

ISOENZYME VARIATION AND SPECIES RELATIONSHIP IN THE GENUS *PIPER*

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Abstract: Eleven species of *Piper* including *Piper nigrum* were studied for variations in isoenzymes of three enzymes viz., peroxidase, esterase and glutamate oxaloacetate transaminase. On grouping based on isoenzyme similarity, *P. nigrum* Linn., *P. pseudonigrum* Velayudhan and Amalraj, *P. bababudani* Rahman and *P. galeatum* DC formed one group while *P. argyrophyllum* Miq. and *P. attenuatum* Buch-Ham constituted the second group and *P. chaba* Hunter, *P. hapnium* Miq. and *P. colubrinum* Link emerged as the third group. *P. betle* Linn. and *P. longum* Linn. showed their distinctness from the rest of the species. Least similarity was observed between *P. colubrinum* on one side and *P. pseudonigrum* and *P. bababudani* on the other side.

Key words: Isoenzyme variation, *Piper* spp., similarity index.

INTRODUCTION

The genus *Piper*, which is considered as one of the most difficult genera to classify, contains several economically important species such as *Piper nigrum*, *Piper betle* and *Piper longum*. Isoenzyme variation is being employed as a successful tool to study the extent of relationship at species level in many plant genera. Although, a large number of species are included in the genus *Piper*, no detailed systematic study has so far been made to assess the depth of similarities / variations among them. Therefore, the investigations reported herein were undertaken with the objective to study the dynamics of variability existing at the isoenzyme level in the genus *Piper* and to elucidate the species relationship in the genus *Piper*.

MATERIALS AND METHODS

The study was conducted at the College of Horticulture, Vellanikkara, Trichur during the period 1992-94. The materials for the investigation included 11 species of *Piper* as detailed below:

- | | |
|---------|---|
| Sl. No. | Name of species |
| 1 | <i>P. pseudonigrum</i> Velayudhan and Amalraj |
| 2 | <i>P. nigrum</i> Linn. |
| 3 | <i>P. bababudani</i> Rahman |
| 4 | <i>P. galeatum</i> C. DC |

- | | |
|----|-------------------------------|
| | <i>P. longum</i> Linn |
| | a) Type Sheemathippali |
| | b) Type Panniyur |
| | c) Type Mulayam |
| | d) Male type |
| 6 | <i>P. colubrinum</i> Link |
| 7 | <i>P. hapnium</i> Miq. |
| | <i>P. chaba</i> Hunter |
| | <i>P. betle</i> Linn. |
| | a) Type 1 |
| | b) Type 2 |
| | c) Type 3 |
| 10 | <i>P. attenuatum</i> Buch-Ham |
| | a) Type 1 |
| | b) Type 2 |
| | c) Type 3 |
| 11 | <i>P. argyrophyllum</i> Miq |
| | a) Type 1 |
| | b) Type 2 |
| | c) Type 3 |
| | d) Type 4 |

Polyacrylamide gel electrophoresis (PAGE) was conducted using vertical slab gel unit of M/s. Biotech. Samples were homogenised in 0.1 molar Tris chloride at pH 7.6 and centrifuged at 15000 rpm at 4 C. Supernatant was used for the analysis. Electrophoresis was carried out at 5 C maintaining a constant current of 25 mA per slab throughout the run. Bromophenol blue in imidazol buffer was used as tracer dye. Three enzymes namely, peroxidase (PRX), esterase (EST) and glutamate oxaloacetate transaminase (GOT) were ana-

Table 1. Similarity indices for peroxidase in *Piper* spp.

