Effects of the Dwarfing Gene from Dee-geo-woo-gen and Three Others on High Temperature Tolerance at Flowering

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The effects of four dwarfing genes on the high temperature tolerance at flowering were investigated. The genes were *d-47(t)* (dee-geo-woo-gen dwarf), *d-12* (yukara dwarf), *d-18^k* (kotaketamanishiki dwarf), and *d-35(t)* (tanganbozu dwarf). The following lines were used: the isogenic lines of Shiokari, i.e., *d-47* line, *d-12* line, *d-18^k* line and *d-35* line, and the isogenic line of *d-47(t)* of Taichung 65, i.e., *d-47(T65)* line. At 35°C, *d-47* line and *d-18^k* line followed by *d-12* line showed higher spikelet fertilities but *d-35* line showed a lower spikelet fertility than Shiokari. At 37°C, *d-47(T65)* line had some fertile spikelets (8.8%), although Taichung 65 had almost no fertile spikelet. It is recognized that *d-47(t)* enhanced the high temperature tolerance under the two genetic backgrounds with different tolerances. The following trend was shown: the more the germinated pollen grains per spikelet, the higher the spikelet fertility.

KEY WORDS: *Oryza sativa* L., dwarf, high temperature tolerance, sterility, pollen

Introduction

In rice, the high temperature damage at flowering is the most serious through all growth stages (NISHIYAMA and SATAKE 1981). There are several reports on occurrences of the high temperature damage at flowering in rice: on cultivations in deserts of Iraq (ITO 1964), Egypt (SATO 1982) and Sudan (MASTUSHIMA *et al.* 1982), and on the dry season crop in the Central Plain of Thailand (OSADA *et al.* 1973). The latter was due to the high temperature during April and May, i.e., the last two months of the dry season. At the most of the meteorological observatories in Southeast Asia except Indonesia, April or May is the month of the highest temperature in the year (Japan Meteorological Agency 1990). Hence, the dry season crop involves the risk of suffering the high temperature damage. According to NISHIYAMA and SATAKE (1981), the gross area in which such damage occurred was estimated to be 4,000,000 ha, and it was foreseen that this would expand with the increase of double and triple croppings. Short culm, photoperiod insensitive and early maturing cultivars, e.g., several of the IR-number cultivars, are used for the dry season crop in many cases. Most of them inherit the dwarfing gene which originates from Dee-geo-woo-gen. It was tentatively designated as *d-47(t)* (KINOSHITA and SHINBASHI 1982, and FUTSUHARA *et al.* 1986). In this study, the authors investigated whether *d-47(t)* and three other dwarfing genes affect the high temperature tolerance at flowering or not, by using isogenic lines.

Received May 28, 1991.

Accepted November 8, 1991.

Material and Methods

1. Experiments by isogenic lines of Shiokari

The names of the dwarfing genes in this study and their gene symbols follow the proposal by the Committee on Gene Symbolization, Nomenclature and Linkage Groups (FUTSUHARA *et al.* 1986).

Shiokari, and its isogenic lines of *d-47(t)* (dee-geo-woo-gen dwarf), *d-12* (yukara dwarf), *d-18^k* (kotaketamanishiki dwarf) and *d-35(t)* (tanginbozu dwarf) were used. They were abbreviated to *d-47* line, *d-12* line, *d-18^k* line and *d-35* line with their gene symbols. The number of backcrosses was 7 in 1984 and 8 in 1985 for both of *d-47* line and *d-12* line, 11 in 1984 and 12 in 1985 for *d-18^k* line, and 10 for *d-35* line. The recurrent parent, Shiokari is of intermediate culm length and one of the leading varieties of Hokkaido around 1970 (Hokkaido Central Agricultural Experiment Station 1979). The traits of the four dwarf lines in morphological and floral characters were reported in detail by MURAI *et al.* (1982), MURAI and KINOSHITA (1983), MURAI *et al.* (1990), and MURAI *et al.* (1991). According to MURAI *et al.* (1990), *d-12* is the same as or allelic of the dwarfing gene of Fukei 71 which is resistant to lodging (FUTSUHARA 1968). *d-18^k* seems to somewhat enhance the cool temperature tolerance at the booting stage (MURAI *et al.* 1991). Tanginbozu (or Ginbozumidashi), the gene source of *d-35(t)*, recorded a high yield (857.5 kg/10a) in 1951 (UCHIYAMADA 1981), but it and its derivatives were not widely cultivated (OKADA *et al.* 1967).

