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Full Length Research Paper

Elongation of the uppermost internode for Shuangdi Pei eS, TGMS rice with eui gene

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Shuangdi Pei eS, a thermo-sensitive genic male sterile (TGMS) rice line with *eui* gene is derived from TGMS rice line Shuangdi S by irradiation with 350 Gy⁶⁰Co γ-ray. To elucidate the uppermost internode elongation of TGMS line with *eui* gene, Shuangdi Pei eS and its original TGMS line Shuangdi S were used to study the effects of temperature on panicle exsertion. At 25°C, the uppermost internode of Shuangdi Pei eS had the quickest elongation from the 4th day before flowering to 0 day (flowering), being 2.1-fold as that of Shuangdi S, whereas it elongated slowly during the 12th day to the 4th day before flowering and the 1st to the 3rd day after flowering. The uppermost internode of Shuangdi Pei eS exserted from the flag leaf sheath at 23, 25 and 27°C, respectively, and the length of elongated uppermost internode increased with the decreasing temperatures. At 30°C, though the panicles of Shuangdi Pei eS were still enclosed in the leaf sheath, the degree of panicle enclosure was significantly lower compared with Shuangdi S. Cytological studies on Shuangdi Pei eS which showed that the uppermost internode elongation was attributed to the increase of cell number and cell elongation, and the latter was more significant. Moreover, the numbers of outermost and innermost parenchyma cells and the cell length of the uppermost internode reduced with the increasing temperatures.

Key words: Rice (*Oryza sativa* L.), thermo-sensitive genic male sterility (TGMS), elongated uppermost internode gene (*eui*), panicle exsertion, temperature.

INTRODUCTION

Rice is an important cereal crop. In China, it is planted in 15.3 to 16.8 million ha, constituting nearly 30% of the cereal crop planting areas and contributing 42% of the total grain production. Yield of rice is 45% higher than the average yield of all other grain crops, due to the introduction of hybrid rice, which is one of the top achievements of modern crop breeding (Luo, 2002). The rice male sterile lines commonly have a defect in the growth of internodes, especially, the uppermost internode, which causes panicle enclosure in leaf sheaths at the heading stage. The genetic defect of male sterile lines has a serious effect on both multiplication of male sterile lines and the production of hybrid seeds. In agriculture, exogenous GA3 treatment has been applied to stimulate panicle emergence. However, the GA₃ application not only requires skilled practice, proper spraying time and clear weather conditions, but also increases seed production cost, along with environmental pollution and seed quality reduction (Yang, 1999).

The poor panicle exsertion of rice male sterile lines has been paid close attention. Rutger and Carnahan (1981) firstly found an elongated uppermost internode recessive mutant named 76:4512 in an M9/Terso F₃ population, and named the recessive gene as elongated uppermost internode gene (eui). After that, the eui stock was introduced and engaged in further studies (Shen et al., 1987; He and Shen, 1991). Yang et al. (2002) obtained several eui mutants by γ-ray irradiation, such as Zhenshan 97eA, Zaoxian 121eA, Pei'ai 64eS, Xiegingzao eA (1) and II-32eA (1). Shuangdi Pei eS, a thermosensitive genic male sterile (TGMS) line with eui gene, is derived from Shuangdi S by irradiation with 350 Gy⁶⁰Co y-ray, and its panicle exsertion was 13.8 cm longer than that of Shuangdi S (Xu et al., 1999; Zhou et al., 2000). The critical temperature inducing sterility of Shuangdi Pei eS is 22°C. The thermo-sensitive period induces the expression of thermo-sensitive gene and eui gene during

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the pollen mother cell formation to pollen mother cell meiosis stages in Shuangdi Pei eS (Xiao et al., 2005). The genetic analysis revealed that Shuangdi Pei eS were controlled by a single recessive gene, and the *eui* gene was allelic to the *eui1* in Xieqingzao eB-1 (Liang et al., 2004). In this study, we chose Shuangdi Pei eS as material to elucidate the elongation process and the cytological mechanism of the elongated uppermost internode.

