

Utilization of *eui* Gene from a Recessive Tall Rice Mutant 02428h in Breeding

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Abstract: In order to improve the panicle extrusion of photo- and thermo-sensitive sterile line 'Pei'ai 64S' by using elongated uppermost internode (*eui*) gene of the wide compatibility rice mutant '02428h', a new photo- and thermo-sensitive sterile line 'P8hS' characterized with elongated uppermost internode was developed by transferring the *eui* gene into Pei'ai 64S through three successive backcrossing. Compared with Pei'ai 64S, the plant height of P8hS was 35.6 cm higher resulted from the elongation of the uppermost and the second internodes from the top. The panicle extrusion of Pei'ai 64S was completely improved and positive effects were found on the main economic characters of P8hS and its hybrids by introducing *eui* gene into Pei'ai 64S.

Key words: rice (*Oryza sativa*); recessive tall; elongated uppermost internode gene; inheritance; backcrossing

Usually, tall plant is a dominant trait over dwarf in rice. In 1978, a recessive tall mutant LM1 with elongated lowermost internode was first obtained from Norin 18 treated with γ -ray by Okuno and Kawai^[1-2]. In 1981, Rutger and Carnahan^[3] found an elongated uppermost internode recessive mutant 76:4512 in Mg/Terso F₃ population, and named the recessive gene as elongated uppermost internode (*eui*). After that, several *eui* mutants were found, such as Grlc obtained by Liao et al^[4], Mh-1 identified by Wu and Zhang^[5], 02428h obtained from somaclonal variation of a wide compatibility japonica variety 02428 by Li and Sun^[6-7], eB-1 and eB-2 originated from M₂ population of Xieqingzao B by Yang et al^[8], 02428ha from 02428h by Wang et al^[9], Gh-2 and Gh-3 from Gang 46B and Shuangkezao treated with γ -ray by Zhu et al^[10] and *eui* mutant found in Luoshami by Zhang et al^[11]. Gao et al^[12] also found *eui* mutants from somatic cell lines of 26 Zaizao, 85-511 and Milyang 46.

The genetic analysis of *eui* revealed that the trait of the above mutants were a recessive character which controlled by a single gene^[3-9, 13-14]. Rutger and Carnahan regarded the *eui* gene as the fourth genetic element in addition to the cytoplasmic male sterility

(CMS) line, maintainer line and restorer line. They thought that the utilization of *eui* gene can not only solve the panicle enclosure of CMS lines, but also improve pollination efficiency of restorer lines by developing tall restorer lines with *eui* gene due to reduced GA₃ application, which decreased seed production cost, improved seed quality and reduced environment pollution^[3, 15-17].

The line 02428 is a wide compatibility japonica rice cultivar with good agronomic characters. Its *eui* mutant 02428h has special value in the utilization of inter-subspecies heterosis between indica and japonica. The genetic characteristics of 02428h have been reported by Li et al^[6], Sun et al^[7] and Chen et al^[13], but its utilization in breeding hasn't been reported yet. In this study, the *eui* gene of 02428h was transferred into a thermo-sensitive genic male sterile (TGMS) line Pei'ai 64S by backcrossing and the heredity and utilization value of *eui* gene in reducing the panicle enclosure of male sterile lines were examined.

MATERIALS AND METHODS

Transfer of *eui* gene

The TGMS line Pei'ai 64S was used as the female parent to be crossed with male parent 02428h. Then successive backcrossings were conducted three times with Pei'ai 64S as the recurrent parent. In BC₁F₁

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and BC₂F₁ generations, the plants used for backcrossing were selfed and harvested for investigating the segregation of *eui* gene in next generation in order to enhance the breeding efficiency. The BC₃F₁ plants similar to Pei'ai 64S were selected for developing selfed-progeny to purify the *eui* gene.

Genetic analysis

Segregations of *eui* gene in F₂, BC₁F₂, BC₂F₂ and BC₃F₂ populations were investigated.

Comparison of agronomic traits

The main agronomic traits of the new TGMS line P8hS with *eui* gene and hybrid combination P8hS/9311 and their controls of Pei'ai 64S and Liangyoupeijiu (Pei'ai 64S/9311) were observed and compared.

