Responses of Rice Genotypes Carrying Different Dwarf Genes to 
* Fusarium moniliforme* and Gibberellic Acid

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Abstract: A total of 32 rice genotypes carrying different dwarf or semi-dwarf genes were inoculated with the fungus *Fusarium moniliforme* Sheldon or treated with 50 mg l⁻¹ GA₃ in order to select resources resistant to rice bakanae disease from the dwarf materials. The length of the elongated seedlings was measured, and the percentage of death of the seedlings after transplanting to field was also counted. A significant correlation was found between the length of the seedling treated with GA₃ and disease injury by bakanae fungus. Rice materials carrying dwarf gene such as *sd₁* were not only sensitivity to GA₃ but also susceptive to rice bakanae disease. Materials carrying dwarf gene *d₁* were insensitive to GA₃ but susceptive to bakanae. On the other hand, all materials carrying *d₂₉*, *sd₆* or *sdq(t)* genes showed resistance to bakanae. The present study indicated that dwarf and semi-dwarf rice materials might be useful resources for improvement of bakanae resistance in rice breeding programs.

Key words: Bakanae disease, Dwarf gene, Gibberellic acid, *Oryza sativa* L., Rice, *Sd₁*. 

Rice bakanae, also called foolish seedling disease, caused by *Fusarium moniliforme* Sheldon, is a major disease at seedling stage of rice, spreading all over the world but mostly occurring in Asia (Ou, 1985; Rood, 2004). Seedlings infected by the fungus elongate and become weak with yellowish leaves, perishing before or after transplanting, or at early tillering stage. Rice plants after transplanting may also be infected, resulting in weak tillering and poor grain filling capacity (Ou, 1985; Jeff, 2001). Disease at a later stage usually causes a yield loss of 10~20%, and the loss could be higher than 30% at outbreak of the disease (Ito and Kimura, 1931; Ou, 1985; Rood, 2004). During the past decade, with the extension of hybrid rice and the use of new methods for seedling raising, especially the increasing application of dry seed-bed raising for hybrid rice, rice bakanae disease has become more and more serious (Li and Luo, 1997; Yang et al., 2003).

Seed treatment with the fungicides has been the most common means to control rice bakanae for a long time. In some countries such as Japan and China, seeds are soaked in water containing Carbendazim, Prochloraz, Trifumezol, Thiram or other fungicides during germination. However, these fungicides cannot prevent the bakanae infection after transplanting. Moreover, with the long-term usage of fungicides, pathogens have become resistant, leading to inefficiency of the fungicides (Zhou et al., 1994; Bhalli et al., 2001).

Great efforts have been made to identify and utilize rice germplasms with bakanae resistance. Some resistant varieties have been identified, but they appear to account for a tiny proportion of the rice germplasms. Li et al. (1993) found only one variety with high resistance to bakanae and 12 with moderate resistance. None of the 204 rice accessions analyzed by Zheng et al. (1993) showed high resistance to bakanae and only 2.5% of them had moderate resistance. Similarly, a few accessions were reported to have high resistance to bakanae in other studies (Li et al., 1994; Lx, 1994; Khokhar and Jaffrey, 2002).

One obvious symptom caused by *Fusarium moniliforme* is spindly growth of plant seedlings, which results from the secretion of gibberellic acid (GA₃) from the fungus. It is well known that rice lines carrying the semidwarf allele *sd₁* are sensitive to GA₃ at the seedling and tillering stages (Kumar, 1984; Li et al., 2005), and this allele is present in most of the modern *indica* rice varieties, hybrid rice as well as half of the *japonica* varieties (Zhu and Li, 1984). Nonetheless, more than 60 dwarf genes and 12 semidwarf genes that are non-allelic to *sd₁* have been reported (Kinoshita, 1995); some of which are insensitive to GA₃. These germplasms may provide a valuable resource for the screening of materials showing resistance to bakanae.
disease. However, whether rice materials with different dwarf or semidwarf genes are resistant to bakanae is unknown.

