EVALUATION OF DISEASE SEVERITY ON RICE GENOTYPES TO BACTERIAL BLIGHT USING AMINO ACID CONTENT ANALYSIS

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Abstract

The bacterial blight (BB) disease severity on two rice genotypes i.e.; BP 4110-2d-33 (backcross between Ciherang x Angke; containing *Xa-4, xa-5)* and BP 3688e-23 (sister lines derived from cingri/memberamo//widas///IRBB 8; containing *xa-8*) were lower compare with TN-1 (containing *Xa-14*). The total amino acid content in cultivar's TN-1 was accounted for about one third to about a half of total amino acid than those of other rice genotypes where the total amino acid was ranging from 1.95% to 4.22%. In BP 3688e-23, and BP 3688e-22 genotypes more amino acid levels were decline although these advance lines showing *xa-8* background. BB resistant gene carried by BP 4110-2d-33 and BP 3688e-23 were stable, whilst BP 3688e-22 was less effective to inhibit BB disease severity. Overall, amino acids were not found to be related to the level of BB resistance; where correlation between amino acid content and BB disease severity is not significant. The slower growth of Xoo on rice genotypes BP 4110-2d-33 and BP 3688e-23 may probably due to other than nutritional factors. The degree of resistance in rice genotypes infected by races of pathogen; as well as the resistance gene possessed by genotype BP 3688e-23 need to be further determined.

Keywords: amino acid content, bacterial blight, rice genotypes, resistance

1. Introduction

In Indonesia pests and diseases were causing yield losses of 212,984 ton of rice in each annual planting season [1]. Among the main diseases, bacterial blight (BB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is considered as one of the major problems for the rice cultivation in both lowland rice and upland rice [2-3]. To date, BB disease become the most important diseases in most of rice growing countries due to its high epidemic potential and destructiveness on high-yielding cultivars. Infected area by BB is second largest after rice tungro disease. Yield loss was estimated at about 20% to 50% in severely infected field; and up to 10% to 20% when the disease infected rice at maximum tillering stage [4].

Current control strategies of Xoo pathogen mostly make use of disease resistant cultivars, which is an economical and effective method of control [5-6], but great variability of pathogenic bacteria leads to frequent breakdown in resistance [7]. Extensive screening work has led to the identification of several sources of resistance. The background of the resistance of plants has been attributed to various factor such as plant character (morphology and biochemical) as well as the nutritional content i.e., glucosinolates content [8]. In addition, it was reported that amino acid production is associated with defense to bacterial invasion [9].

Based upon race identification in screen house study, it was reported that the severity caused by BB on Indonesian differential varieties i.e.; Tetep (*Xa-1, Xa-2, Xa-16*), PB 5 (*Xa-1, Xa-12*), Kuntulan (*Xa-3*), Java 14 (*Xa-1, Xa-3, Xa-12*) and Kencana (no resistant gene) was ranging from 27.5% to 87.5% [10]. Near isogenic lines based on the known resistance genes identified in Japan and the Philippines has been developed and proposed them as international differential genotypes. These IRBB lines represent isogenic lines functioning as differential varieties, so that effective resistant gene to overcome Xoo races pathogen in the region can be traced [11-12]. Isogenic lines can also be served as donor for resistant in studying the mechanism of resistance. The development of new genotypes caries gene for resistant to BB has been continuously carried out. Currently, BB resistant genes were already carried by the derived lines of crossing between improved varieties with *xa5, Xa-7* and *xa8.* Resistance cultivars are strongly associated to the genetic variation.

Resistance gene combinations can increase the effectiveness of resistance genes [13]. In the Philippines *Xa-7* is consistent to control BB infection, whilst *Xa-10* were less effective to BB [14]. In China, it was reported that the incorporation gene *Xa-21* could improved the resistance of the restorer line and the hybrid [5]. In relation to this aspect rice genotypes *viz*. BP 4110-2d-33 (backcross between Ciherang/Angke which inherited the resistance gene from its donor parent Angke that contain resistant gene *Xa-4, xa-5);* BP 3688e-23, and BP 3688e-22. (backcross between cingri/memberamo// widas///IRBB 8 containing resistance gene *xa-8)* were previously developed to confer resistant against BB disease. Genotype TN-1 (backcross DWG/Tsai Yuan chung) confers resistance to BB containing *Xa-14* mostly used to study virulence of BB.

