Physiological Aspects of Catechin Biosynthesis in Tea Plants

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Catechins are a member of flavonoid compounds which have a C_6-C_3-C_6 skeleton together with anthocyanidins and flavones. Of the chemical components of tea leaves, catechins are contained most abundantly, and they exhibit astringency of tea[1]. Catechin content of tea leaves varys with strains and leaf age, and is influenced by environmental factors such as light and temperature. An enzyme, phenylalanine ammonia-lyase (PAL), which is closely involved in the biosynthesis of flavonoid, is also known with other plants to be influenced by various internal and external factors[2].

Pathway of catechin biosynthesis has been considered to be similar in many aspects to that of flavonoid compounds. However, Zaprometov and Bukhlaeva[3] indicated that shikimic acid-^{14}C was more efficiently incorporated into the tea leaf catechins than phenylalanine-^{14}C. This result is not consistent with the present knowledge on flavonoid biosynthesis, suggesting a pathway not including phenylalanine.

Therefore, in order to re-examine the result of Zaprometov and Bukhlaeva, comparison of shikimic acid and phenylalanine was carried out by using tea leaves of different clones and age. On the other hand, relation between the activity of PAL and catechin content or catechin synthesis in tea leaves was examined. Based on these research results, the role of phenylalanine in the biosynthesis of catechin in tea leaves was discussed.

PAL activity and catechin content in tea leaves

Young shoots were divided into different portions, as shown in Table 1, and PAL activity and catechin content were determined[4]. Younger leaves at the top of the shoots showed higher PAL activity per fresh weight, but with stems the lower part tended to show higher activity. PAL activity per each portion of a shoot showed an increase from the bud to the third leaf, and then a decrease with aged leaves. The portion with high activity contained much catechins, showing a proportional relationship between PAL activity per fresh weight and catechin content with the second leaf through to the fifth leaf. Relationship between enzymes related to shikimic acid pathway, i.e., 5-dehydroquinate hydro-lyase and shikimate: NADP oxidoreductase, and leaf age was also examined, and the trend was similar to that of PAL.

Catechin biosynthesis in leaves having different PAL activities

When plucked shoots were kept standing in a vessel containing water, PAL activity in the shoots decreased rapidly, while the activity in old leaves began to increase at 5–8 hr after plucking and continued to increase for more than 30 hr. By use of the phenomena[4], tea leaves with different PAL activities can be obtained. Young shoots and old leaves taken from the clone, Hatsumomiji, were used for the material immediately after plucking and on the next day of plucking. Shikimic acid-^{14}C

Table 1. Distribution of PAL activity and catechin content in tea shoots*

Part	Fresh wt (mg)	Enzyme activity** (units/g fresh wt.)	Enzyme activity** (units/part)	Catechin content*** (%)
Bud	37	9.21	0.34	20.4
First leaf	61	9.48	0.58	26.1
Second leaf	130	5.39	0.70	25.5
Third leaf	271	3.89	1.05	23.0
Fourth leaf	368	1.76	0.65	20.5
Fifth leaf	314	1.06	0.33	18.5
First and second internodes	83	1.42	0.12	16.5
Third internode	120	1.52	0.18	12.0
Fourth internode	153	2.50	0.38	9.2

* The clone used was Benifuji harvested in August.
** A unit of activity is defined as the amount of enzyme catalyzing the formation of 1 μ mole of *trans*-cinnamic acid per hour at 40°C (pH 8.8).
*** Dry weight basis.

Table 2. Incorporation of shikimate-^{14}C or phenylalanine-^{14}C into catechins in the leaves having different activities of PAL

Material	Young shoots				Old leaves			
Time on storage (hr)	17.5		1		1.5		25	
Enzyme activity*	2.3		15.5		36.9		79.3	
Compound fed**	SA	PA	SA	PA	SA	PA	SA	PA
Specific activity (dpm/μ mole)								
(−) Epicatechin	285	105	1,480	924	4,720	2,390	9,000	5,120
(−) Epigallocatechin	145	39	908	126	112	142	449	354
Gallic acid	515	9	922	15	38	6	65	11
Incorporation (%)***								
(−) Epicatechin	0.67	0.19	3.1	1.7	9.3	5.3	22.6	10.5
(−) Epigallocatechin	1.5	0.32	8.9	1.0	0.92	1.2	3.6	2.4
Gallic acid	3.5	0.05	5.9	0.08	0.17	0.02	0.27	0.04