RESULTS

The breeding process of P8hS

The combination Pei'ai 64S/02428h was made in

1999 in Nanjing, Jiangsu Province. The F₁s were planted and backcrossed with Pei'ai 64S as a female parent during winter season in Hainan Province. In the succeeding generations, the plants with plant type similar to Pei'ai 64S were selected for backcrossing with the recurrent parent Pei'ai 64S. Through generations, the plants possessing a pollen fertility over 99% and agronomic characters like Pei'ai 64S were selected for ratooning and the seeds were harvested. In BC₃F₅, all the plants were recessive tall and pollen sterile. Among them, 5 lines possessed stable agronomic characters with 100% of male sterility and were named as 'P8hS'. The detailed breeding process of P8hS is shown in Fig. 1.

Sterility identification of P8hS

After three backcrossing, the evaluation of sterility and fertility segregation in BC₃F₁ and BC₃F₃ populations was conducted in Nanjing, Jiangsu Province. The plants with 99-100% pollen sterility were selected for ratooning. The selfed seeds were harvested and planted in Hainan Province. The seed setting rate in ratoon plants was above 50%, while for BC₃F₂ and BC₃F₄ populations planted in Hainan, it

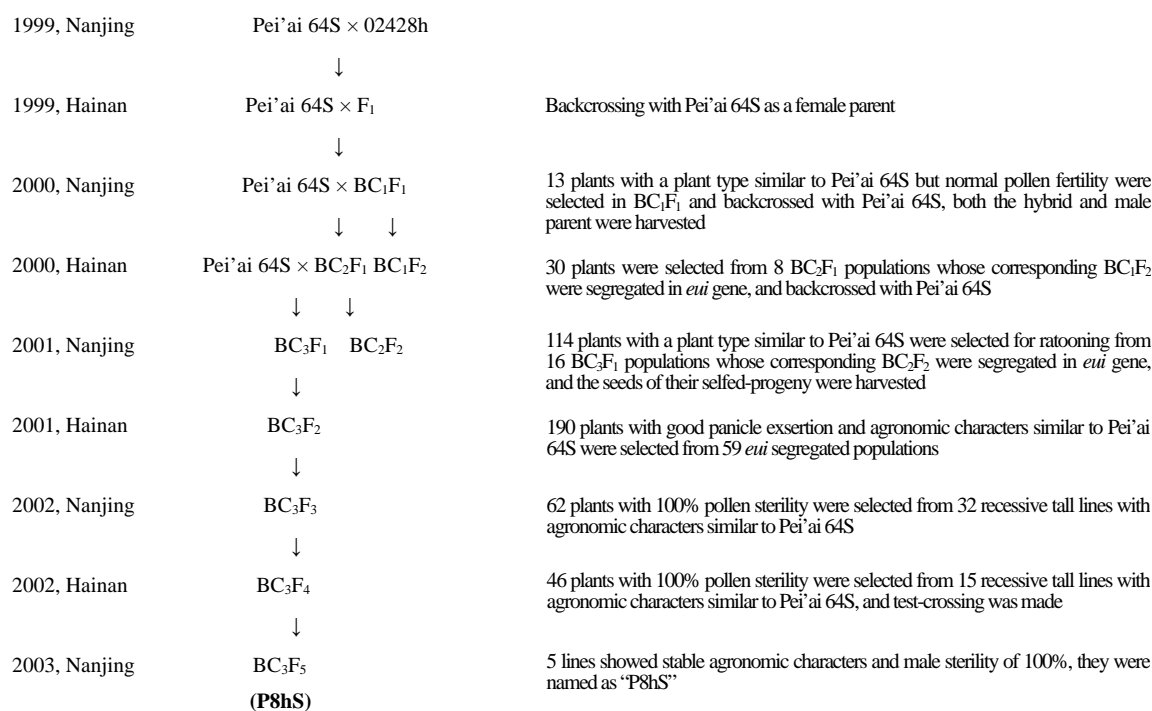


Fig. 1. Breeding process of P8hS.