	1	2	3	4	5a	b	c	d	6	7	8	9a	b	c	10a	b	c	11a	b	c	d
1	1																				
2	0.91	1																			
3	1.00	0.91	1																		
4	0.75	0.82	0.75	1																	
5a	0.23	0.25	0.23	0.15	1																
b	0.25	0.27	0.25	0.17	0.80	1															
c	0.25	0.27	0.25	0.17	0.80	1.00	1														
d	0.17, 0.18	0.17	0.18	0.60	0.75	0.75	1														
6	0.15	0.17	0.15	0.08	0.29	0.33	0.33	0.17	1												
7	0.27	0.30	0.27	0.18	0.60	0.75	0.75	0.50	0.40	1											
8	0.36	0.27	0.36	0.17	0.50	0.60	0.60	0.40	0.33	0.75	1										
9a	0.27	0.29	0.27	0.20	0.30	0.33	0.33	0.22	0.20	0.38	0.33	1									
b	0.17	0.18	0.17	0.11	0.25	0.27	0.27	0.18	0.17	0.30	0.27	0.64	1								
c	0.28	0.29	0.28	0.22	0.21	0.23	0.23	0.15	0.14	0.25	0.23	0.54	0.57	1							
10a	0.43	0.46	0.43	0.46	0.40	0.30	0.30	0.20	0.18	0.33	0.30	0.31	0.19	0.24	1						
b	0.62	0.67	0.62	0.67	0.36	0.27	0.27	0.18	0.16	0.30	0.27	0.29	0.18	0.29	0.73	1					
c	0.64	0.69	0.64	0.69	0.31	0.23	0.23	0.15	0.14	0.25	0.23	0.25	0.16	0.26	0.62	0.83	1				
11a	0.57	0.62	0.57	0.62	0.33	0.25	0.25	0.17	0.15	0.27	0.25	0.27	0.17	0.28	0.67	0.91	0.92	1			
b	0.38	0.42	0.38	0.42	0.50	0.38	0.38	0.25	0.22	0.43	0.38	0.25	0.21	0.19	0.60	0.70	0.58	0.64	1		
c	0.29	0.31	0.29	0.31	0.50	0.38	0.38	0.25	0.22	0.43	0.38	0.25	0.21	0.19	0.78	0.55	0.46	0.50	0.75	1	
d	0.44	0.47	0.44	0.47	0.31	0.23	0.23	0.15	0.14	0.25	0.23	0.25	0.16	0.26	0.75	0.69	0.60	0.64	0.46	0.59	1

-lysed. Staining was done as per the technique outlined by Shaw and Koen (1968).

Measurement of similarity

Similarity was calculated by making pair-wise comparison of genotypes using the method suggested by Sokel and Sneath (1963).

RESULTS AND DISCUSSION

Peroxidase (PRX)

In peroxidase zymogram, a total of 31 isoenzymes were found in the species studied and were numbered serially as PRX-1 to PRX-31 (Fig 1). Among the species, PRX-1 to PRX-3 were recorded only in *P. betle* types.

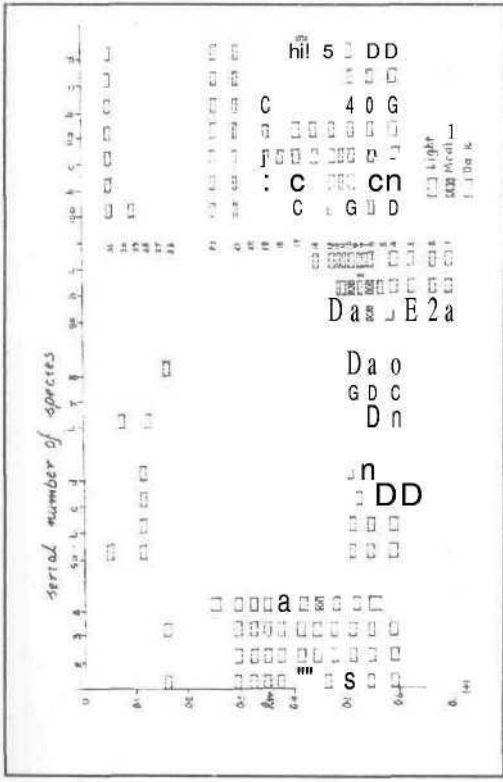


Fig 1. Zymogram of peroxidase in *Piper* spp.

PRX-4 was present in all taxa except *P. galeatum* and *P. longum* male type. PRX-6 was present in all the species whereas PRX-10 was lacking in *P. colubrinum*, a South American species. PRX-7, 8 and 9 were present only in *P. belle* types.