* The activity was expressed as unit in 24 hr per g fresh weight of the material.
** SA and PA denote shikimate-^{14}C and phenylalanine-^{14}C, respectively.
*** Incorporation = 100 (activity in the product)/activity fed.

or phenylalanine-^{14}C was administered to the material[9]. Then the material was allowed to metabolize for 24 hr in a growth cabinet of 25°C. Catechins were extracted, and catechin gallates were hydrolysed by the action of tannase. With paperchromatography, (−)-epicatechin, (−)-epigallocatechin and gallic acid were isolated.

As shown in Table 2, incorporation of shikimic acid-^{14}C into the plant material was always higher than that of phenylalanine: the former was 4.4 times of the latter with shoots, and 1.8 times with old leaves. This result indicates that shikimic acid is more effective than phenylalanine as a catechin precursor. This is in accord with the result of Zaprometov and Bukhlaeva[3].

Incorporation of these precursors into (−)-epicatechin showed an increase in proportion to PAL activities, but such proportional relationship was not observed with (−)-epigallocatechin. Particularly with shikimic acid, shoots at 1 hr after plucking showed the highest incorporation whereas the incorporation was 1/10 and 2/5 with old leaves which have high PAL activity. Specific activity was always higher with (−)-epicatechin than with (−)-epigallocatechin, and ratio of specific activity [(−)-epicatechin/(−)-epigallocatechin] was 3.4 in shoots and 23.4 in old leaves in an average, suggesting that the hydroxylation of (−)-epicatechin to (−)-epigallocatechin became less active in old leaves. It is also apparent from Table 2 that gallic acid, a component of two kinds of catechin gallates, is mainly synthesized from shikimic acid. Thus, the pathway of catechin synthesis from phenylalanine is not considered important, although Zaprometov and Bukhlaeva[3] claimed its significance.

Clonal variation of PAL activity and catechin biosynthesis

PAL activity and catechin content of shoots of 9 clones are shown in Table 3[4]. It is well known that black tea clones contain more catechins than green tea clones. In this experiment also catechin content and PAL activity of black tea clones were 1.3 times and 1.6 times those of green tea clones, respectively. The correlation was again observed between catechin content and PAL activity.

Incorporation of shikimic acid and phenylalanine into catechins was determined with shoots of 5 clones (Table 4). Extent of the incorporation varied considerably with clones, because growth stage of shoots was different with clones. Therefore, ratio of shikimic acid incorporation to phenylalanine incorporation (SA/PA) was used for comparison. The result showed that, except the clone Yabukita, SA/PA was always higher than 1, indicating that shikimic acid is a more effective precausor of catechins. The ratio of SA/PA was almost proportional to catechin content.

From these results, it can be considered that in the clone Yabukita the main pathway of catechin biosynthesis is shikimic acid→phenylalanine→catechins, whereas another pathway not including phenylalanine may possibly exist in other clones rich in catechins.

Table 3. Clonal variation of PAL activity and catechin content in young shoots

Clone*	Enzyme activity (units/g fresh wt.)	Catechin content (%)
Yabukita (G)	3.33	14.3
Tamamidori (G)	3.53	15.8
Yutakamidori (G)	4.08	15.2
Asatsuyu (G)	4.96	16.2
Z-1 (G)	5.22	18.4
Benifuji (B)	5.15	23.8
Benitachiwase (B)	5.79	19.4
Hatsumomiji (B)	5.90	21.8
Benihomare (B)	9.91	21.0

* G and B denote a clone for green tea and a clone for black tea, respectively.

Table 4. Clonal variation of ^{14}C incorporation from shikimate-^{14}C or phenylalanine-^{14}C into catechins in young shoots

Clone	Catechin content (μ moles/g fresh wt.)	Incorporation (%)					
		Simple catechins			Total catechins		
		SA fed	PA fed	SA/PA*	SA fed	PA fed	SA/PA*
Yabukita	54	2.7	4.1	0.7	5.5	6.4	0.9
Tamamidori	76	1.8	1.0	1.8	4.8	1.8	2.7
Benifuji	89	3.8	2.3	1.7	10.3	4.7	2.2
Sayamamidori	95	2.3	1.2	1.9	8.4	2.6	3.2
Hatsumomiji	111	4.3	1.5	2.9	11.4	2.0	5.7

* SA/PA is the ratio of ^{14}C incorporation from shikimate to that from phenylalanine.

