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水稻黄叶突变体叶绿体超微结构与突变基因定位

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摘 要：对黄叶突变体-黄玉 B 的叶绿体超微结构和遗传特性进行研究。结果表明，野生型和突变体在相同的叶龄期，叶片内叶绿体个数差异不显著(P<0.05)，但野生型的叶绿体基质浓厚、基粒片层堆叠比较整齐、有序，突变体基质较淡、基粒片层堆叠比较松散。遗传分析表明，该突变体黄叶性状受 1 对隐性基因控制，命名为 *xl(t)*;用 SSR 分子标记将 *xl(t)*定位在第 11 染色体 RM5349 和 RM21 之间，遗传距离分别为 0.82 cM 和 2.34 cM。

关键词：水稻；黄叶突变体；叶绿体超微结构；基因定位
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Gene mapping and chloroplast ultrastructure of a xantha rice mutant

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Abstract: The chloroplast ultrastructure and genetic characteristics of a xantha rice mutant were studied in this paper. The result showed that the chloroplast number in leaves of mutant was not significantly different from its wild parent (P<0.05). Compared to its wild type parent, chloroplasts of the mutant had thin stroma and disorderly stacked grana lamellae. Genetic analysis demonstrated that the xantha leaf mutation was controlled by a single recessive gene named as *xl(t)* and found to be located between RM5349 and RM21 on chromosome 11 with the genetic distance of 0.82 cM and 2.34 cM, respectively.

Key words: Rice (*Oryza sativa* L.); xantha mutant; chloroplast ultrastructure; gene mapping

Chlorophyll-deficient mutation is a relatively common phenomenon in plant. It is generally believed that the chlorophyll-deficient mutation might be caused by two factors, one is that the mutation of a chlorophyll synthase-encoding gene hinders chlorophyll synthesis, while the chlorophyll deficiency conversely affects the development of chloroplast structure; the other is that the mutation of a chloroplast structural protein-encoding gene hinders the development of chloroplast structure, thereby resulting in the decrease of chlorophyll synthesis[1-2]. Other related researches also show that leaf-color mutation is mostly controlled by recessive genes, especially a recessive nuclear gene [3-4]. It is further demonstrated that leaf-color mutation is controlled by two recessive nuclear genes or cytoplasmic genes [5]. At present, 80 rice leaf-color mutants have been reported at home and abroad, while only a small number of genes controlling leaf-color mutant trait are cloned [6-8]. In order to comprehensively investigate the formation, development and related molecular mechanisms of chloroplasts, more leaf-color mutants are supposed to be tested.

In this study, the xantha mutant WT, which is yellow during the whole growth stage with chlorophyll content accounting for only one-fourth of the wild type, was obtained from ^{60}Co-γ-treated maintainer line Longtefu grown widely for agriculture production [9], and used as our experimental material. The developmental dynamics of chloroplasts of WT were observed by using electron microscopy to analyze relevant genetic characteristics. Our research tries to lay a foun-

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dation for clarifying the yellowing mechanism of the xantha mutant WT.

1 Materials and methods

1.1 Materials

R3027, II32B, mutant WT and its parent Longtefu were used as our experimental materials.

1.2 Electron microscopic observation of chloroplast ultrastructure

Fully expanded leaves of the mutant and wild type controls at 2-leaf stage and 6-leaf stage were collected. Samples were treated with the osmic acid fixation method and observed under JEM-1230 transmission electron microscope [10].

1.3 Construction of F_2 populations and genetic analysis of the xantha mutant

F_1 hybrid seeds were obtained by hybridization of WT with R3027 and II32B; F_1 seedlings were individually planted in Lingshui in winter and selfed by bagging to obtain F_2 seeds. F_2 population hybridized by WT and R3027 was adopted for gene mapping.

1.4 DNA extraction

DNA was extracted from rice seedlings and leaves in accordance with the DNA extraction method simplified by Lu et al[11].

1.5 SSR analysis

According to SSR information published on http://www.gramene.org, 358 SSR markers covering 12 rice chromosomes were selected for primary location mapping of the determinant gene.