P. bababudani and *P. pseudonigrum* possessed identical peroxidase pattern with a similarity index of 1.0 (Table 1). *P. nigrum* was also found to be very closely related to these species with similarity index 0.91. *P. galeatum* differed from *P. bababudani* and *P. pseudonigrum* in the absence of PRX-4 and PRX-26 and presence of PRX-23. Similarity of *P. galeatum* with *P. nigrum* is 0.82 and that with other two species was 0.75. The peroxidase profiles of *P. argyrophyllum* and *P. attenuatum* were similar which support a high level of morphological similarity between the species. Within species variation was observed in *P.*

belle types as in the case of *P. nigrum*. It was observed that *P. chaba* and *P. hagnium* are closely related with similarity index 0.75. The difference was only in the presence of an additional band PRX-26 in *P. chaba*. Morphologically similar species *P. hagnium* and *P. longum* showed a similarity index ranging from 0.50 to 0.75 among the different types of *P. longum* in peroxidase pattern.

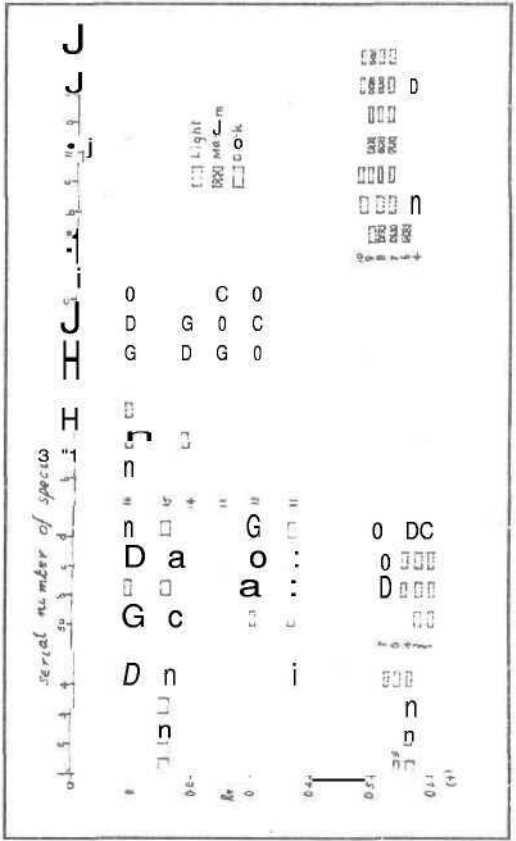


Fig 2. Zymogram of esterase in *Piper* spp.

Esterase (EST)

Different species differed much widely in esterase pattern, unlike peroxidase (Table 2 and Fig 2). However, certain exceptions were also noticed. *P. argyrophyllum* and *P. attenuatum* showed a similarity index as high as 1.0 when different types such as *P. attenuatum* type 3 and *P. argyrophyllum* Type 4 were considered, confirming their high morpholo-

Table 2. Similarity indices for **esterase** in *Piper* spp.

	1	2	3	4	5a	b	c	d	6	7	8	9a	h	c	10a	h	c	11a	b	c	d	
1	1																					
2	0.67	1																				
3	0.67	1.00	1																			
4	0.50	0.33	0.33	1																		
5a	0.13	0.14	0.14	0.33	1																	
b	0.10	0.11	0.11	0.40	0.75	1																
c	0.10	0.11	0.11	0.40	0.75	0.10	1															
d	0.25	0.29	0.29	0.44	0.63	0.50	0.50	1														
6	0.00	0.00	0.00	0.17	0.17	0.13	0.13	0.40	1													
7	0.00	0.00	0.00	0.14	0.14	0.11	0.11	0.13	0.50	1												
8	0.00	0.00	0.00	0.17	0.17	0.13	0.13	0.14	1.00	0.50	1											
9a	0.00	0.00	0.00	0.11	0.25	0.20	0.20	0.22	0.25	0.50	0.25	1										
b	0.00	0.00	0.00	0.11	0.25	0.20	0.20	0.22	0.25	0.50	0.25	1.00	1									
c	0.00	0.00	0.00	0.13	0.29	0.22	0.22	0.25	0.33	0.25	0.33	0.15	0.75	1								
10a	0.17	0.00	0.00	0.25	0.00	0.09	0.09	0.10	0.00	0.00	0.00	0.00	0.00	0.00	1							
b	0.17	0.20	0.20	0.25	0.00	0.09	0.09	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.33	1						
c	0.00	0.00	0.00	0.11	0.00	0.09	0.09	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.60	0.60	1					
11a	0.00	0.00	0.00	0.11	0.00	0.10	0.10	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.75	0.40	0.75	1				
b	0.00	0.00	0.00	0.13	0.00	0.10	0.10	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.75	0.40	0.75	1.00	1			
c	0.14	0.17	0.17	0.22	0.00	0.08	0.08	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.80	0.80	0.80	0.60	0.60	1		
d	0.00	0.00	0.00	0.11	0.00	0.09	0.09	0.10	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.60	1.00	0.75	0.85	0.80	1	