The objective of this study is to compare the response of different dwarf or semidwarf lines of rice to inoculation with Fusarium moniliforme and treatment with gibberellic acid for facilitating selection of genetic resources with the resistance to bakanae disease from the materials with dwarf or semidwarf genes nonallelic to sd1.

Materials and Methods

1. Rice materials

A total of 32 rice genotypes in two sets were used in this study (Table 1). One set consisted of 24 lines, including 10 pairs of lines carrying a single dwarf or semidwarf gene sd1, d1, d29, d32, sdg, sdq(t), sdg(t), D53, sd6 and dct(t), one pair carrying the gene combination sd1, sdq(t) and sd1, sdq(t), and one pair carrying multiple dwarf genes. The other set consisted of eight lines (Table 2), including three semidwarf lines carrying sd1, Gui-chao 2, Gang 46B and Zhe-fu 802, their near isogenic lines carrying both sd1 and another recessive tall gene, eui, which is responsible for elongated upmost internode, Me-1, Gh-2 and Eui 802, and two wild-type tall lines Nan-jing No. 6 and Bei-ken-zi (Ma et al., 2003).

2. Pathogen inoculation and GA3 treatment

Stems of the rice plants showing symptoms of

Table 1. Response of 24 rice lines with different dwarf and semidwarf genes to GA3 and to inoculation with bakanae fungus.

| Rice accession | Abb. | I/J | Dwarf gene | Control (cm) | GA3 (cm) | Increment (%) | Bakanae (cm) | Increment (%) | Seedling mortality (%) |
|----------------|------|-----|------------|-------------|----------|--------------|-------------|--------------|------------------------|
| Taichung 65-sd1| T65- | J sd1| 2.88       | 4.81        | 67.0 g    | ** 64.6 b    | 80.3 ab      |
| Dee-gco-woo-gen| DGWG| I sd1| 4.54       | 9.43        | 107.7 cd  | 70.4 ** 55.1 c | 63.3 c-e    |
| Xue-heai-zao | XHA | J d1 | 3.16       | 3.86        | 22.2 mn   | 3.82         | 20.9 k     | 76.7 b-d   |
| Xue-heai-zao improvement | XHA | I d1 | 3.85       | 4.1        | 6.5 n    | 3.09         | -19.7 o | 100 a      |
| KL803-29      | KL803| J d29| 8.53       | 12.97 *    | 52.1 j1   | 9.37         | 9.9 k-n | 0 f        |
| KL803-29 improvement | KL803 | I d29 | 7.27       | 9.92 *     | 36.5 l-m  | 8.48         | 16.6 k-m | 0 f        |
| KL804-32      | KL804-32 | J d32 | 3.67       | 5.57 **    | 51.8 j1   | 4.93 *       | 34.3 fh   | 80 bc      |
| KL804-32 improvement | KL804 | I d32 | 6.56       | 11.31 **   | 72.4 f-g  | 8.66 *       | 32.0 gh   | 76.7 b-d   |
| Xin-gui-ai    | XGA | I sdg| 2.51       | 4.16 *     | 65.7 h-j  | 2.78         | 10.8 h-n | 0 f        |
| Jing-gui-ai   | JGA | J sdg| 4.1        | 6.25 *     | 65.5 j1   | 5.88 *       | 43.4 d-f  | 0 f        |
| Xin-qian-ai   | XQA | I sdq(t) | 6.3 | 9.10 ** | 44.4 kl | 6.63 | 5.2 n | 0 f |
| Xin-qian-ai 2 | XQA2 | I sdq(t) | 5.57 | 8.75 * | 57.1 i-k | 6.5 | 16.7 k-m | 0 f |
| Xin-teai      | XTA | I sde(t) | 7.77 | 14.63 * | 88.3 d-f | 10.10 * | 30.0 g | 76.7 b-d |
| Xin-teai 2    | XTA2 | I sde(t) | 6.63 | 13.06 ** | 97.0 c-e | 9.28 ** | 40.9 e-g | 63.3 c-e |
| CBR653        | CBR653 | J D53 | 2.6 | 5.37 * | 106.4 cd | 3.83 ** | 47.3 cd | 100 a |
| CBR653 improvement | CBR653 | I D53 | 4.77 | 8.98 * | 88.3 ef | 7.23 ** | 51.6 c-e | 100 a |
| CBR34        | CBR34 | I sd6 | 4.96 | 6.21 | 25.2 m | 5.3 | 6.9 mn | 0 f |
| CBR34 improvement | CBR34 | I sd6 | 2.97 | 3.67 | 23.6 mn | 3.37 | 13.5 k-n | 0 f |
| Conglie-ai   | CLA | I dct(t) | 5.05 | 12.80 ** | 153.5 b | 8.64 ** | 71.1 a | 100 a |
| Conglie-ai improvement | CLA-1 | I dct(t) | 3.68 | 10.91 ** | 196.5 a | 7.86 ** | 113.6 b | 100 a |
| Qian-nong    | QN | I sd1sdq(t) | 7.18 | 13.26 ** | 84.7 e-g | 10.42 ** | 45.1 c-e | 50 c |
| Teai        | TA | I sd1sdq(t) | 6 | 12.53 ** | 108.8 c | 7.70 * | 28.3 h-j | 60 de |
| 83N1041      | 83N1041 | I Multi-dwarf | 6.63 | 11.73 ** | 76.9 f-h | 8.63 * | 30.2 g | 63.3 c-e |
| B5580A       | B5580A | I Multi-dwarf | 3.16 | 3.91 | 23.7 mn | 3.77 | 19.3 j-l | 0 f |

