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Full Length Research Paper

Influences of the disease resistance conferred by the individual transgenes, *Pi-d2, Pi-d3* and *Xa21*, on the transgenic rice plants in yield and grain quality

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To research possible influences of the disease resistance conferred by different trans-resistance genes on the transgenic rice plants in their yields and grain quality, three transgenic rice lines, including two with the resistance genes *Pi-d2* and *Pi-d3,* respectively, for rice blast, and one with the resistance gene *Xa21* for rice bacterial blight, which all showed the highest resistance to their respective pathogen races, were used to analyze and measure their respective characters in yield and grain quality as compared to those of the transgenic control line with the empty vector that was used to transfer *Pi-d2, Pi-d3* and *Xa21*, respectively. Both yield and grain quality of all three transgenic materials with the respective trans-resistance genes decreased significantly. Grain weight per plant of *Pi-d2, Pi-d3* and *Xa21* transgenic individuals decreased by 7.7%, 29.6 and 44.4%, respectively, compared to the control. Grain quality of *Pi-d2 and Pi-d3* transgenic plants were both the third class according to the Industrial Standard, 'Grain Quality of Edible Rice Variety' and one class worse than that of the control, but the third class still belongs to edible grains. However, grain quality of *Xa21* transgenic plants was too bad to be edible.

Key words: *Oryza sativa*, resistance gene, transgenic plant, resistance, yield, grain quality, rice blast, rice bacterial blight.

INTRODUCTION

Rice blast and rice bacterial blight, the two most devastating fungal and bacterial diseases across the rice-growing world, respectively, are main disease factors that restrict the rice yield. Chemical control increases production cost and harms environment, thus now, utilizing rice resistance genes to breed resistant varieties is still the most economical, effective and friendly environmental method that controls these two diseases (Jena, 2006; Sundaram et al., 2009). Transgene technique, an important method that improves rice varieties, can effectively change genetic characters of plants (Bajaj and Mohanty, 2005; Ishizaki and Kumashiro, 2008; Xiao et al., 2009). Usually, in the examination and approval of a new rice variety, its disease resistance and its yield and grain quality as well are all required. However, it has been known that there are some varieties that show high resistance but low yield that is not beneficial to settle starvation. Moreover, more people like to eat rice of good grain quality, but there exist some varieties that are good in grain quality but susceptible to rice blast and/or rice bacterial blight, or vice versa, high resistant but bad in grain quality. Therefore, high yield and good grain quality, as well as high resistance are the long-term aims for rice breeders, even through the transgenic approach.

By now many disease resistance genes, including eleven rice blast (Wang et al., 1999; Bryan et al., 2000; Qu et al., 2006; Chen et al., 2006; Zhou et al., 2006; Liu et al., 2007; Lin et al., 2007; Ashikawa et al., 2008; Lee et al., 2009; Shang et al., 2009) and six rice bacterial blight resistance genes (Song et al., 1995; Yoshimura et al., 1998; Chu et al., 2004; Gu et al., 2004; Iyer and

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Table 1. The characters of yield of rice transgenic plants.

Transgenic line	Number of effective panicles per plant	Number of grains per panicle	Seed setting rate (%)	1000-grain weight (g)	Grain weight per plant (g)	Reductive yield rate (%)
Pi-d2	5.9 ± 1.4	163.3 ± 22.9	81.8 ± 10.5	22.9 ± 0.9	18.1 ± 5.2	7.7
Pi-d3	5.1 ± 1.3	116.2 ± 16.4	91.0 ± 2.0	26.1 ± 0.7	13.8 ± 3.6	29.6
Xa21	4.2 ± 1.0	106.9 ± 18.4	78.3 ± 7.0	30.5 ± 0.8	10.9 ± 3.8	44.4
Control	5.6 ± 1.3	149.8 ± 23.6	91.5 ± 3.1	25.7 ± 0.8	19.6 ± 5.9	—

Reductive yield rate (%) = (Y_{contol}-$Y_{resistance\ gene}$)/ $Y_{control}$ ×100%, and 'Y' is referred to grain weight per plant.
The control was transgenic plants with the empty vector only.
Each value was the mean of three independent plots with 12 individuals per plot, and standard deviations were indicated.