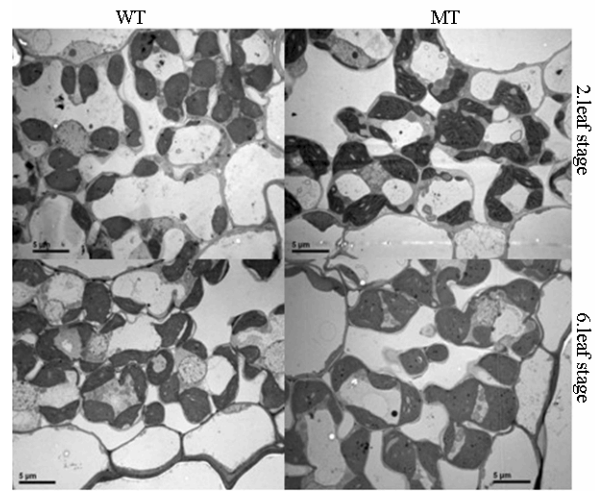
1.6 Data analysis

Experimental data were analyzed using EXCEL2003 and linkage analysis of SSR molecular markers and mutant genes, and the calculation of genetic distance and mapping were conduced using JoinMap3.0 software.

2 Results

2.1 Chloroplast number and ultrastructure

Changes of the chloroplast number in plant leaves are considered as an important physiological phenomenon, which is regulated by developmental stages and demand of other plant tissues for nutrient elements [12]. In this experiment, the chloroplast number in the leaves of wild type and the mutant planted in the same seedbed both increased with their rising leaf ages; however, at the same leaf age, the chloroplast number in mesophyll cells of wild type and their mutant showed no significant difference ($P > 0.05$) (Figure 1 and Table 1), indicating that the leaf yellowing of the mutant does not result from the decrease of chloroplast number in mesophyll cells, nutrient deficiency or any other environmental factors.



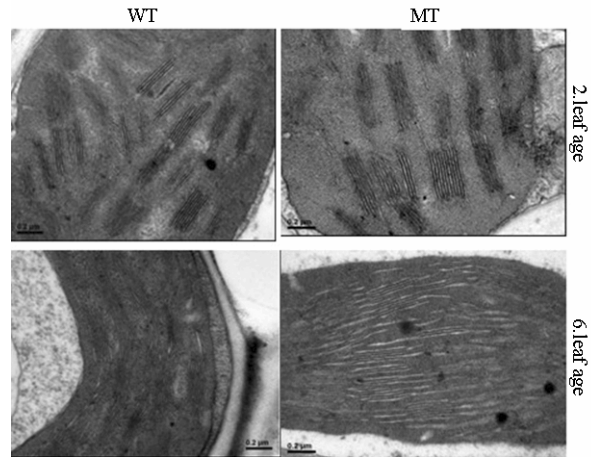
WT, wild type; MT, mutant

Figure 1 Chloroplasts in mesophyll cells of fully expanded leaves of rice at different ages (× 4 000)

Table 1 Changes of the average number of chloroplasts in a mesophyll cell of fully expanded leaves of rice at different ages

Variety	Chloroplast number	
	2-leaf stage	6-leaf stage
Wild type	30±2.78^A	35±2.03^A
Mutant	31±1.53 ^{A}	33±3.15^A

Note: Data are shown as mean±SD. with triplicates. The same capital letter in the same column indicates no significant difference (P>0.05).



WT, wild type; MT, mutant

Figure 2 Chloroplast grana lamellae of fully expanded leaves of rice at different ages (×80 000)

Generally, chloroplasts in plant cells are developed from proplastids, lamellar membranes stacked as

grana with the formation and accumulation of chlorophyll. With the increase of leaf age, the number of chloroplast grana lamellae in mesophyll cells of wild type and their mutant gradually increased, with dense stroma and osmiophilic granules; however, at the same leaf age, the chloroplast of wild parent showed dense stroma and regularly stacked grana lamellae, while that of the mutant showed thin stroma and disorderly stacked grana lamellae, as shown in Figure 2.