The experiment in 1984 was conducted in the Arid Land Research Center, Tottori University. Shiokari and its isogenic lines except *d-35* line were grown in a vinyl house from April to June. Six seedlings were separately transplanted to a 1/5,000 a cylindrical pot in a circle. The total of fertilizers applied per pot corresponded to 0.42, 0.27 and 0.30 g for N, P₂O₅ and K₂O, respectively. High temperature treatments were conducted in artificial light growth rooms which were illuminated at about 27,000 lx from 6 a.m. to 7 p.m. Different temperatures, viz. 26, 35 and 38°C were set from 9 a.m. to 6 p.m.; the relative humidity was kept at about 70% during the nine hours. At night (7 p.m. to 6 a.m.), the temperature was set at 20°C for all the rooms of the treatments. The length of each treatment was 5 days. The treated pots were taken into and out from the rooms at about 8 p.m. The following panicles of main culms and tillers were investigated: 1) the panicles which initiated flowering on the first day of the treatments, and 2) the panicles which initiated flowering on the day just before the treatments. The spikelets which flowered before the treatments were marked with an oleaginous fet pen. The number of panicles per plant was about 5 for all the lines.

All of the experiments in this study with the exception of that in 1984 were conducted in the college of Agriculture and Veterinary Medicine, Nihon University, Tokyo. In 1985, twelve seeds were sown along the brim of a 1/10,000 a cylindrical pot which was manured with a chemical fertilizer at a level of 0.14 g for N, P₂O₅ and K₂O each. The lines were grown in natural light growth cabinets from June to September, which were set at 26°C (5 a.m. to 6 p.m.) and 20°C (6 p.m. to 5 a.m.). A high temperature treatment at 35°C from 9 a.m. to 5 p.m. for 5 days was conducted with a natural light growth cabinet in which

the average relative humidity was 77%. The panicles of the main culms, which initiated flowering on the first day of the treatment, were investigated. One to 5 spikelets per panicle which flowered after the treatment were observed on some of the panicles, and were marked with an oleaginous felt pen. On the first, third and fifth days during the treatment, about 40 spikelets per line were sampled after 6 p.m. and stored in a paper bag. The pistil in a spikelet was picked out, and was set on a slideglass. Pollen grains on stigmas were stained with cotton blue, and the number of pollen grains adhering on stigma hairs were counted under a microscope ($\times 400$). The following pollen grains were judged to be germinated: 1) a pollen tube was shown on a pollen grain, 2) an empty pollen grain because its content seemed to go out due to the elongation of its pollen tube, and 3) more than half of the content of a pollen grain seemed to go out (see Fig. 1). It was frequent that no pollen tube was shown in the cases of 2) and 3).

2. Experiments by the isogenic line of Taichung 65

The other isogenic line of *d-47(t)* was developed by the backcross of Taichung 65. The donor of *d-47(t)* was Taichung Native 1. The number of backcrosses was 7. Taichung 65 is a Japonica type cultivar developed in Taiwan before the Second World War. This line is designated as *d-47(T65)* line in this paper. Its morphological and floral characteristics were reported by MURAI *et al.* (1991).

In 1986, 12 seeds were sown along the brim of each 1/10,000 a cylindrical pot which was fertilized at a level of 0.53 g for N, K_2O_5 , and P_2O_5 each. A slow-release coated fertilizer, 15% of each element of which is readily available, was used. It was presented by MITSUI-TOATSU-FERTILIZERS, INC. They were grown in natural light growth cabinets which were set at 25°C (5 a.m. to 6 p.m.) and 19°C (6 p.m. to 5 a.m.), from May to October. A high

Fig. 1. A pollen grain, more than half of the content of which went out, in consequence of the elongation of its pollen tube (indicated by an arrow).

temperature treatment at 35°C from 9 a.m. to 5 p.m. for 4 days was conducted with a natural light growth cabinet in which the average relative humidity at 35°C was 78%.

In 1987, 8 seeds were sown along the brim of each 1/10,000 a cylindrical pot which was manured at a level of 0.49 g for N, K₂O and P₂O₅ each. Another slow-release coated fertilizer, only 5% of each element of which is readily available, was used. They were grown in natural light growth cabinets of the same temperature setting as in 1986. A high temperature treatment at 37°C from 9 a.m. to 5 p.m. for 3 days was conducted in an artificial light growth room which was illuminated at about 20,000 lx from 5 a.m. to 8 a.m. and at about 60,000 lx from 8 a.m. to 6 p.m. The relative humidity at 37°C was kept at about 77%. During the treatment, a pot of another type was set under each pot of *d*-47 (T65) line, and the positions of its panicles were raised up to those of Taichung 65.