McCouch, 2004; Sun et al., 2004) have been cloned in rice, and their functions were identified using transgenic individuals. Nevertheless, few studies have been done on the influences of the disease resistance on the transgenic rice plants in their yields and grain quality. Such study has been conducted only once in *Arabidopsis* by Tian et al. (2003). They introduced the resistance gene *RPM1* that confers resistance to *Pseudomonas syringae* into susceptible *Arabidopsis* plants, and found that the resistant individuals (*RPM1*$^+$ transgenic line) tend to have fewer siliques and seeds per silique, as well as lower shoot biomass than the paired susceptible individuals (*RPM1*$^-$ transgenic line). More importantly, *RPM1*$^+$ plants suffer, on average, a 9% decrease in total seed production relative to their counterparts, *RPM1*$^-$ transgenic line. If the resistance in the transgenic rice plants with a disease resistance gene is similar to the *RPM1* resistance in *Arabidopsis*, that is, negatively correlate with yield or grain quality in rice, we should not only consider the disease resistance of the transgenic rice plants but ignore their yields and grain quality, and must take everything into account. Unfortunately, such studies have been performed in rice yet. This paper analyzed the influences of the disease resistance conferred by *Pi-d2* (Chen et al., 2006) and *Pi-d3* (Shang et al., 2009) for rice blast, as well as *Xa21* (Jiang et al., 2004) for rice bacterial blight, respectively, on the corresponding transgenic rice plants in their yields and grain quality.

MATERIALS AND METHODS

Transgenic lines and planting

The rice blast- and rice bacterial blight-susceptible variety, TP309 (*Oryza sativa* L subsp. *Japonica*), was used for transformation material. The four transgenic rice lines, with the transgene *Pi-d2, Pi-d3* and *Xa21* as well as the empty vector, respectively, were provided by Lihuang Zhu in the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences (Beijing, China), which all originated from TP309. The empty vector is the binary vector pCAMBIA1300, which had been used as a transgene vector to introduce *Pi-d2, Pi-d3* and *Xa21* into TP309 (Jiang et al., 2004; Chen et al., 2006; Shang et al., 2009). The three transgenic lines with *Pi-d2, Pi-d3* and *Xa21*, respectively, all exhibited the highest resistance to their respective rice blast or rice bacterial blight pathogen races (L. H. Zhu, personal communication), whereas the transgenic line with the empty vector was susceptible to rice blast and rice bacterial blight as TP309. Transgenic plants were segregatively grown, with thirty six individuals per transgenic line and twelve individuals per plot, respectively, in a net house. Pathogens and pests were prevented during the whole planting. Each value was the mean of three independent replicates and standard deviations were indicated.

Measurement of yield and grain quality

The harvested individuals at yellow ripening stage were measured for number of effective panicles per plant and number of grains per panicle, as well as seed setting rate. Grain weight per plant and 1000 g weight were determined after natural drying of seeds. The characters of grain quality were investigated in the Rice Product Quality Supervision and Inspection center of the Ministry of Agriculture, China National Rice Research Institute, according to the Industrial Standard, 'Grain Quality of Edible Rice Variety' (The Ministry of Agriculture of the People's Republic of China, 2003).

RESULTS AND DISCUSSION

The influences of the resistance conferred by individual transgenes on the transgenic rice plants in yield

Rice yield mainly includes four aspects, number of effective panicles per plant, number of grains per panicle, seed setting rate and 1000 g weight. Of note, yields of the three transgenic lines with *Pi-d2, Pi-d3* and *Xa21* respectively, tested in this experiment decreased (Table 1). Although *Pi-d2* and *Pi-d3* are both blast resistance genes, they had significantly different influences on these two transgenic lines in yield reduction. Seed setting rate and 1000 g weight of *Pi-d2* plants were lower than those of the control, but number of effective panicles per plant and number of grains per panicle were higher; as a result, the grain weight per plant in *Pi-d2* transgenic individuals only decreased by 7.7% compared to the control. Whereas the number of grains per panicle of *Pi-d3* plants was significantly lower than that of the control, which resulted in a 29.6% decrease in grain weight per plant.

Pi-d2, cloned from the rice indica variety Digu, represents a new type of plant resistance gene that encoding a receptor-like kinase protein with a predicted extracellular

Table 2. The characters of grain quality of rice transgenic plants.