2.2 Genetic analysis of the xantha mutant

WT showed yellow leaves from the emergence of the first leaf to mature (Figure 3), and the yellow-leaf trait was stably expressed during the whole growth period. After many years of experiments, this yellow-leaf trait is stably expressed at different places, showing consistent performance among various plants, which is significantly different from that of normal-leaf rice plants.

F_1 hybrid seeds from WT crossed with R3027 or II32B were sown, and the plant leaves were all green during the whole growth period, indicating that the yellow-leaf trait of WT is recessive. F_2 populations showed segregation of yellow and green-leaf plants in two coupling groups, which is consistent with the theoretical segregation ratio of 3:1, indicating that the inheritance of xantha mutant gene is controlled by a single recessive gene (Table 2), which was named as *xl(t)*.

Table 2　The segregation rate of yellow and green-leaf plants in rice F_2 populations

F_2 population	Total	Green-leaf plant	Yellow-leaf plant	Green/ Yellow	χ^2	$\chi^2_{0.05,\ 1}$
WT / R3027	3 925	2 980	945	3.15:1	1.73	3.84
WT / II32B	5 116	3 860	1 256	3.07:1	0.52	

Figure 3　Leaf color phenotype of WT

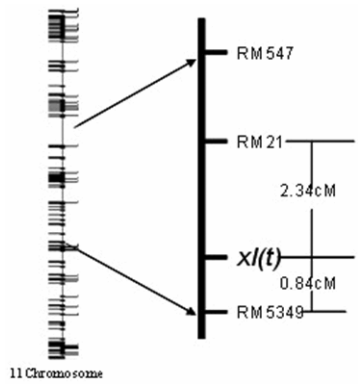


Figure 4　Location of the xantha leaf mutant gene *xl(t)* on chromosome 11

2.3 Mapping of mutant gene

Polymorphism screening of WT and R3027 was carried out using 358 SSR markers covering 12 rice chromosomes. After three rounds of preliminary screening, a total of 16 pairs of markers showed differences from their two parents. Gene pools were respectively constructed with equal amount of DNA of 10 green-leaf plants and 10 yellow-leaf plants in $F_{2:3}$ populations and amplified using the 16 pairs of markers, whose results showed that RM5349 and RM21 markers on chromosome 11 had differences between the two gene pools. Furthermore, 218 yellow-leaf individuals in $F_{2:3}$ of WT / R3027 were adopted for final verification, the results were again consistent with the amplification of gene pools, indicating that RM5349 and RM21 markers on chromosome 11 had significant linkage relationship with the yellow-leaf trait of the experimental materials. Accordingly, the xantha mutant locus was deduced to be located between RM5349 and RM21 with the genetic distance of 0.82 cM and 2.34 cM (Figure 4).

3 Discussion

Biosynthesis of chlorophyll b in green plants from glutamate involves 16 step reactions, which are catalyzed by 16 different enzymes encoded by more than 20 genes [13]. Any mutation in the enzymes in the chlorophyll synthesis pathway may hinder the biosynthesis of chlorophyll and change the proportion of pigment in the chloroplasts, thereby probably results in the change of leaf color. The chloroplast structure of xantha mutant has been reported, and the analysis the chloroplast ultrastructure of a xantha wheat mutant showed that its chloroplast structure contains a small amount of grana with few or small grana lamellae, while lacks rich, highly organized endomembrane system, as observed in normal wheat control. Tang et al[14] investigated the subcellular structure of leaf cells in Nan-huang barley, an albino mutant, and the result showed that the chloroplast grana lamellae of the mutant was significantly reduced, but the lipoid granules

was increased. Sun et al[15] observed the development of plastids of xantha sugarcane mutant and found that the xantha sugarcane mutant plastidsonly contains some small bubbles, a small number of lamellae, some ribosomes and DNA-like fibrils, and unusual stacking and swelling thylakoid structure. However, in this study, the chloroplast numbers of wild type and the xantha mutant at the same leaf age are not significantly different, and the mutant has normal chloroplast shape and osmiophilic granules. However, the mutant has thin stroma and loosely stacked grana lamellae, while the wild type has dense stroma and regularly stacked grana lamellae.

