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Abstract The use of a thermosensitive genic male sterility (TGMS) system in two-line hybrid rice breeding is affected greatly by the sterility instability of TGMS lines caused by temperature fluctuation beyond their critical temperatures for fertility reversion. To prevent seed production from self contamination, we have developed a system to secure seed purity using a herbicide-sensitive TGMS mutant, M8077S, obtained by radiation. Genetic analysis, using the F1, F2 and F3 populations derived from this mutant and other normal varieties, revealed that bentazon lethality/sensitivity was controlled by a single recessive gene, which was named bel. The mutant can be killed at the seedling stage by bentazon at 300 mg/l or higher, a dosage that is safe for its F1 hybrids and all other normal varieties. This mutant is also sensitive to all the tested sulfonylurea herbicides. Response of segregating plants to these two types of herbicide indicated that sulfonylurea sensitivity was also controlled by bel. By crossing this mutant with Pei-Ai 64S, an F2 population was developed for genetic mapping. Surveying the two DNA pools from sensitive and non-sensitive F2 plants identified four markers that were polymorphic between the pools. The putative linked markers were then confirmed with the F2 population. The bel locus was located on chromosome 3, 7.1 cM from the closest microsatellite marker RM168. Phenotypic analysis indicated that the bel gene had no negative effect on agronomic traits in either a homozygous or heterozygous status. The mutant M8077S is valuable in the development of a TGMS breeding system for preventing impurity resulting from temperature fluctuation of the TGMS. Several two-line hybrid rice crosses using this system are under development.

Keywords Herbicide sensitivity · Genetic mapping · Mutation · Seed production · Microsatellites · Hybrid rice (Oryza sativa L.)

Introduction

Hybrid rice breeding has been very successful in China since the 1970s. With the development of photo-thermosensitive genic male sterility (P/TGMS) or environment-sensitive genic male sterility (EGMS) lines, a two-line breeding system has been developed as a simplified alternative to the traditional three-line breeding (Yuan 1992), that requires a male-sterile line, a sterility maintainer line and a fertility restorer. The two-line breeding system is much simplified since an EGMS line can serve as a sterile line under one environmental condition and can propagate itself under different environmental conditions. The ability to maintain sterility makes EGMS lines practicable as a female to cross with any other commercial rice variety. In recent years, a number of two-line hybrids have been commercialized in China, and several Asian countries have also established hybrid breeding programs using EGMS lines (Lu et al. 1994; Li and Yuan 2000).

However, most EGMS lines require a specific temperature to maintain their sterility. Abnormal weather could bring the temperature down below the critical level that is required for conversion of TGMS lines from sterility to fertility, simply called fertility conversion, which makes EGMS lines fertile or partially fertile in the location where they are supposed to be sterile in normal years. This results in a potential problem for seed production of two-line hybrid rice, i.e. an EGMS line producing seeds from selfing. The mixture of real hybrids with selfed seeds from the EGMS line cannot be used in...
rice production, resulting in a great loss to seed producers or to rice producers once false hybrid seeds are used in rice production. As an insurance for seed production, marking the seeds from EGMS lines using genetic markers could help remove the false hybrids from the mixture.

Morphological and chemical markers have been investigated as genetic markers to identify specific seeds/plants from a mixture. In rice, several morphological markers such as pale leaves (Dong et al., 1995) and purple leaves (Mou et al., 1995) have been employed for marking EGMS lines. These markers can be used to identify real F1 hybrids from selfed seeds (false hybrids) at the seedling stage. However, removing false hybrid seedlings must be made by hand, which is labor-intensive, and cannot ensure that false hybrids have been completely cleaned up.

Selective responses of plants to a chemical product can be used to identify a specific type of plant from a mixture and thus this selective chemical can be exploited as a marker. Herbicides can be one of these selective chemicals. There are two different types of herbicide that can be used for removing false hybrids in rice and other crops, herbicides that can selectively kill rice and ones that are safe for rice. When a herbicide can selectively kill normal rice varieties, a mutant with dominant resistance to this herbicide will be free from killing. The herbicide can kill the seedlings from a normal variety but it does not work on a variety that has the resistance gene. Zhang et al. (1998) transferred the bar gene into a restorer line of hybrid rice to make it resistant to the herbicide BASTA, which can be used to selectively kill false hybrids. When a herbicide is safe for normal rice varieties, recessive sensitivity to the herbicide can be used to selectively kill the seedlings from the selfed seeds of the female once it possesses sensitivity. Mori et al. (1984) obtained a herbicide-sensitive mutant through radiation, which was lethal to bentazon. The lethality was found to be controlled by a recessive gene. Maruyama et al. (1991) discussed the possibility of using this mutant in seed production by mixed planting.