Transgenic line	Grain quality character (class)										Total level
	Brown rice rate	Milled rice rate	Head milled rice rate	Chalky grain rate	Chalkiness	Transparency	Alkali spreading value	Gel consistency	Amylose content	Protein content	
Pi-d2	Third	Fourth	Second	Third	Third	First	Second	Second	First	First	Third
Pi-d3	Third	Third	First	Fourth	Third	Second	First	Third	First	First	Third
Xa21	Third	Fourth	Second	Fifth	Out	Fourth	First	Second	First	First	Out
Control	Third	Third	First	Third	Second	First	First	Third	First	First	Second

The control was transgenic plants with the empty vector only.
The characters of grain quality and total level were indicated according to the Industrial Standard, 'Grain Quality of Edible Rice Variety', in which a higher quality class num ber means a worse grain quality. 'Out' is referred to the grains that is not edible.

domain of a bulb-type mannose specific binding lectin and intracellular serine-threonine kinase domain. A single amino acid difference at position 441 of *Pi-d2* distinguishes the resistant and susceptible alleles of *Pi-d2* (Chen et al., 2006). *Pi-d3* is a blast resistance gene, identified in the indica variety Digu and belongs to NBS-LRR type resistance genes (Shang et al., 2009). The different structure between *Pi-d2* and *Pi-d3* may cause different defence pathways, which may exert different influences on these two transgenic plants in yield decrease, and it seems that NBS-LRR structure has more serious effect on yield as *RPM1* in *Arabidopsis* is also a NBS-LRR type resistance gene (Grant et al., 1995).

Xa21 transgenic plants had the highest 1000 g weight, but number of effective panicles per plant and number of grains per panicle as well as seed setting rate were all significantly lower than those of the control, and caused a serious 44.4% decrease in grain weight per plant. It is suggested that the resistance conferred by *Xa21* should has very bad effect on yield. We also found that seed germination rate and plant height, as well as spike length of the three transgenic lines with resistance genes were all lower than those of the control (data not shown).

The influences of the disease resistance conferred by individual transgenes on the transgenic rice plants in grain quality

Grain quality of all three transgenic lines with respective resistance genes decreased (Table 2). According to the Industrial Standard, 'Grain Quality of Edible Rice Variety', both of the grain quality of *Pi-d2* and *Pi-d3* transgenic plants belong to the third class, and one class worse than that of the control, but the third class still belongs to edible grains. However, the grain quality of *Xa21* transgenic plants was too bad to be edible.

Besides, milled rice rate and head milled rice rate as well as gel consistency of *Pi-d2* and *Xa21* transgenic plants were all one class worse than those of the control. Chalky grain rate and chalkiness of *Pi-d2, Pi-d3* and *Xa21* transgenic plants were all worse than those of the control, especially *Xa21* transgenic individuals. Transparency of *Pi-d3* and *Xa21* transgenic plants were worse compared to the control, especially *Xa21* transgenic individuals was three classes worse than that of the control. It is suggested that the resistance in transgenic rice plants, with resistance genes *Pi-d2* and *Pi-d3* for rice blast, as well as *Xa21* for rice bacterial blight, should have very bad effects on grain quality, especially *Xa21* transgenic plants can not be applied to rice production.

Breeders often used non-transgenic plants as the control to study the function of a transgene in transgenic plants, in such cases, the different phenotypes of transgenic plants from non-transgenic plants could be attributed to effects of both the transgene and the exogenous vector. Using the transgenic plants, with the same empty vector as resistance gene transformation, as the control to research influence of resistance in transgenic rice plants with resistance genes on yield and grain quality, in this paper, can avoid the effect caused by transgene action.

Both yield and grain quality of all three transgenic materials with resistance genes decreased in this experiment, especially *Xa21* transgenic plants, and *Pi-d3* plants also significantly decreased the yield. It should be noted that all transgenic lines with the respective resistance genes used in this study, exhibited highest resistance to rice blast or rice bacterial blight pathogen races. Therefore, we suggest that, in any transgenic rice breeding programs, the three important factors, disease resistance and yield, as well as grain quality, should be always taken into account in a proper balance, and that the rice transgenic line that has gained an adequate resistance rather than the highest would be one of the best candidates for further breeding. The present study was conducted in a small scale, the experiments that the influences of the disease resistance conferred by more resistance genes on the transgenic rice plants in yield and grain quality are needed in future.

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