MATERIALS AND METHODS

Rice materials

The rice material Shuangdi Pei eS, a TGMS line with *eui* gene, is derived from the TGMS rice line Shuangdi S by irradiation with 350 Gy⁶⁰Co γ-ray. The original TGMS line Shuangdi S was used as the check, which was received from the Hunan Institute of Biology, Changsha city, Hunan Province, P.R. China.

Temperature treatments

The pot experiment was conducted in 2010 at the Department of Life Science, Hunan University of Arts and Science, Changde, China. The rice materials Shuangdi Pei eS with eui gene and its original line Shuangdi S were sown on May 20th and transplanted into the 30 cm diameter pots with 10 plants per pot on 15 June. There were 180 pots for each rice material. The panicle developmental stages were determined according to the method of Ding (1961). During the pollen mother cell formation and meiosis stages, both the experimental materials were treated for six days (from 29th July to 3rd August) with the constant temperatures respectively and illumination of 1000 µmol/(m²·s) in a plant growth chamber. Among them, 20 pots for each material were treated at 25°C, and 8 pots for each material were at 23, 27 and 30°C, respectively. Each treatment had three replications. Before and after treatment, the materials were grown under natural high temperature, and the natural temperature treatment was regarded as control. The natural daily mean temperature was 29.30 ± 1.86°C from 20th July to 13th August, 2010. At 25°C, the length of the 1st internode from the top (the elongated uppermost internode) was measured at the 12th, 8th and 4th day before flowering, 0 day (flowering) and the 3rd day after flowering. Under the treatments of 23, 25, 27 and 30°C, the anthers were collected just before flowering, and the pollen grains suspended in 2% potassium iodide solution (I2-KI). The pollen with normal spherical shape and dark blue color was referred to as fertile, otherwise as sterile. The panicles with 95% sterile pollen grains were tagged, and then the flowering date was recorded with proper level on plants. When the 1^{st} internode from the top ceased elongating, the panicle exsertion, the lengths of the 1^{st} , 2^{nd} , 3^{rd} top most internodes were measured, from 50 culms per material.

Preparation and observation of internode sections

The elongated uppermost internodes of the treated Shuangdi Pei eS and Shuangdi S were excised into several segments (1.5 cm per segment). They were fixed in Formalin-Acetic acid-Alcohol (FAA) solution, dehydrated in ethanol, cleared in xylene, and embedded with paraffin. In order to observe the samples clearly, they were then cut into sections before dyeing with 1% safranine and aniline blue. According to the mean length of parenchyma cells, the internodes were divided into three sections according to the method

as follows: namely the middle section (the mean length of parenchyma cells >50 μm), the top section (from the middle to the top node, the mean cell length <50 μm) and the basal section (from the middle to the second node from the top, the mean cell length <50 μm). The parenchyma cells in the samples were observed under a microscope, and the number of cells recorded, as well as, the lengths of cells were measured with a micrometer, and then the representative cells were photographed with an OLYMPUS BH-2 micro-pickup camera (Zhong et al., 2005).

Data analysis

Data of the internode length, panicle exsertion, stainable pollen rate, selfed seed setting rate, cell length were gained with standard statistics methods. The comparisons of the internode length, panicle exsertion, stainable pollen rate, selfed seed setting rate, the number of cells and cell length were conducted by *t*-test between Shuangdi Pei eS and Shuangdi S. Duncan's multiple range test was done for the number and length of cells of elongated uppermost internodes under different temperature treatments for Shuangdi Pei eS.

