

so expressed field resistance to BI and BB. According to the PRRT BI resistant test in 1988 and SCRRT BB resistant test in 1990, Shuangchao 25

showed resistant reactions of scale 1 to BI and 3 to BB.

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### A new saline-alkali tolerant rice line 647-4 selected by tissue culture

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We have conducted experiments for the selection of saline-alkali tolerant rice by tissue culture for years. Now a series of tissue culture materials have been selected, designated as "D rice". Japonica rice cultivars Mudanjiang 17, Liaogeng 10 and Tengxi 138 were used as parents and their mature embryos as explants. The calli induced from the embryos were cultured in stress medium (MS + 2,4-D 2 mg/l + NaCl 1% + NaHCO<sub>3</sub> 0.5%) for 15-20 d and transplanted to fields or pots when the seedlings with three leaves developed from the stressed calli. The seeds obtained when they matured were called "D rice".

The "D rice" lines were planted in the sodic soil with about pH 8 in Zhaoyuan County,

Heilongjiang Province. Ninety lines were discarded on the basis of the growth observation in seedling and reviving stages. The rest were tested further.

Through screening of saline-alkali tolerance and yield evaluation in the field and pot cultivation for several years, 7 lines were selected from 119 lines of "D rice", of which line 647-4 was the most promising which had the highest yield, 6.76 t/ha, 13.4% more than CK (cv. Mudanjiang 17). Its filled grain percentage was 75.2% and 1000-grain weight 26.2 g with the character of high saline-alkali tolerance, high-yielding potential and disease resistance.

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### Activities and distribution of Chitinase and $\beta$ -1,3-Glucanase in rice induced by *Pyricularia Oryzae*

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Potential inhibitors of fungal growth, Chitinase and  $\beta$ -1,3-glucanase, are widespread in higher plants. However, it has not been reported in rice plants. We studied the possibility of Chitinase and  $\beta$ -1,3-glucanase against BI pathogens in rice. ZG<sub>1</sub> (a physiological race of *P. oryzae*) distributing widely in China and rice cultivars showing obvious resistant or susceptible reactions to ZG<sub>1</sub> were chosen as experimental materials, such as Tetep resistant to ZG<sub>1</sub> and Heigu susceptible to ZG<sub>1</sub>. Activities of Chitinase and  $\beta$ -1,3-glucanase in 11 rice cultivars were assayed. The results showed that activities of the hydrolases were very low in healthy plants, and no significant difference was observed among these cultivars. However, activi-

ties of Chitinase and  $\beta$ -1,3-glucanase increased by six to nine and three to five times respectively in seven days in the cultivars infected by spores of *P. oryzae* or treated with its mycelia cultured filtrate. By assaying enzyme activities both in intercellular fluids extracted by vacuum-infiltration and in protoplasts isolated from rice leaves, it was indicated that there were Chitinase and  $\beta$ -1,3-glucanase in both intercellular and intracellular spaces of rice, but specific activities of the enzymes in intercellular spaces were much higher than those in intracellular spaces. In resistant cultivars, specific activities of Chitinase and  $\beta$ -1,3-glucanase in intercellular spaces were 9 and 28 times respectively of those in intracellular spaces, where-

as in susceptible cultivars, 10 and 16 times. Chitinases in Heigu treated with mycelia cultured filtrate were partially purified on an affinity chromatography column of regenerated chitin. On SDS-PAGE plate, there were two proteins (MW. 37.1 and 28.0 kD) having Chitinase activity. The partially purified Chitinases could release N-acetylglucosamine (GlcNAc) from cell walls of ZG<sub>1</sub> and inhibit the germination of ZG<sub>1</sub> spores as well.

Since Chitinase and  $\beta$ -1, 3-glucanase in rice could be induced by *P. oryzae*, and the induced

Chitinase could inhibit the fungal growth *in vitro*, furthermore, the enzymes existed in both intra- and extracellular spaces, and the specific activities were much higher in extracellular spaces than in intracellular spaces, we believe that these two enzymes could theoretically prevent the growth of fungi in rice tissues. However, Heigu could still be infected by *P. oryzae*, indicating that Chitinase and  $\beta$ -1,3-glucanase required the appropriate condition for playing their roles in rice resistant to Bl pathogens *in vivo*. Therefore, it is necessary to further studies.

### Comparison of methods for identification of cold tolerance of rice

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Under low temperature stress, the plasmamembrane and chloroplast of plant cells are impaired, resulting in electrolyte leakage and alteration of chlorophyll fluorescence which can be measured with conductometer and fluoremeter. The cold tolerance of excised leaves of several rice varieties were determined with these two instruments at seedling and heading stages to compare with that of the whole plant treated in phytotron. The experiment had three replications.

The materials were grown in pots. They were sampled at seedling age of 21 days or at heading stage. (1) Method of phytotron: at seedling stage, the materials were treated at 8.5°C temperature in average within the range of 5.5°C -12.5 °C , 12 h sunshine, 1,000-2,000 lux intensity and RH > 90%. The injuries were divided into 9 grades based on the method of A. Coly. The cold tolerance at heading stage was expressed with seed-setting rate of plants treated with 16°C mean temperature of five days. (2) Method of conductometer: the blades or their fragments of functional leaves were immersed in deionized water, then treated with low temperature (0°C) in the dark. The relative conductivity was used as electrolyte leakage ratio. (3) Method of flouremeter: 2 cm leaf fragments taken from functional leaves of the main

stem were treated with 0°C and saturated humidity in the dark. The flourescence was measured with SF-10 Plant Productivity Fluoremeter (made in Canada) controlled by a microcomputer. The maximal rate of chlorophyll fluorescence rise (Fr) was used as cold tolerance index.

Results are shown in Table 1 and 2. Variance analysis revealed that the cold tolerance at both

Table 1. Cold tolerance of rice at seedling stage

Variety	Conductometer T50 (h) <sup>a</sup>	Fluoremeter T'50 (h) <sup>b</sup>	Phytotron grade <sup>c</sup>
G.Y.Q.	184.2	45.3	6.9
Nongken 58S <sup>d</sup>	154.9	80.1	6.3
Teqing	167.9	38.9	8.7
KS-9 × Teqing	111.5	40.9	7.2
N98S	125.4	56.2	7.8
Shanyou 63 <sup>d</sup>	111.8	54.9	7.6
W6154S × Teqing <sup>d</sup>	96.0	43.6	7.6
KS-9	94.0	61.3	7.8
W6154S 312	75.9	40.9	7.3
W6154S	72.0	53.3	8.8

<sup>a</sup>Treating time when 50% electrolyte leaked out; <sup>b</sup>Treating time when Fr decrease to 50%; <sup>c</sup>Nine d after treatment;

<sup>d</sup>Variety with same grade of cold tolerance in three methods.

stages determined by these three methods showed very significant differences among varieties, meaning these methods were all capable of identifying