Table 3 Key enzymes and genes involved in chlorophyll biosynthesis and degradation in *Arabidopsis thaliana* and rice

Enzyme	Gene	Annotation for *Arabidopsis thaliana* [16-17]	Annotation for rice
Glutamyl tRNA reductase	*HEMA1*	At1g58290	Loc_Os10g35840
	HEMA2	At1g09940	
	HEMA3	At2g31250	
Glutamate-1-semialdehyde aminotranase 2,1Aminomutase	*GSA1(HEML1)*	At5g63570	
	GSA2(HEML2)	At3g48730	Loc_Os08g41990
5-Aminolevlinate dehydratase	*HEMB1*	At1g69740	
	HEMB2	At1g44318	
Porphobilinogen deaminase	*HEMC*	At5g08280	Loc_Os02g07230
Uroporphyrinogen III synthase	*HEMD*	At2g26540	Loc_Os03g08730
UroporphyrinogenIII decarboxylase	*HEME1*	At3g14930	Loc_Os01g43390
	HEME2	At2g40490	Loc_Os03g21990
Coproporphyrinogen oxidase	*HEMF1*	At1g03475	Loc_Os04g52130
	HEMF2	At4g03205	
Protoporphyrinogen oxidase	*HEMG1*	At4g01690	Loc_Os01g18320
	HEMG2	At5g14220	Loc_Os04g41260
Mg-chelatase	*ChL11*	At4g18480	
	ChL12	At5g45930	Loc_Os03g36540
	ChLD	At1g08520	Loc_Os03g59640
	ChLH	At5g13630	Loc_Os03g20700
			Loc_Os07g46310
Mg-protoporphyrin IX methyltransferase	*ChLM*	At4g25080	Loc_Os06g04150
Mg-protoporphyrin IX monomethylaste cyclase	*CHL27*	At3g56940	Loc_Os01g17170
8-vinyl reductase	*DVR*	At5g18660	Loc_Os03g22780
NADPH-protochlorophyllide oxidoreductase	*PORA*	At5g54190	Loc_Os04g58200
	PORB	At4g27440	Loc_Os10g35370
	PORC	At1g03630	Loc_Os10g35730
Geranylgreanyl reductase	*ChlP*	At1g74470	
Chlorophyll synthase	*ChLG*	At3g51820	Loc_Os05g28200
Chlorophyll a oxygenase	*CAO(CHL)*	At1g44446	Loc_Os10g41780
Chlorophyllase	*AtCLH1*	At1g19670	
	AtCLH2	At5g43860	Loc_Os10g28370
Pheophorbide a oxygenase	*ACD1*	At3g44880	Loc_Os03g05310
Red chlorophyll catabolite reductase	*ACD2*	At4g37000	Loc_Os10g25020
			Loc_Os10g25030
			Loc_Os10g25040

Genes responsible for leaf-color mutation are widely distributed on different rice chromosomes. However, only about 10 leaf-color mutant genes of rice are located using molecular markers technology

(http://www.gramene.org/newsletters/rice_genetics/rgnl 21v12p93.html), and only three xantha mutant genes of rice are mapped on chromosomes 2, 5 and 10 [6, 18]. In this study, genetic analysis shows that the xantha mutation trait of the mutant is controlled by a recessive nuclear gene as the most leaf-color mutants does. By using SSR markers, xantha mutation-related gene *xl(t)* is located between loci RM5349 and RM21 on chromosome 11, with the genetic distance of 0.82 cM and 2.34 cM, respectively. Three mutant genes *z2*, *v9* and clb_{GZ} are located on chromosome 11, respectively control zebra-leaf, pale green-leaf and green-revertible albino leaf traits [10, 19], which are significantly different from the yellow-leaf phenotype of *xl(t)*. In addition, based on the related information of genes cloned in chlorophyll synthesis pathway in *Arabidopsis thaliana*, using the NCBI data information, the corresponding homologous chlorophyll synthetase genes in rice were obtained (Table 3). Our results also showed that there is no chlorophyll synthesis-related gene on rice chromosome 11. Therefore, *xl(t)* in this study is a new rice leaf-color mutant gene or mutant locus.

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