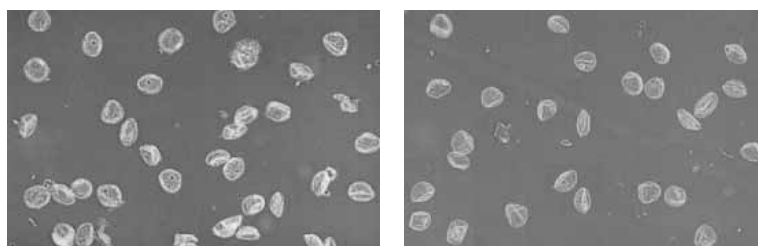


Fig. 2. Pollen fertility of P8hS (left) and Pei'ai 64S (right) in sterile period.

was normal. In BC₃F₅, all of the 46 selected lines were sterile. Among them, 5 lines showed stable sterility with 100% sterile plants and 100% pollen sterility. All the sterile pollens were typical abortive as same as Pei'ai 64S (Fig. 2).

Comparison of main agronomic traits between P8hS and Pei'ai 64S

The plant height of the new TGMS line P8hS with *eui* gene was 35.6 cm higher than that of Pei'ai 64S (Table 1). The increasing in plant height was mainly due to the elongation of the uppermost and the second internodes from the top. Compared with Pei'ai 64S, the length of the uppermost and the second internodes was 28.7 cm and 12.3 cm longer, respectively. However, the third, fourth and fifth internodes from the top were shorter. In Pei'ai 64S, 54% of the panicle was enclosed in the sheath of the flag leaf, while in P8hS, the whole panicle was exerted from the sheath of the flag leaf with a panicle neck length of 13.8 cm

due to the significant elongation of the uppermost internode (Fig. 3). The panicle length of P8hS was 2.2 cm longer with 35 more spikelets per panicle than Pei'ai 64S, but no differences were noted between the number of panicles per plant as well as the number of primary and secondary branches. In their hybrids crossed with 9311, P8hS/9311 showed significant increases in plant height, panicle length, panicle neck length, number of secondary branches, number of spikelets per panicle and number of grains per panicle compared with Pei'ai 64S/9311 (Table 2). The grain size of P8hS/9311 was larger than that of Pei'ai 64S/9311 (data not shown). The above results showed that introducing of the *eui* gene to Pei'ai 64S could not only solve the panicle enclosure problem, but also improve the main economic traits of Pei'ai 64S and its hybrid crossed with 9311.

Heredity of recessive tall in 02428h

During the entire breeding process of P8hS the

Table 1. Comparison of main agronomic traits between P8hS and Pei'ai 64S (2002, Nanjing).

Character	P8hS	Pei'ai 64S (CK)	Difference	<i>t</i> -value
Plant height (cm)	112.6	77.0	35.6**	16.32
No. of panicles per plant	10.6	10.8	-0.2	-0.18
Panicle length (cm)	25.3	23.1	2.2**	3.53
Panicle neck length (cm)	13.8	-12.6	26.4**	16.79
Length of the 1 st internode counted from the top (cm)	49.4	20.7	28.7**	20.77
Length of the 2 nd internode counted from the top (cm)	27.1	14.8	12.3**	18.94
Length of the 3 rd internode counted from the top (cm)	8.9	12.8	-3.9*	-2.38
Length of the 4 th internode counted from the top (cm)	2.6	4.0	-1.4	-2.11
Length of the 5 th internode counted from the top (cm)	0.9	1.8	-0.9*	-2.74
No. of primary branches	12.4	12.0	0.4	0.78
No. of secondary branches	49.4	46.8	2.6	1.21
No. of spikelets per panicle	242.2	206.8	35.4*	2.39

$t_{(0.05, df=8)}=2.306$, $t_{(0.01, df=8)}=3.355$.