1 The six lines with the word “improvement” were developed by transferring the corresponding dwarfing allele to the tall mutant Zhong-xuan 5 T through backcrossing.

2 Abb=abbreviation.

3 I/J = indica/japonica subspecies.

4 * and ** indicated significant difference between the control and treatment at 0.05 and 0.01 levels, respectively.

5 Increment by GA3 (%) = (GA3 treatment – Control)/Control x 100.

6 Increment by Bakanae fungus (%) = (Inoculation with bakanae fungus – Control)/Control x 100.

7 Different letter in the column indicated significant difference at P<0.05 level.
bakanai disease caused by *Fusarium moniliforme* were collected in the summer of 2004 in the paddy field at the China National Rice Research Institute (CNRRI), Hangzhou, China. They were cut into 3 cm pieces, surface sterilized with 0.1% aqueous solution of mercuric chloride, rinsed twice with distilled water and then grown on potato dextrose agar medium. The plates were incubated at 30 ± 2°C for four days. The isolated fungus was identified according to Booth (1971) and Nelson et al. (1983). The spores of *Fusarium moniliforme* were washed with distilled water and filtrated through gauze. The concentration of the spores was measured with spectrophotometer at 560 nm according to the method of Ahmed et al. (1986) who indicated that the concentration was 1.50 × 10^5 conidia/ml.

The rice seedlings were inoculated with the fungus spores and treated with GA 3. In each growth chamber and germinated at 32°C for 36 hours in Carbendazim solution diluted in 5000 ml

Different letter in the column indicated significant difference at P < 0.05 level.

### Results

#### 1. Response of the rice lines with different dwarf genes to GA3 and bakanae fungus

Table 1 shows the response of the 24 rice lines with different dwarf genes to GA3 and bakanae fungus. The elongation was not significantly promoted by GA3 secreted from *Fusarium moniliforme* or by the disease, seedlings were transferred to the field after measurement of the length. The number of dead seedlings was counted every day. The basal nodes of the dead seedlings were examined under microscope for the fungus.

#### 3. Data analysis

Analysis of variance (ANOVA) and t-test were conducted using DPS software (Tang and Feng, 1997). Least significant differences for comparison of means were computed at P < 0.05.