RESULTS

Comparison of the uppermost internode lengths between Shuangdi Pei eS and Shuangdi S

Under the 25°C treatment, the uppermost internodes began to elongate at the 12th day before flowering. From the 8th day before flowering to 0 day (flowering), the elongation rate of uppermost internodes for Shuangdi Pei eS was faster than that of Shuangdi S. The fastest period for the elongation of internodes in Shuangdi Pei eS and Shuangdi S was from the 4th day before flowering to 0 day (flowering). During this period, the increased length in Shuangdi Pei eS was 5.55 cm per day and contributed 62.71% of the total length, while those in Shuangdi S were 2.65 cm per day and 56.68%, respectively. The internode elongation rate decreased significantly after flowering and ceased elongating on the 3rd day after flowering (Table 1). It indicated that the difference of the uppermost internode elongation between Shuangdi Pei eS and Shuangdi S was mainly from the 4th day before flowering to flowering, and consequently increased the length of the uppermost internode for Shuangdi Pei eS 2.1 times per day by contrast with the Shuangdi S.

Comparison of the number of parenchyma cells and the length of uppermost internodes between Shuangdi Pei eS and Shuangdi S

The differences in the number of parenchyma cells and the length of the uppermost internode between Shuangdi Pei eS and Shuangdi S were not significant on the 12th day before flowering (Table 2). With the panicle development, the differences between the two materials were significant on the 4th day before flowering and highly significant after flowering. During the 12th day before flowering to 0 day (flowering), the parenchyma cells of the

Table 1. Lengths of the uppermost internode at different stages under 25°C treatment.

| Developmental stage | Shuangdi Pei eS | | | Shuangdi S | | | |
|-------------------------------|--|--------------------------|-----------------------|---------------------------------|--------------------------|-----------------------|--|
| | Uppermost internode length (cm) | Increasing length (cm/d) | Increasing rate a (%) | Uppermost internode length (cm) | Increasing length (cm/d) | Increasing rate a (%) | |
| The 12th day before flowering | 0.17±0.12(0.12±0.02) ^b | - | - | 0.15±0.16(0.13±0.07) | - | - | |
| The 8th day before flowering | 2.30±0.13(1.54±0.09) | 0.53(0.36) | 6.02(5.61) | 1.40±0.18(1.20±0.12) | 0.31(0.27) | 6.68(5.94) | |
| The 4th day before flowering | $(12.40\pm0.35)*(8.58\pm0.61)*$ | 2.53(1.76) | 28.53(27.83) | $7.00\pm0.47(6.07\pm0.45)$ | 1.40(1.23) | 29.95(27.06) | |
| 0 day (flowering) | $(34.60 \pm 1.29)^{**}(24.74 \pm 1.34)^{**}$ | 5.55(4.04) | 62.71(63.87) | 17.60±1.52(15.30±1.53) | 2.65(2.31) | 56.68(51.28) | |
| The 3rd day after flowering | $(35.40 \pm 1.68)^{**}(25.30 \pm 1.46)^{**}$ | 0.20(0.14) | 2.26(2.21) | 18.70±1.75(18.00±1.84) | 0.28(0.68) | 5.88(15.00) | |

^a The increasing rate indicates the proportion of the mean increasing length of internodes between the two different developmental stages to the whole length of internodes; ^b Data in brackets are results under natural conditions with a natural daily mean temperature of 29.30 ±1.86°C from 20 July to 13th August, 2010; * and ** indicate significant difference at 0.05 and 0.01 levels, respectively, by *t* test, between values under the 25°C treatment and the natural conditions.

Table 2. Numbers and length of parenchyma cells at different developmental stages under 25°C treatment.

| Developmental stage | Material | Length of uppermost internode (cm) | Number of parenchyma cells (entries) | Length of parenchyma cell (µm) | |
|---|-----------------|------------------------------------|--------------------------------------|--------------------------------|--|
| The 10th day before flavoring | Shuangdi Pei eS | 0.22±0.02 | 221 | 10.6±0.53 | |
| The 12 th day before flowering | Shuangdi S | 0.19±0.04 | 198 | 10.2±0.38 | |
| The Oth day he fam flavoring | Shuangdi Pei eS | 2.38±0.10 | 1452 | 16.3±0.66 | |
| The 8 th day before flowering | Shuangdi S | 1.82±0.11 | 1133 | 16.1±0.68 | |
| The 4 th day before flowering | Shuangdi Pei eS | 13.28±0.82* | 2692* | 49.2±0.58* | |
| | Shuangdi S | 7.82±0.44 | 1962 | 39.6±0.71 | |
| O day (flavoring) | Shuangdi Pei eS | 33.38±2.22** | 4042** | 82.2±1.41** | |
| 0 day (flowering) | Shuangdi S | 16.18±1.55 | 3251 | 49.5±1.18 | |
| The Ord days the Alexander | Shuangdi Pei eS | 35.82±2.33** | 4249** | 84.1±1.65** | |
| The 3 rd day after flowering | Shuangdi S | 18.82±1.87 | 3518 | 53.2±1.52 | |