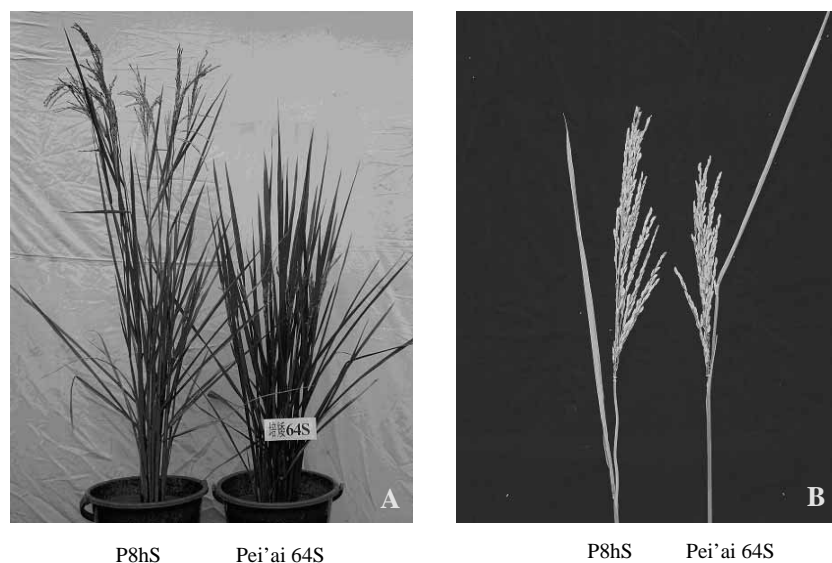


Fig. 3. Comparison of the plant type (A) and panicle extrusion (B) between P8hS and Pei'ai 64S

heredity of *eui* was also studied. In 2000, the segregation of *eui* in the F_2 population of 'Pei'ai 64S/02428h' was investigated in Nanjing and Hainan. The ratio of 680 normal to 239 *eui* and 238 normal to 86 *eui* in two populations were fit to the theoretical ratio of 3:1 by χ^2 test. Similar results were observed in the 9 BC_1F_2 and 18 BC_2F_2 populations in 2000 in Hainan and in 2001 in Nanjing (Table 3). Segregations of *eui* and normal plants in 4 test cross lines ($BC_1F_1/02428h$) in Hainan during 2000 were all fit to the 1:1 ratio (Table 4). Further more, the segregations of *eui* and normal plants in the populations of BC_1F_2 , BC_2F_2 and test cross $BC_1F_1/02428h$ and the ratio of segregated lines and non-segregated lines were 1:1 (Table 5). The above results showed that the heredity

of *eui* in 02428h was in accordance with the genetic law of a recessive gene, which is in agreement with previous reports [6-7, 13]. So the transfer of *eui* gene to other varieties by backcross would be easier. In this study, the new TGMS line P8hS was developed by three backcrossing, indicating a great efficiency in breeding program.

DISCUSSION

Utilization of the recessive tall gene *eui* in rice breeding

The *eui* gene is significantly important in

Table 2. Comparison of main agronomic traits between P8hS/9311 and Pei'ai 64S/9311 (2003, Nanjing).

Character	P8hS/9311	Pei'ai 64S/9311(CK)	Difference	<i>t</i> -value
Plant height (cm)	128.6	123.7	4.9*	2.724
No. of panicles per plant	6.3	6.2	0.1	0.075
Panicle length (cm)	25.0	22.7	2.3*	3.345
Panicle neck length (cm)	10.5	2.5	8.0**	8.726
No. of primary branches	13.9	13.4	0.5	0.700
No. of secondary branches	73.5	52.0	21.5**	7.055
No. of spikelets per panicle	253.4	176.2	77.2**	5.588
No. of grains per panicle	211.6	154.4	57.2**	4.449
Seed setting rate (%)	83.5	87.7	-4.1	-1.498
1000-grain weight (g)	25.38	25.33	0.05	0.144
Grain weight per plant (g)	20.2	17.9	2.3	0.520

$t_{(0.05, df=13)}=2.160$, $t_{(0.01, df=13)}=3.012$.

Table 3. Segregations of *eui* gene in F₂ generations.