### Table 2. Response of the *eui* recessive and wild-type tall-plant genotypes rice materials to GA3 and to inoculation with bakanae fungus

| Rice accession | Abb. | I/J | Dwarf gene | Control (cm) | GA3 (cm) | Increment (%) | Bakanae (cm) | Increment (%) | Seedling mortality (%) |
|----------------|------|----|------------|-------------|----------|--------------|-------------|--------------|------------------------|
| Mh-1           | Mh1  | I  | eui/sd1    | 5.27        | 10.90 ** | 106.8 b      | 7.95 **     | 50.9 b       | 76.8 ab               |
| Gui-chao No.2  | GC2  | I  | sd1        | 6.7         | 8.73 *   | 30.3 f       | 8.63 *      | 28.8 d       | 43.3 b                |
| Gh-1           | Gh1  | I  | eui/sd1    | 6.15        | 11.80 ** | 91.9 c       | 10.30 **    | 67.5 a       | 73.3 ab               |
| Gang 46B       | G46B | I  | sd1        | 4.94        | 11.40 ** | 130.8 a      | 6.43 *      | 30.2 d       | 50.0 b                |
| Eui802         | Eui802 | I | eui/sd1    | 7.92        | 13.70 ** | 73.0 d       | 11.10 **    | 40.2 c       | 83.5 a                |
| Zhe-fu 802     | ZF802 | I  | sd1        | 6.7         | 15.00 ** | 123.9 a      | 8.07 *      | 20.4 e       | 60.0 ab               |
| Nan-jing No.6  | NJ6  | I  | Wild type  | 7.17        | 11.00 ** | 53.4 e       | 7.53        | 5.0 g        | 0 c                   |
| Bei-ken-zi     | BK   | J  | Wild type  | 5.31        | 10.46 ** | 97.0 c       | 5.95        | 12.1 f       | 0 c                   |

1 Abb=abbreviation.
2 I/J=j-indica/japonica subspecies.
3 * and ** indicated significant difference between the control and treatment at 0.05 and 0.01 levels, respectively.
4 Increment by GA3 (%) = (GA3 treatment − Control)/Control × 100.
5 Increment by Bakanae fungus (%) = (Inoculation with bakanae fungus − Control)/Control × 100.
6 Different letter in the column indicated significant difference at P < 0.05 level.
two lines carrying sdq (t) (XQA and XQA –2) and one of the two lines carrying sdg (XGA). The elongation was significantly promoted in the remaining 14 lines, including the five pairs of lines carrying sd1, d32, sde (t), D53 and dc (t) genes, the two lines carrying double-dwarf genes, one line carrying sdg and one line carrying multi-dwarf genes (Table 1). The percentage of increment of seedling length caused by bakanae fungus was much higher in CLA and CLA-I with dc (t) gene, T65-sd1 with sd1 gene, CRH6 and CRH-I with D53 gene, and QN with sd1/sdg(t) double genes than in other genotypes (Table 1). Seedling mortality of 13 lines (except JGA) was higher than 50% after they were transplanted to the field, which suggested that bakanae resistance could be evaluated during seedling stage from the elongated length of seedlings after inoculation.

Note that most seedlings of XHA and all seedlings of XHA-I, which carried the dl gene were insensitive to both GA3 and bakanae, but they died after transplanting. Observation under a microscope indicated that the seedlings were truly infected with Fusarium moniliforme. It is possible that the expression of dl gene induced the lack of α-subunit of the heterotrimeric G-protein (Ga) for GA signal transduction, thus retarded the transmission of GA3, (Ueguchi-Tanaka et al., 2000), and did not promote the elongation of seedlings.

2. The response of eui recessive and wild-type genotypes to GA3 and bakanae disease

The response to GA3 was significant (P<0.05) in the semidwarf variety GC2 and highly significant (P<0.01) in all the other genotypes (Table 2), whereas the response to bakanae disease varied greatly among different genotypes. The elongation promoted by the fungus inoculation was not significant in the wild-type tall-plant genotypes, but significant in the genotypes carrying sd1, and highly significant in the eui recessive tall-plant genotypes. Similar variations were observed in the seedling mortality after transplanting to the field. All the seedlings of the wild-type tall-plant genotypes grew normally, whereas the semidwarf genotypes and eui genotypes had high mortalities of 43.3~60.0% and 73.3~83.3%, respectively (Table 2). This indicated that all the six rice lines carrying sd1 were susceptible to bakanae disease, but the wild-type tall genotype was resistant.

