Resistance to Bacterial Leaf Blight in a Somaclonal Rice Mutant HX-3 at Cellular Level

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Abstract: The interaction between rice host and its pathogen Xanthomonas oryzae pv. oryzae (Xoo) at cellular level was studied by using a resistant somaclonal mutant HX-3 and its susceptible donor Minghui 63. After inoculation with Xoo strain Zhe 173 (Chinese pathotype IV), the activity of superoxide dismutase (SOD) and peroxidase (POD) in the callus of Minghui 63 was increased dramatically, and the active oxygen(\(O_2^-\)) was produced at a higher rate; Meanwhile, the callus grew slowly with the reduction of protein content. Compared to the activity of SOD and POD, the production rate of \(O_2^-\) and the fresh weight in HX-3 callus varied little after the inoculation. It could be proposed that there were great differences between the resistance of HX-3 and Minghui 63 at cellular level. There was no difference detected concerning resistance to bacterial leaf blight in HX-3 between the plant and the callus.

Key words: bacterial leaf blight; somaclonal mutant; active oxygen; resistance; cell level

During the past 30 years, significant progress has been made in in vitro selection of plant somaclonal variants from embryo cultures, and now this technology has become an important research field in plant cell engineering [1]. Certain distinguished merits have been demonstrated in this technology. For example, the selection for objective plants can be made at cell level instead of plant level; also, some target characteristics can be selected during the course of culturing rather than in the end of the cultivation. As this technique is more efficient than traditional selection methods, it has been widely employed in related fields, especially in the screening of disease resistant somaclonal mutants, as well as other plant mutants with adverse environment tolerance [1-5].

Resistances at cell and plant level are two indispensable aspects included in the plant disease resistance. Although most studies [3, 5] proved that the disease resistance at the two levels in the plant were in accordance with each other, however, reverse example did exist [6]. Therefore, understanding the correlation of disease resistance at plant and cell level can provide an important theoretical basis for the application of in vitro selection of disease-resistant mutants. As disease resistant somaclonal mutants are derived from cells and have resistance at plant level, they can provide as the desired materials for the related research. However, few studies have been done on this aspect.

HX-3 is a somaclonal mutant with resistance to bacterial leaf blight that we have successfully obtained through in vitro selection from the mature embryo of Minghui 63, a hybrid rice restorer highly susceptible to bacterial leaf blight. It has proved that HX-3 carries a new resistance gene (Xa-25) to bacterial leaf blight [7]. In this paper, both HX-3 and Minghui 63 were studied on their resistance to bacterial leaf blight at cell and at plant level, with the purpose to enrich the theoretical data on mutant selection in vitro, and also, to enhance our understanding on the resistance mechanism of the new resistance gene Xa-25 in HX-3.

MATERIALS AND METHODS

Materials

Rice varieties: Resistant somaclonal mutant HX-3 and its susceptible donor Minghui 63 were provided by Laboratory of Rice Biotechnological Breeding, Institute of Agrobiological Genetics and Physiology, Jinagsu Academy of Agricultural Sciences.

Bacterial leaf blight strain: Zhe 173 (Chinese pathotype IV), a typical bacterial leaf blight strain in the lower reaches of the Yangtsu River were used in our study. This strain was provided by Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences.

Methods

Induction and subculture of rice callus

Callus of HX-3 and Minghui 63 were induced, respectively from their mature embryos. The media used for callus induction and subculture were the same, which
was comprised of a basal medium M8 supplemented with 1 mg/L 2,4-D, 2 mg/L 6-BA, 5% sucrose and 7 g/L agarose. Four weeks after induction, the calli were transferred to a fresh medium (20 calli per flask) for subculture. Then the calli were inoculated with bacterial leaf blight except for the control. Samples of the callus were taken before and after (at 5, 9 and 13 days) the inoculation of the pathogen. The fresh weight of these samples was measured by a Digital Balance.

**Bacterial leaf blight inoculation**

Bacterial leaf blight was cultured and inoculated to the callus according to Sun et al. [4].