^{*} and ** indicate significant difference at 0.05 and 0.01 levels, respectively, between Shuangdi Pei eS and Shuangdi S by t test.

uppermost internode for Shuangdi Pei eS were 791 more in number and 32.7 μ m longer in length than those for Shuangdi S; after flowering, they were 731 more and 30.9 μ m longer for Shuangdi

Pei eS than those for Shuangdi S. The increases in the number and length of parenchyma cells of uppermost internodes were slow after flowering, and stopped at the 3rd day after flowering. It

suggested that the elongation of the uppermost internode in Shuangdi Pei eS was attributed to cell division and elongation, especially, the cell elongation from the 12th day before flowering to

Table 3. Comparison of panicle exertions, lengths of internode between Shuangdi Pei eS and Shuangdi S under different artificial temperature conditions.

| Treatment | Length of the 1st internode from the top (cm) | Length of the 2 nd internode from the top (cm) | Length of the 3 rd internode from the top (cm) | Panicle exertion(cm) | Stainable pollen rate (%) | Selfed seed setting rate (%) |
|--|---|---|---|----------------------|------------------------------|------------------------------|
| 23°CShuangdi Pei eS | 37.62 ± 1.25** | 19.60 ± 1.03* | 11.33 ± 0.58 | 7.35 ± 2.14** | 1.43 ± 1.47 | 0.00 ± 0.00 |
| Shuangdi S | 23.10 ± 1.69 | 15.25 ± 1.13 | 10.10 ± 0.61 | -8.30 ± 0.67 | 1.35 ± 1.25 | 0.00 ± 0.00 |
| 25°C Shuangdi Pei eS | 35.42 ± 1.24** | 19.30 ± 1.35* | 9.87 ± 0.68 | 4.49 ± 1.86** | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Shuangdi S | 21.20 ± 1.59 | 15.12 ± 1.45 | 9.65 ± 0.59 | -11.20 ± 1.35 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 27°C Shuangdi Pei eS | 32.30 ± 1.22** | 19.20 ± 1.46* | 9.91 ± 0.49 | 1.01 ± 0.87** | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Shuangdi S | 20.36 ± 1.61 | 14.97 ± 1.51 | 9.05 ± 0.50 | -14.44 ± 0.85 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 30°C Shuangdi Pei eS | 26.30 ± 1.26 * | 18.64 ± 1.55* | 9.65 ± 0.78 | -5.65 ± 2.25** | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Shuangdi S | 17.95 ± 1.45 | 14.68 ± 1.57 | 8.29 ± 0.71 | -16.55 ± 1.35 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Natural condition ^a Shuangdi Pei eS | 25.25 ± 1.16* | 18.47 ± 1.60* | 9.59 ± 0.67 | -5.56 ± 2.24** | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Shuangdi S | 17.93 ±1 .35 | 14.46 ± 1.59 | 8.17 ± 0.61 | -16.65 ± 1.26 | 0.00 ± 0.00 | 0.00 ± 0.00 |

^{*} and ** indicate significant at 0.05 and 0.01 levels, respectively, between. Shuangdi Pei eS and Shuangdi S by t test. Natural daily mean temperature was 29.30°C±1.86°C from 20 July to 13 August, 2010.

flowering.

