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Development of DNA Marker to Identify Candidate Line Containing High Content of Curcumin in Turmeric

## Hiroshi HAYAKAWA<sup>1,2\*</sup>, Tetsuya KOBAYASHI<sup>2</sup>, Yukio MINAMIYA<sup>1,2</sup>, Katsura ITO<sup>3</sup>, Akira MIYAZAKI<sup>2</sup>, Tatsuya FUKUDA<sup>2</sup>, Yoshinori YAMAMOTO<sup>2</sup> (<sup>1</sup>Unit. Grad. Sch. Agr. Sci., Ehime Univ., <sup>2</sup>Fac. Agr. Kochi Univ., <sup>3</sup>JST Innovation Satellite Kochi)

ウコン属植物における高クルクミン含有率系統識別マーカーの開発 早川宗志<sup>1,2\*</sup>・小林徹也<sup>2</sup>・南谷幸雄<sup>1,2</sup>・伊藤桂<sup>3</sup>・宮崎彰<sup>2</sup>・福田達哉<sup>2</sup>・山本由徳<sup>2</sup> (<sup>1</sup>愛媛大学大学院連合農学研究科・<sup>2</sup>高知大学農学部・<sup>3</sup>JSTイノベーションサテ ライト高知)

The dried or fresh rhizomes of spice turmeric (*Curcuma longa*) are well known as traditional medicine and the curcumin is widely used in various areas. Although there are differences in the amount of curcumin within this species, it is difficult to identify the candidate line by rhizomes because the morphological characters are very similar among them. To conduct an accurate and easy identification of high content line of curcumin in this species, we analyzed some sequences of chloroplast DNA (cpDNA).

[Materials and Methods] <u>Cultivation</u> We cultivated nine *C. longa*, two *C. aromatica* and one *C. zedoaria* (Table 1). The fresh weights of shoot biomass and rhizome yield were measured. We extracted curcumin by 80% of ethanol from ground samples and measured curcumin content (mg/100g) using HPLC. <u>DNA analysis</u> We amplified and sequenced four regions, namely *matK*, *rpl16* intron2, *petB* intron1 and *petB* intron2 in cpDNA.

[Results and Discussion] *C. longa* (Indonesia A) had the highest curcumin content in the cultivated *Curcuma* species (Table 1). To find the appropriate out-group taxa to conduct infraspecific analyses of *C. longa*, we reconstructed molecular phylogenetic tree of *C. longa* and its allied species using *matK* sequences, indicating that *C. aromatica* and *C. zedoania* are close related to *C. longa*. Next, to develop the molecular marker to identify high content line of curcumin of this species, network analysis using chloroplast microsatellite regions was conducted (Fig. 1). The result is that unique haplotype within *C. longa* (Haplotype C) was agreed with the high content line of curcumin. These results indicate that our chloroplast microsatellite regions allow us to find out high content line of curcumin within various cultivars of this species.

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Species	Shoot biomass <sup>1)</sup> (g hill <sup>-1</sup> )	Rhizome yield <sup>1)</sup> (g hill <sup>-1</sup> )	Curcuminoids content <sup>2)</sup>	Haplotype
<i>C. aromatica</i> (Tanegashima)	293	602	36.3	В
<i>C. longa</i> (Kochi)	1170	998	357.6	D
<i>C. longa</i> (Tanegashima)	710	803	391.7	D
<i>C. longa</i> (Wakayama A)	848	926	388.3	D
<i>C. longa</i> (Wakayama C)	585	961	395.5	D
<i>C. longa</i> (Okinawa A)	845	939	364.0	D
<i>C. longa</i> (Okinawa B)	848	716	373.1	D
<i>C. longa</i> (Indonesia A)	309	304	2677.8	С
<i>C. longa</i> (Indonesia B)	835	864	336.6	D
<i>C. longa</i> (Wakayama B)	358	662	0.7	Е
C. zedoaria	573	738	1.0	Α

Table 1. Shoot biomass, rhizome yield and curcumin content at maturity in 2008.

<sup>1)</sup> Fresh weight.

<sup>2)</sup> Content of curcumin in primary branch rhizome (mg/100g).

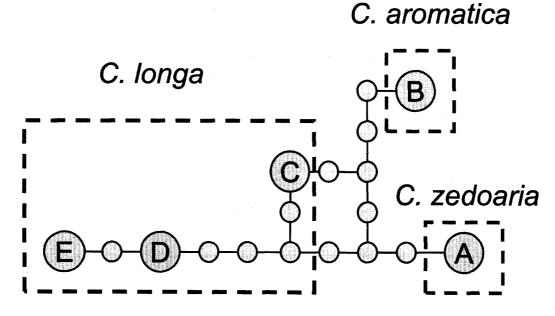


Fig. 1 Parsimony network of *C. longa*, *C. aromatica* and *C. zedoaria* based on *rpl1*6 intron2, *petB* intron1 and *petB* intron2 of cpDNA. Haplotype letters correspond to

those in Table 1. Each species is boxed by dashed line. Large circles indicate the taxa in which haplotypes were found. Small circles between haplotypes represents a mutational step.