gical similarity. *P. bababudani* showed a similarity index of 1.0, with *P. nigrum* and 0.67 with *P. pseudonigrum*. *P. pseudonigrum* had a similarity index of 0.67 with *P. nigrum* and 0.50 with *P. galeatum*. Intra-species variation was very less in *P. betle* types but much high in *P. attenuatum*, *P. longum* and *P. argyrophyllum*. EST-13 was present only in *P. betle* types. *P. colubrinum*, *P. hapnium* and *P. chaba* differed much from the rest of the spe-

ties in esterase pattern. *P. chaba* and *P. colubrinum* possessed only EST-16.

Glutamate oxaloacetate transaminase (GOT)

A total of 24 isoenzymes were observed in GOT zymogram for *Piper* spp. and numbered as GOT-1 to GOT-24 (Fig 3). The similarity index ranged from 0 to 1.0 (Table 3), the

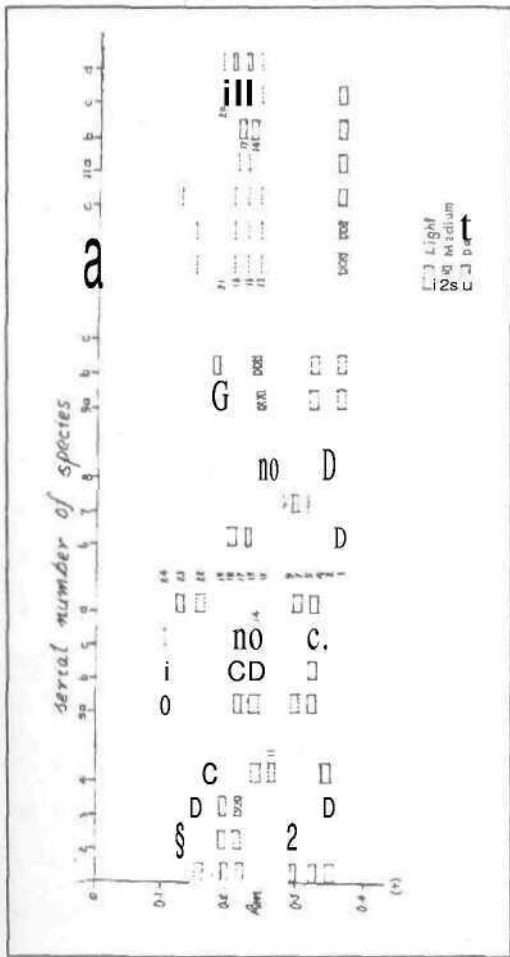


Fig 3. Zymogram of GOT in *Piper* spp.

maximum being among *P. betle* types. Among the species studied, the highest similarity index of 0.67 for GOT was observed among, *P. attenuatum* and *P. argyrophyllum* types and *P. pseudonigrum* and *P. bababudani*. *P. pseudonigrum* and *P. nigrum* had a similarity index of 0.43 and that for *P. nigrum* and *P. bababudani* was 0.33. *P. colubrinum*, an exotic species showed a comparatively higher similarity index (0.33) with *P. attenuatum* and *P. argyrophyllum* types. *P. belle* also showed a similarity of 0.33 with *P. galeatum* and *P. longum* types. *P. longum* male type which is popular in North India showed its difference

from South Indian types with similarity index ranging from 0.14 to 0.29.