Year	Location	Combination	Normal plants	Tall plants	$\chi^2_{(3:1)}$	<i>P</i>
2000	Nanjing	Pei'ai 64S/02428h F ₂	680	239	0.44	0.50-0.75
2000	Hainan	Pei'ai 64S/02428h F ₂	238	86	0.33	0.50-0.75
2000	Hainan	Pei'ai 64S ² /02428h F ₂	387	125	0.07	0.75-0.90
2001	Nanjing	Pei'ai 64S ³ /02428h F ₂	1280	384	3.18	0.05-0.10

Table 4. Segregations of *eui* gene in test cross of Pei'ai 64S//Pei'ai 64S/02428h with 02428h (Hainan, 2000).

Line No.	Normal plants	Tall plants	$\chi^2_{(1:1)}$	<i>P</i>
H005127	31	47	2.88	0.05-0.10
H005129	34	27	0.59	0.25-0.50
H005132	36	27	1.02	0.25-0.50
H005134	53	49	0.09	0.75-0.90
Total	154	150	0.03	0.75-0.90

Table 5. Segregations of *eui* gene in test cross and F₂ lines.

Year	Location	Combination	Segregated lines	Non-segregated lines	$\chi^2_{(1:1)}$	<i>P</i>
2000	Hainan	Pei'ai 64S ² /02428h F ₂	9	4	1.23	0.25-0.50
		Pei'ai 64S//Pei'ai 64S/02428h//02428h	4	5	0.00	>0.90
2001	Nanjing	Pei'ai 64S ³ /02428h F ₂	18	12	0.83	0.25-0.50

genetics, breeding, phytophysiology and biochemistry research. Due to independent heredity of *eui* gene with other genes controlling the plant height in rice, the transfer of *eui* gene into other varieties by backcrossing is convenient. Shen et al [16], Virmani et al [18], and Liang et al [19] solved the panicle enclosure of CMS lines by transferring the *eui* gene to CMS lines via backcrossing. In previous research Yang et al [17], and Zhou et al [20] developed a breeding method to obtain maintainer, restorer lines and TGMS lines with *eui* by radiation.

Among the *eui* mutants yet developed, 76:4512, 02428h and 02428ha were japonica type, Grlc, Mh-1, eB-1 and eB-2 were indica type, and Mh-1, eB-1 and eB-2 were obtained through radiation. 02428h was a mutant obtained from somaclonal variation of a wide compatibility japonica variety 02428. The 02428h possessing both recessive *eui* and wide compatibility gene (WCG), plays an important role in heterosis between indica and japonica. The utilization of inter-subspecies heterosis would be possible through the development of wide compatibility sterile line with *eui* gene by transferring *eui* gene and WCG into

CMS line. Such CMS line could not only solve its panicle enclosure, but also widen the spectrum of crossing parents.

Significance of P8hS in rice breeding

Pei'ai 64S is an elite TGMS line with excellent agronomic characters and less critical temperature shifting from sterile to fertile. The crossing of Pei'ai 64S with other restorer lines resulted in development of several hybrids such as Liangyoupeijiu, Liangyou E32. Liangyoupeijiu is known as the pioneer of "super hybrid rice" in China, while Liangyou E32 possesses the ideal plant type of super hybrid rice. However, in Pei'ai 64S a serious kind of panicle enclosure problem was observed. In the seed production of the hybrid, Pei'ai 64S displayed that half of the panicles would be enclosed in the sheath of the flag leaf and the seed setting rate would be very low if the plant was not treated with GA₃. Therefore, the GA₃ must be used to improve the panicle extrusion of Pei'ai 64S so as to increase the seed setting rate during hybrid seed production. However, Pei'ai 64S is insensitive to GA₃,

so the application rate of GA₃ must be more than 450 g/ha to effectively overcome the panicle enclosure problem. This increased the cost of hybrid seed production and the farmer's burden, reduced seed quality and brought environmental pollution. In this research we found that the TGMS line P8hS with *eui* gene had a better panicle exertion, with similar traits on main agronomic characteristics and pollen fertility to Pei'ai 64S. The hybrid combination P8hS/9311 had better agronomic traits than Liangyoupeijiu. Therefore, the development of P8hS will bring a great technical progress in hybrid seed production of Pei'ai 64S.

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