**Preparation of enzyme extract**

Enzymes were prepared following the method of Laval-Matin et al. [8]. Callus samples were homogenized in an ice bath with the extraction buffer containing 50 mmol/L Tris-HCl (pH 7.8), 0.5% PVP (W/V), 7 mg/L PMSF, 50 mmol/L EDTA. The homogenate was centrifuged at 10 000 r/min for 20 min at 4°C. The supernatant was then used as enzyme extract.

**Soluble protein assay**

Contents of the soluble protein in the enzyme extract were measured by the method of Bradford [9] using BSA as a standard.

**POD activity**

POD activity was assayed following the method of Zhang [10]. Absorbance change of the brown guaiacol at 460 nm was recorded for calculating POD activity.

**SOD activity**

SOD activity was assayed by the photochemical method described by Giannopolitis and Ries [11]. One unit SOD activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of NBT (p-nitro blue tetrazolium chloride) reduction measured at 560 nm.

**Content of O₂⁻**

The content of O₂⁻ was assayed according to the description of Wang and Luo [12].

Identification of rice resistance at plant level

It was performed according to Fang [13].

**RESULTS**

**Resistance in HX-3 and Minghui 63 at plant level**

Identification on the resistance in HX-3 and Minghui 63 to bacterial leaf blight showed apparent difference between them at plant level (Table 1). HX-3 was highly resistant to the disease at seedling, tillering and adult stages, it means that the resistance of HX-3 showed whole growth duration resistance. On the contrary, Minghui 63 was highly susceptible in all the developmental stages tested.

**Influence of inoculation on the growth of rice callus**

Compared with the control without the inoculation of bacteria, the gained weight of the inoculated callus of Minghui 63 were much smaller. For example, the weight gain of the callus was only 66% of the control at the 13th day after inoculation. This indicated that the pathogen inhibited the growth of callus to a great extent. Differently, only small weight changes were detected in the callus of HX-3 after the inoculation of the pathogen compared with the control (Fig.1).

**Influence of inoculation on protein content in rice callus**

The contents of soluble protein in the control of both HX-3 and Minghui 63 increased at first. The content maintained at a high level for a quite long time (12 days), then decreased gradually as the time extended to 13 days.

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**Table 1. Resistance reactions to bacterial leaf blight (Zhe 173) in HX-3 and Minghui 63 at seedling, tillering, adult stages.**

<table>
<thead>
<tr>
<th>Rice material</th>
<th>Seedling stage</th>
<th>Tillering stage</th>
<th>Adult stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lesion length</td>
<td>Reaction</td>
<td>Lesion length</td>
</tr>
<tr>
<td>Minghui 63</td>
<td>6.8 ± 0.8</td>
<td>S</td>
<td>13.8 ± 1.1</td>
</tr>
<tr>
<td>HX-3</td>
<td>1.9 ± 0.3</td>
<td>R</td>
<td>2.5 ± 0.7</td>
</tr>
</tbody>
</table>

Reaction: R, Resistant; S, Susceptible.
As protein is critical in the normal development and proliferation of cells, the reduction in protein content would surely have adverse influence on its development, this had been demonstrated in the former results. It was also found that the protein content declined more rapidly in Minghui 63 than in HX-3, indicating that the pathogen had influenced the susceptible variety more severely.

Influence of inoculation on the activity of SOD and POD in the callus

The activity of SOD in the callus of Minghui 63 increased gradually after the inoculation of bacteria. It reached the highest peak at the 11th day after inoculation (about 5 times as high as the control). The activity of SOD in HX-3 was similar between the treated callus and the control. The SOD activity rose gradually at the 9th day after inoculation, then it reached the maximum level at the 13th day, which was 30% higher than that of the control (Fig. 3). Furthermore, it was discovered that the activity of SOD in the control callus of Minghui 63 and HX-3 (without the inoculation) was quite different. This result could partly be explained by the genotypic factor.

In the calli of Minghui 63, the activity of POD rose dramatically after the inoculation of bacteria (1.4 times as high as the control). The high activity of this enzyme remained until the 13th day (1.7 times as high as the control). POD activity in the calli of HX-3 presented a similar pattern between the control and the inoculated samples. Its activity declined at the 9th day, then reached the lowest level at the 13th day (75% as high as the control). These results implied that the dynamic pattern of POD activity were also different in Minghui 63 and HX-3 (Fig 4).