Discussion

In General, modern rice cultivars carrying the sd1 semi-dwarf gene are sensitive to GA3, while lines carrying dwarf gene dl are insensitive to GA3, at least at a low GA concentration (Kumar, 1984; Ueguchi-Tanaka et al., 2000; Li et al., 2005). No matter whether other semidwarf genes or the recessive tall gene eui were present (Tables 1 and 2), all lines carrying sd1 were highly sensitive to GA3 in the present study. Nevertheless, four lines carrying either sdq (t) or sdg were also sensitive to GA3, which was different from the results of Li et al. (2005). This may have resulted from different GA treatment methods: we treated the seeds at germination stage by soaking them in 50 mg l⁻¹ GA3, whereas Li et al. (2005) treated the seedling at 2-leaf stage or adult stage by spraying 50 to 400 mg l⁻¹ of GA3. The inconsistency suggested that the response of rice plants to GA3 treatment might be varied depending on the growth stage of rice, the concentration of GA3 and the method of GA3 treatment.

The GA3-sensitive rice plants displayed similar symptom, i.e. elongated but weak plant, when inoculated with the fungus Fusarium moniliforme Sheldon., because GA was secreted by the fungus (Table 2). Thus, highly significant correlation between the response to GA3 and bakanae inoculation was observed among the 24 rice lines with different semidwarf genes. Except for the wild-type tall-plant genotypes (NJ6 and BK) and the two carrying dwarf gene dl (XHA and XHA-I), all other lines sensitive to GA3 were also susceptible to bakanae disease (Tables 1 and 2). He and Li (1994) reported that rice lines carrying both eui and sdl were sensitive to GA3 treatment, and furthermore had higher levels of the endogenous GA3 and IAA than other genotypes. It seems that the sensitivity to GA3 treatment of eui and sdl were additive, which may account for significant elongation caused by bakanae fungus in the three rice carrying eui/sd1 (Table 2).

Microscopic observation confirmed that the death of the plants was truly caused by infection with bakanae fungus. The bakanae fungus is known to release fusaric acid, hydro-fusaric acid and other toxic substances to retard the growth of the seedling, and ultimately result in the death of the seedlings (Ahmed et al., 1986; Zheng et al., 1993; Jeff, 2001).

Screening for resistance to rice bakanae disease has been underway since 1990s. A few lines were reported to have high resistance to bakanae in the studies of Li et al. (1994), Lv (1994) and Khokhar and Jaffrey (2002). According to our results, no matter whether other semidwarf genes or the recessive tall gene eui were present, rice lines carrying sdl were sensitive to GA3 and susceptible to bakanae disease. This may be the main reason why it is difficult to identify rice lines highly resistant to bakanae disease from germplasms with the sdl gene because this allele is present in most of the modern indica rice varieties, indica hybrid rice as well as half of the japonica varieties (Zhu and Li, 1984).

For the same reason, Lv (1994) found that indica rice showed higher resistance than indica rice, and Li et al. (1994) also found that half of japonica materials displayed moderate resistance when evaluated during the budding stage and the flowering stage. Therefore, screening and characterization of the disease resistant resources from rice germplasm carrying dwarf or
semidwarf genes rather than sd1 may be an efficient way to breed for bakanae resistance.

The present study indicated that the rice accessions carrying d29, sd6 and sdq(t) genes display resistance to bakanae disease. We also found that there is no linkage between the three dwarf genes and other negative characters such as small grain and sterility (Mackill and Rutger, 1979; Ma et al., 2003). Thus, these materials might be useful resources in rice breeding program for the improvement of resistance to bakanae.

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