In both years, the panicles of the main culms, which initiated flowering on the first day of the treatment and on the day just before the treatments, were investigated. The spikelets which flowered before and after the treatments were marked with black and red oleaginous felt pens, and were not involved in calculating the spikelet fertility of each panicle. On each day during the treatments, 40~50 spikelets per line were sampled after 6 p.m. and stored in a paper bag. Pollen grains were investigated in the same manner as in 1985.

(1) Panicles which initiated flowering on the first day of the treatments.

(2) Panicles which initiated flowering on the day just before the treatments.

Fig. 2. Reactions of Shiokari and its isogenic lines to high temperature in spikelet fertility in 1984.

- *d*-12 line.
- △ *d*-18^k line.
- *d*-47 line.
- Shiokari.

Results

1. Experiments by isogenic lines of Shiokari

Fig. 2 shows the reactions of Shiokari and its isogenic lines to high temperature in spikelet fertility in 1984. Shiokari showed the steepest decrease (about 50%) from 26°C to 35°C, followed by *d-12* line. On the other hand, *d-18^k* line and *d-47* line showed small or no decrease from 26°C to 35°C. At 38°C, almost all the spikelets were sterile in all the lines. In 1985, Shiokari showed a low spikelet fertility at 35°C similar to those in 1984, while *d-18^k* line, *d-47* line and *d-12* line showed higher spikelet fertilities (Fig. 3). The spikelet fertility of *d-35* line was lower than that of Shiokari. Fig. 4 shows a relation between the spikelet fertility in this study and the percentage of fertilized spikelets discerned by "iodine reaction of starch (MATSUSHIMA and TANAKA 1960)." In the former, it was tested by touching with fingers whether there was any endosperm in a spikelet or not. By using the latter method, the spikelets, the pistils of which stopped development as early as one day after fertilization, can be discerned from non-fertilized spikelets. Values in the former and latter almost agreed in all the lines both in the 35°C treatment and the control. Fig. 5 shows the

Fig. 3. Reactions of Shiokari and its isogenic lines to high temperature in spikelet fertility in 1985.

- *d-12* line.
- △ *d-18^k* line.
- × *d-35* line.
- *d-47* line.
- Shiokari.

Fig. 4. Relation between the spikelet fertility and the percentage of fertilized spikelets discerned by the iodine reaction of starch.

Symbols are the same as in Fig. 3.

number of pollen grains adhered on stigma hairs per spikelet, which was divided into 0 (no pollen grain), 1~9, 10~19, 20~29, and more than 29, and the proportion of spikelets belonging to each group was exhibited by the percentage to the total number of spikelets. In

Fig. 5. The number of pollen grains adhered on stigma hairs per spikelet in Shio-kari and its isogenic lines in 1985.

0 (no pollen grain).
 1~9.
 10~19.
 20~29.
 more than 29.
 ← Spikelet fertility.

the percentage of more than 29 pollen grains at 35°C, *d-35* line showed a value distinctly lower than the other lines. In addition, there was 13.9% of no pollen grain. These seem to result in the low spikelet fertility of *d-35* line at 35°C. Further, *d-35* line showed distinctly fewer pollen grains per spikelet in the control than the other lines. On the other hand, *d-18^k* line showed the highest percentage of more than 29 pollen grains at 35°C as well as the highest spikelet fertility at 35°C. As shown in Fig. 6, the percentage of more than 29 pollen grains at 35°C was significantly correlated with the spikelet fertility at 35°C. Fig. 7 shows the number of germinated pollen grains per spikelet, which was divided into 0 (no germinated pollen grain), 1~9, 10~19, and more than 19, and the proportion of

Fig. 6. Correlation between the spikelet fertility and the percentage of spikelets with more than 29 pollen grains on stigma hairs at 35°C in 1985.

*Significant at 0.05 level.

Symbols are the same as in Fig. 3.

spikelets belonging to each group was exhibited by the percentage to the total number of spikelets. *d-35* line showed distinctly fewer germinated pollen grains per spikelet at 35°C than the other lines. There was 22.3% of no germinated pollen grain at 35°C. In the percentage of more than 19 at 35°C, the lines were in the order of *d-18^k* line > *d-47* line > *d-12* line > Shio-kari > *d-35* line. Fig. 8 shows the correlation between the spikelet fertility and the

(1) 35°C.

(2) Control.

