15.68
5	16.16	16.68	16.35	15.93	15.79
10	16.52	16.96	16.69	16.29	15.85
20	16.78	17.54	16.87	16.42	16.15
30	16.59	16.94	16.66	16.26	15.81
40	16.23	16.54	16.33	15.92	15.61

of the tea clones (Table 4-6) and followed by phenylalanine (16.81-18.59%). Lower values of catechin content were recorded by tyrosine even at the optimum concentration used (15.77-18.92). Shikimic acid is the primary substrate involved in the earlier stages of catechin biosynthesis pathway and leads to the formation of gallic acid. Shikimic acid pathway is a common pathway which lend the production of numerous secondary metabolites in higher plants. Stimulation of the synthesis of aromatic amino acids through the shikimate pathway at the time of shoot formation is in keeping with the increased activity of the glycolytic and phosphoenol pyruvate pathway in the tissue[10].

Among the three clones, UPASI-17 registered the highest value of 20.34% for catechin content in all the times of sampling and incubation periods including the control. UPASI-3 registered the least value of 17.12% irrespective of the substrates and their concentrations

Table 3: Total catechin content in UPASI-17 shoots treated with different substrates

Treatments	2 h	5 h	10 h	15 h	20 h
Tyrosine (mM)					
Control	17.20	17.00	17.19	17.16	17.14
1	16.99	17.47	17.21	16.94	16.81
2	17.42	17.79	17.47	17.22	16.95
5	17.67	18.17	17.84	17.50	17.20
10	17.98	18.38	18.14	17.72	17.36
20	18.17	18.92	18.28	18.00	17.66
30	17.90	18.43	18.04	17.84	17.41
40	17.66	18.12	17.76	17.36	17.15
Gallic acid (mM)					
1	17.44	17.92	17.66	17.39	17.26
2	17.87	18.24	17.92	17.67	17.40
5	18.12	18.62	18.29	17.95	17.65
10	18.43	18.83	18.59	18.17	17.81
20	18.62	19.37	18.73	18.45	18.11
30	18.35	18.88	18.49	18.29	17.86
40	18.11	18.57	18.21	17.81	17.60
Glutamic acid (mM)					
1	17.51	17.74	17.63	17.46	17.25
2	17.92	18.11	18.01	17.77	17.51
5	18.21	18.46	18.32	17.91	17.72
10	18.56	18.73	18.64	18.12	17.92
20	18.71	19.00	18.86	18.39	18.26
30	18.49	18.69	18.52	18.20	18.10
40	18.24	18.43	18.38	17.94	17.80
Phenylalanine (mM)					
1	17.64	18.33	18.22	18.05	17.84
2	18.50	18.70	18.60	18.36	18.10
5	18.79	19.05	18.91	18.50	18.31
10	19.14	19.32	19.23	18.71	18.51
20	19.29	19.59	19.45	18.98	18.85
30	19.07	19.28	19.11	18.79	18.69
40	18.82	19.02	18.97	18.53	18.39
Shikimic acid (mM)					
1	18.39	19.08	18.97	18.80	18.59
2	19.25	19.45	19.35	19.11	18.85
5	19.54	19.80	19.66	19.25	19.06
10	19.89	20.07	19.98	19.46	19.26
20	20.04	20.34	20.20	19.73	19.60
30	19.82	20.03	19.86	19.54	19.44
40	19.57	19.77	19.72	19.28	19.14

Table 4: UPASI-3 shoots treated with shikimic acid

Treatments	2 h	5 h	10 h	15 h	20 h	Mean (st)
Control (mM)	14.45	14.55	14.56	14.59	14.66	14.56
1	15.06	15.95	15.64	14.99	14.58	15.25
2	15.47	16.21	16.04	15.42	14.65	15.56
5	15.86	16.41	16.15	15.73	14.91	15.81
10	16.11	16.67	16.45	15.81	15.42	16.09
20	16.49	17.16	16.78	16.19	15.90	16.50
30	15.90	16.69	16.42	15.78	15.48	16.06
40	15.48	16.48	15.59	15.37	15.08	15.60
Mean (Mt)	15.60	16.27	15.96	15.49	15.08	

Table 5: UPASI-9 shoots treated with shikimic acid

Treatments	2 h	5 h	10 h	15 h	20 h	Mean (st)
Control (mM)	15.19	15.22	15.31	15.13	15.09	15.19
1	15.73	16.03	15.88	15.52	15.38	15.71
2	15.87	16.31	16.06	15.70	15.62	15.91
5	16.16	16.67	16.36	15.87	15.73	16.16
10	16.54	16.89	16.71	16.23	15.79	16.43
20	16.76	17.51	16.86	16.36	16.09	16.72
30	16.60	16.96	16.66	16.20	15.75	16.43
40	16.22	16.56	16.38	15.86	15.62	16.13
Mean (Mt)	16.14	16.52	16.28	15.86	15.63	

Table 6: UPASI-17 shoots treated with shikimic acid

Treatments	2 h	5 h	10 h	15 h	20 h	Mean (st)
Control (mM)	17.01	16.94	17.13	17.10	17.08	17.05
1	18.33	19.02	18.91	18.74	18.53	18.71
2	19.19	19.39	19.29	19.05	18.79	19.14
5	19.48	19.74	19.60	19.19	19.00	19.40
10	19.83	20.01	19.92	19.40	19.20	19.67
20	19.98	20.28	20.14	19.67	19.54	19.92
30	19.76	19.97	19.80	19.48	19.38	19.68
40	19.51	19.71	19.66	19.22	19.08	19.44
Mean (Mt)	19.14	19.38	19.31	18.98	18.83	

Table 7: Clonal variation in substrate treated shoots

Clones	2 h	5 h	10 h	15 h	20 h	Mean (st)
UPASI-3	15.33	15.86	15.63	15.21	14.97	15.40
UPASI-9	15.71	16.08	15.88	15.54	15.36	15.71
UPASI-17	18.41	18.76	18.56	18.22	18.00	18.39
Mean(Mt)	16.48	16.90`	16.69	16.32	16.11	16.50
CD	0.05	0.03	1.33	1.29	0.77	
CV	0.13	0.07	3.53	3.49	2.12	

(Table 7). It could be interpreted from the results that an incubation period for 5 h was the optimum time required for production of higher amount of total catechins in all the clones. It was seen that an extended duration of incubation had a negative impact on catechin synthesis. Data generated is in line with the report published by Grisebach[11].

Externally added substrates oozed out from the crop shoots especially from gallic acid treated shoots. But for other substrates there was no leaching from the crop shoots. Oozed out solution contained catechins in ppm levels (data not presented). The quantity of catechin oozed out was not directly proportional to the duration of incubation under light. Gallic acid is presumably esterified with epicatechin and epigallo catechin to form the catechin gallates in young tea shoots. Since shikimic acid was the substrate that accounted for the maximum enhancement in synthesis of total catechins in all the clones tried, it may be concluded that it is the primary pathway for catechin synthesis in tea[12-14].

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