Effect of different temperature treatments to panicle exsertion during thermo-sensitive period

As shown in Table 3, during the thermo-sensitive period, when Shuangdi Pei eS was treated with the constant temperatures of 23, 25, 27 and 30°C respectively, the panicle exsertion was in positive values at 23, 25 and 27°C, respectively. The lower the temperature, the greater the panicle exsertion was. At 30°C, although, some panicles of Shuangdi Pei eS were enclosed in the sheaths of flag leaf, the degree of panicle enclosure was significantly lower than that of Shuangdi S. Whereas Shuangdi S performed poor panicle exsertion under the four temperature treatments. The differences in the panicle exsertion and the length of uppermost internodes between Shuangdi Pei eS and Shuangdi S indicated that the eui gene could express under the treatments

of 23, 25 and 27°C, but was inhibited at a temperature of 30°C. The *eui* gene mainly promoted the elongation of internodes, especially, the uppermost internode.

Comparison of the number and length of cells in the uppermost internode for Shuangdi Pei eS under different temperature treatments

Under the different temperature treatments, the number and mean length of the cells in elongated uppermost internode for Shuangdi Pei eS decreased as elevation of temperature. At 30°C, the outermost and innermost parenchyma cells in internodes were 772 and 294 less in number, and 15.9 and 26.6 µm shorter in length than those at 23°C. The mean lengths of the outermost and innermost parenchyma cells had no significant differences in the basal and top sections at 23, 25, 27 and 30°C, while in the middle section, the mean lengths of outermost and innermost parenchyma cells were 20.0 and 37.4 µm at 30°C,

respectively, shorter than those at 23°C (Table 4 and Figure 1).

The aforementioned results indicated that the positive effect of *eui* gene on the parenchyma cell division and elongation reduces with increasing temperature, and the *eui* gene mainly promoted the elongation of parenchyma cells in the middle section of the uppermost internode.

DISCUSSION

Elongation of rice internodes is one of the most important agronomic traits for hybrid rice production which determines the plant height and underlies the grain yield. It has been shown that the elongation of internodes is under genetic control. The recessive tall characteristics of the elongated uppermost internode (eui) genotype in rice have been paid close attention since it was the eui gene as the fourth genetic element in addition to cytoplasmic male sterile line,

Table 4. Comparisons of parenchyma cell number and length in different sections of the uppermost internode in Shuangdi Pei eS under different temperature treatments.