Influence of inoculation on the production rate of \( \mathbf{O}_2^- \)

Production rate of \( \mathbf{O}_2^- \) remained at a low level in the control callus of Minghui 63 (Fig. 5). For the inoculated callus, the production rate of \( \mathbf{O}_2^- \) increased remarkably at the first day of inoculation (about 4 times as high as the control), then reduced gradually. However, it kept a level much higher than that of the control (It was still 50% higher than the control at the 13th day). Similarly, with the former two enzymes, production of
O$_2^-$ in the callus of HX-3 presented a similar pattern between the control and the inoculated samples. The O$_2^-$ produced at a high rate at first, then reduced gradually. At the 13th day, the production rate of O$_2^-$ in the inoculated callus reduced 30% compared with the control.

DISCUSSION

Up to now, many promising rice germplasm with resistance to disease have been discovered from the mutants of susceptible rice varieties through in vitro somaclonal mutant selection \cite{3-5, 14, 15}. This has proved the utility and efficiency of this technique in exploring novel rice germplasm resources for disease resistance. However, whether the resistance at the plant and the cellular levels are accordant with each other in the mutants, there are still doubts about it. Although studies have showed that the resistance in some mutants expressed at both levels \cite{4, 14}, the work of Yu et al. on the interaction between rice callus and bacterial blight has complex results. It was discovered that among the callus-pathogen groups studied, 4 groups showed different reaction between plant and cell level \cite{6}. In this study, it was detected that after the inoculation of the bacteria, the soluble protein content in the callus of Minghui 63 decreased quickly, indicating that its development was severely inhibited. In comparison, although the soluble protein content reduced a little in the callus of HX-3 after the inoculation of the pathogen, there was no obvious reduction in the weight gain, indicating a stronger resistance to the disease in the variety compared with Minghui 63. It could be concluded from all these results that the resistance reaction of HX-3 to bacterial leaf blight was accordant at the cell and the plant level. Therefore, the in vitro technology that we have established for the selection of resistant somaclonal mutants has been proved to be feasible \cite{4}.

It has been well known that the plant resistance in rice to bacterial leaf blight is correlated to reactive oxygen species (ROS). During the incompatible reaction (resistant reaction) between the plant and the pathogen, the activity of the protective enzymes in the plant always decreases, while the level of ROS increases. Some cells may also show HR-like reactions. These cells can break down rapidly, finally, a systematic or local resistance reaction is induced in the plant. In comparison with the incompatible reaction, no injury of ROS and HR-like reaction can be detected in a compatible interaction (susceptible reaction). Also, the local resistance reaction can’t be induced \cite{16, 17}. In this study, it is discovered that the production of O$_2^-$ in the callus of Minghui 63 increased immediately after the inoculation with strain of bacterial leaf blight, which then was accompanied with marked increase in SOD and POD activity. While for HX-3, the metabolic patterns of O$_2^-$ were similar between the inoculated callus and the untreated control, indicating a slow response for the cells to the pathogen. The resistant reactions demonstrated at the cell level seemed quite different with those at the plant level. The reason for which may include the following two aspects: (1) Different rice varieties have different genotypes, so their respective reaction to the pathogen (bacterial leaf blight) may present differently at the cell level and the plant level. (2) The inoculation quantity on the callus and plant are different in our research. The inoculation quantity on the callus was much higher than that on the plant. Therefore, the slow reaction in the callus of HX-3 to the pathogen may present its resistance at the cell level. The reason might be that the cells of HX-3 can strongly inhibit the infection of the pathogen, or something (such as antitoxin) can be produced in its cells, which may act as an antagonist to the toxic products of the pathogen, thus greatly enhance the resistance in the cell.

In addition, it is worthy to note that the production rates of O$_2^-$ were significantly different between HX-3 and Minghui 63, which were not inoculated with the pathogen. It could be deduced that certain mutations had occurred in HX-3, which resulted in the increasing of O$_2^-$ production in the cells. Further study will be needed to clarify the relationship between this phenomenon and the resistance of HX-3 at the cellular level.

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REFERENCES