Table 5. Effect of shading treatment applied for 8 days on catechin content in young shoots of a clone Yabukita in July

Catechins	Amount of catechins (mg/shoot)		Concentration of catechins (dry wt. %)	
	unshaded	shaded	unshaded	shaded
(−) Epicatechin	0.82	0.48	0.9	0.7
(−) Epigallocatechin	3.82	2.00	4.2	2.9
(−) Epicatechin gallate	1.46	1.10	1.6	1.6
(−) Epigallocatechin gallate	6.37	5.04	7.0	7.3

Effect of light on PAL activity and catechin biosynthesis

To produce high quality tea, shading culture is widely practiced in tea gardens of Japan. Shading treatment reduces total catechin content of tea leaves, but quantitative changes of each catechin had not been well known.

The shading treatment was applied to the clone Yabukita by covering the plants with black cheesecloth for 8 days (with approximately 95% of light cut off)[5]. As shown in Table 5, (−)-epigallocatechin was reduced by 48%, followed by (−)-epicatechin (by 41%). Two gallates were reduced by 20–25%. Therefore the ratio of gallates to simple catechins [(−)-epicatechin and (−)-epigallocatechin] was increased in shaded leaves. Under the shading, growth of shoots was retarded: weight of shoots decreased to 3/4 of that of normal shoots. Therefore, decrease of simple catechins in terms of concentration (dry weight %) was only 20–30%, and concentration of gallates became slightly higher than that of the unshaded control. These results show apparently that light promotes catechin synthesis. Furthermore it is apparent that effect of light on the esterification of catechins with gallic acid is small.

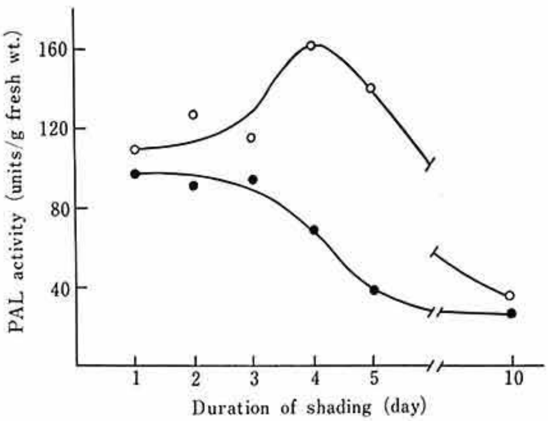

Fig. 1. Effect of light on PAL activity in young shoots of the clone Z-1.
•——• shaded plants, ∘——∘ unshaded plants.

Effect of shading treatment on PAL activity was examined with the clone, Z-1[4]. Changes of the activity in shaded and unshaded shoots are shown in Fig. 1. Apparently PAL activity was lowered by the shading.

Role of phenylalanine in catechin biosynthesis in tea plants

As described above, PAL activity was concentrated to the vigorously growing portion of tea plants such as buds and young leaves at the top of shoots, and the activity was almost proportional to catechin content.

Furthermore the activity was higher with clones with higher content of catechin, and was decreased by the shading treatment which reduced catechin content. All these results indicate that PAL, and consequently phenylalanine, play an important role in the catechin biosynthesis. On the other hand results which are not consistent with the above results were obtained by tracer experiments: namely shikimic acid was incorporated into catechins more efficiently than phenylalanine. This result of tracer experiments suggests that the participation of phenylalanine and PAL to the pathway of catechin biosynthesis may not necessarily be obligatory. Hills and Ishikura[6] and Swain and Williams[7] expressed the similar view.

This contradictory fact as above can be explained by assuming that catechins of tea leaves might be synthesized through at least two pathways, the one including phenylalanine, as presently proposed for flavonoid biosynthesis and the other which is still hypothetic. It is presumed that, in the latter case, phenylpyruvic acid or phenyllactic acid is involved and that 3 acetate units are combined to them producing α-hydroxychalcone,[8] which further convert to catechins.

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