P. hapnium stands distinct from the rest of the species in having only three isoenzymes i.e., GOT-5, 7 and 9. The similarity of *P. hapnium* with other species for GOT zymogram was mostly zero except with *P. nigrum*, *P. pseudonigrum* and *P. longum* types wherein a similarity of less than 0.20 was observed. *P. chaha* showed its distinctness from the rest of the species with a similarity index of zero with most of the species and recorded a low similarity index of 0.20 with *P. colubrinum*, 0.13 with *P. pseudonigrum* and 0.17 with *P. bababudani* and *P. galeatum*. *P. galeatum* showed a GOT pattern which was much different from *P. pseudonigrum*, *P. nigrum* and *P. bababudani* unlike in the case of peroxidase and esterase systems. GOT 11 and 14 found in *P. galeatum* were absent in these three species while GOT-17 and 19 which were present in the later groups were absent in *P. galeatum*.

Similarity index among *Piper* spp. for isoenzyme banding pattern

Average similarity indices for the three enzymes were computed among the eleven *Piper* spp. (Table 4) to study the relative closeness and distinctness of different species. The groups of the species observed to be closely related are:

- Group 1 *P. nigrum*, *P. pseudonigrum*, *P. bababudani* and *P. galeatum* (S.I. 0.38 to 0.78)
- Group 2 *P. argyrophyllum* and *P. attenuatum* (S.I. up to 0.72)
- Group 3 *P. chaha*, *P. hapnium* and *P. colubrinum* (S.I. = 0.30 to 0.51)

P. bababudani (Rahiman, 1981) and *P. pseudonigrum* (Velayudhan and Amalraj, 1992) are the recently described species in the genus *Piper*. These species were found to be very similar to *P. nigrum* except in some minor morphological characters. The highest similarity index of 0.78 was observed between *P.*

Table 3. Similarity indices for GOT in *Piper* spp.

	1	2	3	4	5	a	b	c	d	6	7	8	9a	b	c	10a	b	c	11a	b	c	d	
1	1																						
2	0.43	1																					
3	0.67	0.33	1																				
4	0.11	0.00	0.14	1																			
5a	0.38	0.29	0.13	0.13	1																		
b	0.25	0.14	0.14	0.14	0.80	1																	
c	0.25	0.14	0.14	0.14	0.80	1.00	1																
d	0.43	0.33	0.14	0.00	0.29	0.14	0.14	1															
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1													
7	0.13	0.17	0.00	0.00	0.14	0.00	0.00	0.17	0.00		1												
8	0.13	0.00	0.17	0.17	0.00	0.00	0.00	0.00	0.20	0.00		1											
9a	0.11	0.00	0.00	0.33	0.29	0.33	0.33	0.14	0.17	0.00	0.00		1										
b	0.11	0.00	0.00	0.33	0.29	0.33	0.33	0.14	0.17	0.00	0.00	1.00		1									
c																1							
10a	0.10	0.00	0.13	0.00	0.00	0.00	0.00	0.13	0.33	0.00	0.00	0.13	0.13	-		1							
b	0.10	0.00	0.13	0.00	0.00	0.00	0.00	0.13	0.33	0.00	0.00	0.13	0.13	-	1.00		1						
c	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.13	0.33	0.00	0.00	0.13	0.13	-	0.67	0.67		1					
Ha	0.11	0.14	0.14	0.00	0.13	0.14	0.14	0.00	0.17	0.00	0.00	0.14	0.14	-	0.50	0.50	0.50		1				
b	0.13	0.17	0.17	0.17	0.33	0.40	0.40	0.00	0.20	0.00	0.00	0.40	0.40	-	0.14	0.14	0.14	0.40		1			
c	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.13	0.13	-	0.67	0.67	0.67	0.50	0.14		1		
d	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	-	0.50	0.50	0.50	0.33	0.00	0.80		1	

pseudonigrum and *P. bababudani* which showed their closeness confirming the morphological similarity. *P. nigrum* showed a similarity index of 0.75 with *P. bababudani* and 0.67 with *P. pseudonigrum*. *P. galeatum* was observed to be closer to *P. pseudonigrum* (S.I. = 0.45) and *P. bababudani* (S.I. = 0.41) than *P. nigrum* which showed a similarity index of only 0.38. In earlier studies by Rahiman and Subbaiah (1984), Rahiman and