| Dealth and and all about the dealth. | Temperature treatment | | | | | |
|---|-------------------------|------------------------|------------------------|------------------------|----------------------------------|--|
| Position and cell characteristics | 23°C | 23°C 25°C 27°C | | 30°C | - Natural condition ^a | |
| Top section | | | | | | |
| Length of section (mm) | 26.8 | 24.9 | 22.5 | 15.9 | 16.5 | |
| No. of the outermost parenchyma cells (entries) | 640 ^{aA} | 599aA | 554aA | 482 ^{bA} | 473 ^{bA} | |
| No. of the innermost parenchyma cells (entries) | 585aA | 552aA | 519aA | 422bA | 413 ^{bA} | |
| Length of the outermost parenchyma cell (µm) | 42.9±0.5aA | 42.7±0.7aA | 42.1±0.6aA | 36.8±0.4bA | 36.8±0.8bA | |
| Length of the innermost parenchyma cell (µm) | 47.1±0.2 ^{aA} | 46.5±0.3 ^{aA} | 45.1±0.4 ^{aA} | 42.7±0.3 ^{bA} | 42.6±0.5 ^{bA} | |
| Middle section | | | | | | |
| Length of section (mm) | 329.6 | 307.0 | 278.0 | 224.7 | 218.0 | |
| No. of the outermost parenchyma cells (entries) | 3964aA | 3744aA | 3682aA | 3483 ^{bA} | 3473 ^{bA} | |
| No. of the innermost parenchyma cells (entries) | 2844 ^{aA} | 2841aA | 2838aA | 2816aA | 2804bA | |
| Length of the outermost parenchyma cell (µm) | 81.6±1.7 ^{aA} | 80.5±1.7 ^{aA} | 74.0±1.6 ^{bB} | 61.6±1.7cC | 61.3±1.7°C | |
| Length of the innermost parenchyma cell (µm) | 114.5±1.9 ^{aA} | 106.7±2.1bA | 96.6±1.8cB | 77.1±1.5 ^{dC} | 76.4±1.6 ^{dC} | |
| Basal section | | | | | | |
| Length of section (mm) | 17.8 | 16.4 | 14.8 | 9.7 | 10.5 | |
| No. of the outermost parenchyma cells (entries) | 520a ^A | 507 ^{aA} | 463 ^{aA} | 383 ^{bA} | 361 ^{bA} | |
| No. of the innermost parenchyma cells (entries) | 487 ^{aA} | 477 ^{aA} | 462aA | 381 ^{bA} | 358 ^{bA} | |
| Length of the outermost parenchyma cell (µm) | 35.8±0.6 ^{aA} | 34.2±0.5 ^{aA} | 34.1±0.6aA | 31.4±0.6bA | 32.4±0.5bA | |
| Length of the innermost parenchyma cell (μm) | 38.4±0.7 ^{aA} | 36.4±0.7 ^{aA} | 34.2±0.7 ^{aA} | 31.6±0.5 ^{bA} | 32.7±0.7 ^{bA} | |
| The uppermost internode (panicle neck) | | | | | | |
| Length of internode (mm) | 378.2 | 352.3 | 319.3 | 258.3 | 249.0 | |
| No. of the outermost parenchyma cells (entries) | 5128 ^{aA} | 4854 ^{aA} | 4703aA | 4356 ^{bA} | 4311 ^{bA} | |
| No. of the innermost parenchyma cells (entries) | 3920aA | 3874ªA | 3823aA | 3626 ^{bA} | 3579 ^{bA} | |
| Length of the outermost parenchyma cell (µm) | 72.1±1.6 aA | 71.1±1.6aA | 65.3±1.6bA | 56.2±1.7cB | 55.9±1.7dB | |
| Length of the innermost parenchyma cell (µm) | 94.9±1.1 aA | 89.4±1.1aA | 82.1±1.3bB | 68.3±1.2°C | 67.8±1.4 ^{dC} | |

^a Natural daily mean temperature was 29.3 ± 1.86°C from 20th July to 13th August, 2010; within a row, data followed by the same lowercase and uppercase letters are not significant at 0.05 and 0.01 levels, respectively, by SSR method.

maintainer line and restorer line in hybrid cereal production. The utilization of eui gene can not only solve the panicle enclosure in male sterile lines, but also improve the pollination efficiency in restorer lines (Shen et al., 1987; He and Shen, 1991; Yang et al., 2002). Yang et al. (2002) found several eui mutants from maintainer lines and TGMS or photo-sensitive genic male sterile (PGMS) lines treated with γ-ray, and provided a new path for utilizing the eui gene in hybrid seed production. The eui-T(P)GMS lines can not only be used to produce hybrid seeds when it is sterile and propagate themselves when it is fertile, but also exhibit the elongation of internodes, particularly, the uppermost internode during the heading stage. Shuangdi Pei eS is a TGMS line with the recessive eui gene obtained from radiation-induced mutation, presenting stable sterility and high combining ability. Different temperatures were found inducing the full expression of male sterile gene and eui gene for

Shuangdi Pei eS, that is, the full expression of male sterility gene needs a certain high temperature. When the daily mean temperature was higher than 23°C over temperature, the expression level of male sterility was higher. Inversely, the full expression of eui gene needs a certain low temperature, when the daily mean temperature was lower than 30°C, the lower the temperature, the higher the expression level of eui gene, the better the panicle exsertion. In this study, Shuangdi Pei eS turned to be fertile when treated at 23°C, and its panicles were enclosed in the leaf sheaths when treated at 30°C. Therefore, the range of temperature in inducing the high expression of male sterility gene and eui gene of Shuangdi Pei eS should be from 25 to 27°C. When the TGMS lines with eui gene are utilized in hybrid seed production, it is necessary to select suitable regions and seasons for maintaining the optimum temperature. Many studies have showed that if the daily mean temperature is