We now report a herbicide-sensitive rice mutant obtained from an EGMS line that combines EGMS with bentazon-sulfonylurea sensitivity. The gene has been linked to microsatellite markers that can be used for marker-assisted breeding. Application of this mutant as an insurance for the purity of hybrid seeds in two-line hybrid rice production has been examined.

**Materials and methods**

**Plant materials**

A herbicide-sensitive mutant in rice, M8077S, used in this study, was obtained through a mutation breeding program. Seeds from a temperature-sensitive genic male-sterile rice variety, W6154S, were treated with 350 Rad Co60 in 1996, and plants from treated seeds (M1) were planted in Badong, Hubei, China, at an elevation of 700 m with the temperature below the critical level required for fertility conversion of W6154S. M1 plants were harvested in bulk as M2 seeds, and M2 plants were planted in the same location and harvested. M3 plants were planted by families and some seeds from M3 plants were saved as seed sources. One of the sensitive mutant would be killed by herbicide. Six different herbicides, including NC-311, bentazon, Londax, molinate, facet and Weinong, which are safe to normal rice, were sprayed successively in the M3 planting plot at one- to three-leaf stages. A recommended concentration rate for controlling weeds was used. Among 25,100 M3 families tested, only one of them, numbered 8,077, was completely killed after spraying with bentazon and this mutant was named M8077S. The retained seeds from corresponding M4 plants were then multiplied and used in further experiments.

**Herbicide test**

To screen a herbicide to which M8077S is significantly sensitive, a total of 29 herbicides belonging to 11 different chemical classes was tested using recommended rates for controlling weeds. The tested herbicides include bentazon and six sulfonylurea herbicides, i.e. Londax (bensulfuron), NC-311 (pyrazosulfuron), sulfometuron, metsulfuron, cinssulfuron and chlorosulfuron. Rice plants of M8077S, with the original variety W6154S and a commercial rice variety R1074 as controls, were planted in trays, each containing 50 plants. Based on the response of plants to these herbicides, two of them, bentazon and Londax, were selected for further tests.

A field test was applied to find suitable growth stages and concentration rates for bentazon to kill M8077S. For the concentration test, M8077S and the two control varieties were planted in the field and sprayed with bentazon at the 3-leaf stage with eight different concentrations: 20, 39, 78, 156, 313, 625, 2,500 and 5,000 mg/l. For stage tests, plants were sprayed with 1,250 mg/l of bentazon at different growth stages including seedling, tillering and flowering. Since bentazon is absorbed by plant stems and leaves, the influence of plot size and bentazon concentration in the soil were not considered.

For the concentration test with Londax, M8077S and control varieties were planted in pots of 25-cm diameter, each with ten plants. Each pot was applied with 5 ml of Londax and six different concentrations, 3, 15, 30, 300, 1,500 and 3,000 mg/l, were tested.

**Analysis of genetic pattern and gene effects**

M8077S was used as a female parent, and crossed with the original W6154S and five other indica varieties, Ganghui 2, C64-7, R1073, R1074 and 6175, respectively. Each panicle in F1 and F2 populations was bagged to prevent outcrossing. At the 2-leaf stage, all plants were sprayed with 1,250 mg/l of bentazon. Plants were then scored as normal or dead based on their reaction to the herbicide 7 days after spraying. The segregation ratio of normal to dead plants was calculated for each cross. To investigate multiple effects of herbicide sensitivity on other agronomic traits, two crosses, M8077S/Guhanhui 2 and W6154S/Guhanhui 2, were compared agronomically.

To determine whether reactions of the mutant to two different herbicides, Bentazon and Londax, are controlled by an identical gene, two experiments were designed. (1) The segregating population test: F2 seeds from two crosses, M8077S/R1073 and M8077S/R1074, were planted in 40×40×12-cm trays. At the one-leaf stage, each tray was sprayed with 20 ml of 30 mg/l of Londax. Seedlings were evaluated for growth inhibition 5 days after spraying, and then sprayed with 1,250 mg/l of bentazon. The seedlings were scored for death 7 days later. The correlation between the inhibited growth by Londax and the plant death by bentazon was investigated. (2) The interaction test: 30 M8077S seeds were planted in a pot. At the one-leaf stage, seedlings in the pot were sprayed with 5 ml of 30 mg/l of Londax, and followed by 5 ml of 1,250 gm/l of bentazon. The seedlings were sprayed for 1, 3, 6, 12, 18, 24 and 48 hours respectively, in seven different pots, after spraying with Londax. Interaction between these two herbicides was evaluated by comparison with a control in which only bentazon was sprayed.