Fig. 7. The number of germinated pollen grains per spikelet in Shio-kari and its isogenic lines in 1985.

percentage of more than 9 or 19 germinated pollen grains at 35°C. The spikelet fertility was highly significantly correlated with the percentages, indicating that the more the germinated pollen grains the higher the spikelet fertility at 35°C.

2. Experiments by the isogenic line of Taichung 65

Table 1 shows the spikelet fertilities at 35°C and 37°C in 1986 and 1987, respectively, and those in the controls. Both of *d-47*(T65) line and Taichung 65 showed little decrease of spikelet fertility at 35°C. At 37°C, Taichung 65 had almost no fertile spikelet, but *d-47*(T65) line had some fertile spikelets (8.8%). Fig. 9 shows the number of pollen grains adhered on stigma hairs per spikelet. Both lines showed little decrease at 35°C in the number. A similar result was shown in the number of germinated pollen grains per spikelet (Fig. 10-(1)). At 37°C, on the other hand, the percentages of no pollen grain were 76.9% of Taichung 65 and 37.0% of *d-47*(T65) line (Fig. 9-(2)). Table 2 shows the percentage of pollen germination: the percentage was calculated for the spikelets, the number of adhered pollen grains of which were from 2 to 99. Both lines showed no decrease at 35°C in the percentage. At 37°C, on the other hand, the percentages of both lines were remarkably low.

Discussion

SATAKE and YOSHIDA (1978) conducted several experiments on the high temperature damage at flowering. One of those

Table 1. The Spikelet fertilities (%) of *d-47* (T65) line and Taichung 65 at 35°C and 37°C, and in the controls

Line	1886		1987	
	35°C	Cont.	37°C	Cont.
<i>d-47</i> (T65) line	94.0	95.5	8.8	97.7
Taichung 65	93.1	96.4	0.4	97.9

Table 2. The percentage of pollen germination of *d-47* (T65) line and Taichung 65 at 35°C and 37°C, and in the controls

Line	1886		1987	
	35°C	Cont.	37°C	Cont.
<i>d-47</i> (T65) line	83.0	77.6	25.9	81.1
Taichung 65	82.2	73.7	36.7	82.7

Fig. 8. Correlation between the spikelet fertility and the percentage of spikelets with more than 9 or 19 germinated pollen grains at 35°C in 1985.

*, **Significant at 0.05 and 0.01 levels, respectively. Symbols are the same as in Fig. 3.

showed that the spikelet fertilities of susceptible, moderate and tolerant varieties were about 10, 80 and 90%, respectively, at 35°C, and those of the former two at 38°C were near to 0%. At 35°C in 1985 in this study, the spikelet fertility of Shiokari was 40.5%, indicating that its tolerance is between the susceptible and the moderate. Those of *d-35* line, *d-12* line, *d-47* line and *d-18^k* line were 8.1, 62.9, 65.3 and 66.3%, respectively. In 1984, *d-12* line showed the lower fertility at 35°C than *d-47* line. Accordingly, it is inferred that *d-47*(t) and *d-18^k* followed by *d-12* enhance the high temperature tolerance but *d-35*(t) lowers it. Further, *d-47*(T65) line had some fertile spikelets at 37°C, while Taichung 65 had almost no

Fig. 9. The number of pollen grains adhered on stigma hairs per spikelet in *d-47* (T65) line and Taichung 65.

0 (no pollen grain).
 1~9.
 10~19.
 20~29.
 more than 29.
 ← Spikelet fertility.

Fig. 10. The number of germinated pollen grains per spikelet in *d-47* (T65) line and Taichung 65.

0.
 1~9.
 10~19.
 more than 29.
 ← Spikelet fertility.

fertile spikelet. Taichung 65 showed a fertility higher than 90% at 35°C. Hence, the tolerance of Taichung 65 seems to correspond to the moderate tolerance by SATAKE and YOSHIDA (1978). Thus, it is recognized that *d-47(t)* enhanced the high temperature tolerance under the two genetic backgrounds with different tolerances. However, the following possibility can not be completely denied: a gene which enhances the high temperature tolerance is closely linked with *d-47(t)* on the same chromosome.