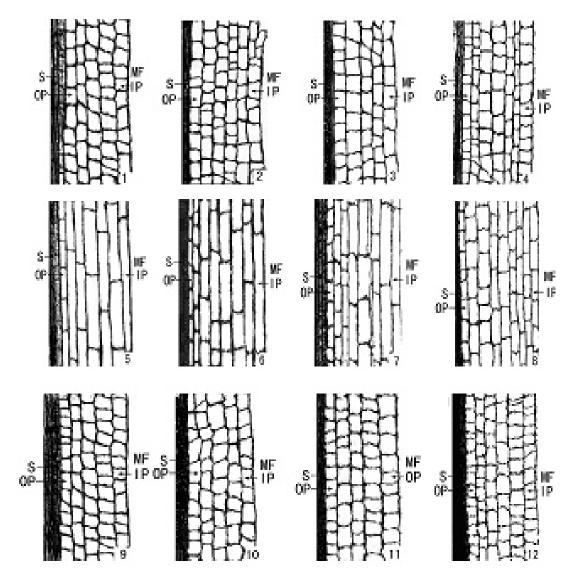


Figure 1. Comparison of uppermost internode cell in different section under different temperature; 1.Top section in 23°C(x150); 2.Top section in 25°C(x150); 3.Top section in 27°C(x150); 4.Top section in 30°C (x150); 5.Middle section in 23°C(x150); 6.Middle section in 25°C(x150); 7.Middle section in 27°C(x150); 8.Middle section in 30°C(x150); 9.Basal section in 23°C x150); 10.Basal section in 25°C(x150); 11.Basal section in 27°C x150); 12.Basal section in 30°C x150); S: sclerenchyma; MF: medullary fistula; OP: outermost parenchyma cell; IP: innermost parenchyma cell.

higher than 28°C during the thermo-sensitive period, the sprayed amount of GA_3 was 30 g/ha for the *eui*-T(P)GMS lines (Zhang et al., 2000; Zhang et al., 2001; Zhang et al., 2001), and 450 to 750 g/ha for Pei'ai 64S (Jiang, 2000; Gao et al., 2001; Chen and Wang, 2004) for elongating the internode in hybrid seed production.

By comparison of the parenchyma cells of the uppermost internodes treated with different temperatures, our research revealed that the parenchyma cells divided and elongated rapidly during the 12th day before flowering to flowering, but slow after flowering. During the rapid cell division and elongation periods, the parenchyma cells of the uppermost internodes divided and elongated faster in

Shuangdi Pei eS than in Shuangdi S, while during the slow cell division and elongation periods, there were no differences between Suangdi Pei eS and Shuangdi S in the increased number and length of cells in the uppermost internode. The results indicated that the period, in which the *eui* gene advanced cell division and elongation of the uppermost internode, was from the 12th day before flowering to flowering. There were no differences in the number and length of parenchyma cells between the basal and top sections of the elongated uppermost internodes in Shuangdi Pei eS under the four temperature treatments, but there were obvious differences in the middle section. Compared with the

outermost parenchyma cells, the elongation and division of innermost parenchyma cells promoted by the eui gene were much more obvious. The aforementioned results indicate that the function of the eui gene has position effect, and the major action site was in the middle section of the uppermost internode. Moreover, it suggests that the function in terms of the elongation of internodes was promoted by low temperature (23°C) and inhibited by high temperature (30°C) is accomplished by promoting or inhibiting expression of the eui gene, which enhances or restrains the cell division and elongation of internodes. especially, the uppermost internodes. In a word, the functional mechanism of the eui gene is complex, and it should be taken into account in both functional stage and site of the eui gene when the molecule mechanism of the gene is studied.

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