Bhagavan (1985) and Ravindran *et al.* (1992) it was reported that *P. galeatum* was closely related to *P. trichostachyon*. Similarly *P. nigrum* was reported to be related to *P. wightii* (Ravindran *et al.*, 1992). Unfortunately, these two species could not be included in the present study. *P. attenuatum* and *P. argyrophyllum* are other two species which were observed to be closely related in the present study. This also confirms the earlier reports by

Table 4. Indices of similarity among *Piper* spp.

	1	2	3	4	5a	h	c	d	6	7	8	9a	h	c	10a	h	c	11a	h	c	d	
1	1																					
2	0.67	1																				
3	0.78	0.75	1																			
4	0.45	0.38	0.41	1																		
5a	0.24	0.23	0.17	0.20	1																	
h	0.20	0.18	0.17	0.24	0.79	1																
c	0.20	0.18	0.17	0.24	0.79	1.00	1															
d	0.28	0.27	0.20	0.21	0.50	0.46	0.46	1														
6	0.05	0.06	0.05	0.08	0.15	0.15	0.15	0.10	1													
7	0.13	0.16	0.09	0.11	0.30	0.29	0.29	0.26	0.30	1												
8	0.16	0.09	0.18	0.17	0.22	0.24	0.24	0.18	0.51	0.42	1											
9a	0.13	0.10	0.09	0.21	0.28	0.29	0.29	0.20	0.21	0.29	0.19	1										
b	0.09	0.06	0.06	0.19	0.26	0.27	0.27	0.18	0.19	0.27	0.17	0.88	1									
c	0.14	0.15	0.14	0.17	0.25	0.23	0.23	0.20	0.24	0.25	0.28	0.64	0.66	1								
10a	0.23	0.15	0.18	0.24	0.13	0.13	0.13	0.14	0.17	0.11	0.10	0.14	0.10	0.12	1							
h	0.29	0.29	0.31	0.31	0.12	0.12	0.12	0.18	0.16	0.10	0.09	0.14	0.10	0.15	0.69	1						
c	0.21	0.27	0.21	0.27	0.10	0.11	0.11	0.13	0.16	0.08	0.08	0.13	0.09	0.13	0.63	0.70	1					
11a	0.23	0.25	0.24	0.25	0.15	0.16	0.16	0.09	0.11	0.09	0.08	0.14	0.10	0.14	0.64	0.60	0.72	1				
h	0.17	0.15	0.18	0.24	0.28	0.29	0.29	0.12	0.14	0.14	0.13	0.22	0.20	0.09	0.50	0.41	0.60	0.68	1			
c	0.14	0.16	0.15	0.18	0.17	0.15	0.15	0.15	0.19	0.14	0.13	0.13	0.11	0.09	0.65	0.67	0.41	0.53	0.50	1		
d	0.15	0.16	0.15	0.19	0.10	0.11	0.11	0.08	0.10	0.08	0.08	0.08	0.05	0.13	0.62	0.59	0.67	0.58	0.40	0.73	1	

various workers. Hooker (1886) and Gamble (1925) had observed the similarity of these species on morphological grounds. Rahiman and Subbaiah (1984) based on flavonoid analysis reported paired similarity of 82 per cent and paired affinity of 33.33 per cent between the two species. Paired similarity of 75 per cent and paired affinity of 30 per cent were considered as indices of closeness. Rahiman and Bhagavan (1985) based on D² analysis showed that these two species are

biometrically related. Results of cluster analysis based on 30 characters by Ravindran *et al.* (1992) also showed close relationship of these two species which formed a single cluster.

ACKNOWLEDGEMENT

The study forms a part of the M.Sc. (Hort.) thesis of the senior author submitted to the Kerala Agricultural University. The authors are

grateful to the Officer in-charge, NBPGR Regional Station, Vellanikkara, Trichur for kindly sparing some valuable germplasm material. Junior Research Fellowship provided to the senior author from an ICAR aided adhoc project is gratefully acknowledged.

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