The study of SATAKE and YOSHIDA (1978) indicated that the percentage of spikelets with more than 9 germinated pollen grains was almost identical with the spikelet fertility on the temperature range from 29 to 38°C; i.e., 10 germinated pollen grains were necessary for fertilization. A similar result was obtained by TOGARI and KASHIWAKURA (1958). It mostly applies to the results of the lines in the controls (Fig. 7 and 10). At 35°C in 1985, however, the spikelet fertility was apparently lower than the percentage of spikelets with more than 9 germinated pollen grains in each of the lines (Fig. 7), indicating that far more than 9 germinated pollen grains per spikelet were needed for fertilization. YAMADA *et al.* (1955) observed that the full length of a pollen tube was reduced on the temperature range from 30 to 40°C. Therefore, there is a possibility that the elongation of a pollen tube after germination was depressed at 35°C in Shiokari and its isogenic lines, increasing non-fertilization.

SATAKE and YOSHIDA (1978) found under high temperature conditions that anthers of the tolerant variety easily dehisced resulting in many pollen grains on a stigma; on the other hand, many anthers of the susceptible variety did not dehisce resulting in no pollen grain on a stigma. In this study, the trend was shown among Shiokari and its isogenic lines at 35°C that the more the pollen grains adhered per spikelet, the higher the spikelet fertility (Fig. 5 and 6). Further *d-47(T65)* line had more pollen grains adhered per spikelet than Taichung 65 (Fig. 9). Therefore, it is inferred that *d-47(t)* acts to somewhat facilitate anther dehiscence under high temperature conditions.

MURAI *et al.* (1991) obtained results indicating that *d-47(t)* lowers the cool temperature tolerance at the booting stage. Accordingly, it is considered that *d-47(t)* originated from *Dee-geo-woo-gen* is suited to the tropics rather than to cold climatic regions.

Acknowledgement

The authors wish to express deepest appreciation to Dr. I. SATO Professor Emeritus, Tottori University for making this study possible. They are grateful to Prof. T. SATAKE, Hokkaido Takusyoku Junior College for his valuable advice, and to Mr. Y. SATO, Mr. N. KOYAMA and Mr. F. TOMIKAWA for their cooperation in carrying out the experiments.

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イネにおける低脚烏尖由来の矮性遺伝子ならびに他の3種の矮性遺伝子が
開花期の高温耐性に及ぼす作用

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d-47 (t) (低脚烏尖矮性), *d-12* (ユーカラ矮性), *d-18^k* (小丈玉錦矮性) および *d-35* (t) (短銀坊主矮性) が開花期の高温耐性に作用を及ぼすか否かを調べた。

供試系統としては, しおかりを反復親とした同質遺伝子系統である *d-47* 系統, *d-12* 系統, *d-18^k* 系統および *d-35* 系統, ならびに, 台中65号を反復親とした同質遺伝子系統である *d-47* (T65) 系統を用いた。

1984年には, 35℃ならびに38℃処理を5日間行った。1985年ならびに1986年には, 35℃処理を5日間ならびに4日間行った。1987年には, 37℃処理を3日間行った。処理時間は, 1984年が午前9時から午後6時の9時間であった以外は, いずれも午前9時から午後5時までの8時間であった。これらの処理は, 自然光もしくは人工光の人工気象室にて行った。

1984年の35℃処理では, 各系統の稔実歩合は, *d-47* 系統 > *d-18^k* 系統 > *d-12* 系統 > しおかり, の順であった (Fig. 2)。また, 1985年の35℃処理では, *d-18^k* 系統 > *d-47* 系統 > *d-12* 系統 > しおかり > *d-35* 系統の順であった (Fig. 3)。故に, *d-47* (t) と *d-18^k* および *d-12* は開花期の高温に対する耐性を高める作用を有し, 反対に *d-35* (t) は低下させる作用を有すると推定された。35℃においては, 柱頭毛に付着する花粉が多いほど稔実歩合が高い傾向がみられた (Fig. 5および6)。また, 発芽花粉数においても同様の傾向がみられた (Fig. 7および8)。

台中65号と *d-47* (T65) 系統は, 35℃において90%以上の稔実歩合を示し, 柱頭上の花粉数や発芽花粉数も減少しなかった (Table 1, Fig. 9および10)。37℃では, 台中65号の稔実歩合が0.4%なのに対して *d-47* (T65) 系統は8.8%であった (Table 1)。そのとき, 台中65号と *d-47* (T65) 系統において, 柱頭毛に花粉の全くない穎花の割合は, それぞれ76.9%と37.0%であった (Fig. 9)。さらに, 両系統とも花粉発芽率の著しい減少がみられた (Table 2)。

以上より, *d-47* (t) は, 異なった遺伝的背景のもとで開花期の高温耐性を向上させたことが認められた。また, 本実験における耐性の差異は, 高温時に葯が裂開しやすいか否かに起因すると推定された。