The mechanism involved in disease suppression remains to be explored combining host resistance and other control measures. Various studies on the mechanisms of rice plants resistance against Xoo have been reported from various aspects, both morphological (physical) and chemical. Identifying new sources of resistance is necessary to provide effective resistance [15-16]. In addition, disease caused by bacteria and other pathogens was reported by other workers could affect amino acid contents. It was reported that on bacterial infected plant, amino acid declined significantly, this may be due to proteolysis [17]. However; it was reported that plant diseases by infectious fungi have been shown to accumulate amino acids [18]. Therefore, more research need be done to evaluate this aspect. In order to improve cultivars/lines against BB disease prior their might be released, degree of amino acid may regarded as an important factor to be studied thoroughly in understanding and improving the mechanism of resistance. In the previous study Kim and Yoo [19] reported that out of twenty one polypeptides exhibited changes in rice plants responding to infection, whereas two polypeptides dramatically increased in level during infection and these polypeptides play an important role in plant-microbe interactions.

This study was undertaken to evaluate rice genotypes against Xoo bacterium and explore amino acid content in rice plants differing in resistance levels to BB to relate any changes of possible shifts in protein metabolism.

2. Methods

Preparation of Rice Cultural Practice and BB Disease Severity Observation. Rice plants were maintained in a rice plot environment at Ciranjang village, Cianjur-West Java (260 m above sea level) during 2007/2008 planting season. Rice seed genotypes (BP 4110-2d-33, BP 3688e-23, BP 3688e-22, and TN-1) were germinated in a rice seed bed containing Ultisol soil type. BP 4110-2d-33 rice genotype was previously developed using backcross between Ciherang/Angke which inherited the resistance gene from its donor parent Angke that contain resistant gene *Xa-4 xa-5;* whilst genotypes BP 3688e-23, and BP 3688e-22. derived from cingri/memberamo//widas///IRBB 8; were suggested resistant to BB disease conferring resistance gene *xa-8*. Genotype TN-1 was used as susceptible check to BB. When the seedlings reach at 21 d-old, they were transplanted in the rice plots (4 m x 5 m). The trials were arranged in a complete randomized block design with six replications, and then they were fertilized with 120 kg N and 50 kg P_2O_5 ha^{-1}, respectively. Rice plants were grown under rice field condition at 26.6 $^{\circ}$C and 82.30% RH under 12: 11 h day and night light regime. The BB severity was observed on rice leaves based upon heavy infection under natural condition in endemic area of Ciranjang-Cianjur.

Amino Acid Content, Data Collection and Analysis. Following collection after its harvest, the samples were stored in freezer until analyzed. Duplicates samples were prepared for total amino acids (AA) assay according to AOAC standard procedure reported previously [20]. Samples preparations for amino acid were as follow: 200 mg of plant extract was ground into mesh powder, macerated in 2 ml HCl 6N and it was spun down at 1500 rpm for 15 min. The supernatant was further vacuum evaporated in hydrolysis tube. Residual filtrate containing amino acid was filtered through a 45 μm filter diluted in one ml HCl 0.1 N, and it was run on the high performance liquid chromatography (HPLC) for analysis. The total amino acids were determined on a HPLC analyzer following the instruction of the manual. Percentage (%) AA content was estimated using formula as follow:

$$AA\ (\%) = \frac{\mu g\ mL^{-1}}{\text{Weight of sample mg } mL^{-1}} \times 100\% \quad (1)$$

Rice plants were scored for its BB disease severity using IRRI-Standard Evaluation System for Rice (SES) scale [21] based on 1 to 9 scale where: scale 1 = 0–3% lesion area, scale 2 = 4–6% lesion area, scale 3 = 7–12% lesion area, scale 4 = 13–25% lesion area, scale 5 = 26 – 50% lesion area, scale 6 = 51–75% lesion area, scale 7 = 76–87% lesion area, scale 8 = 88–94% lesion area and scale 9 = 95–100% lesion area. Observation was made based on 20 fully develop leaves in each replication at generative stage.

Data on total free amino acid composition were subjected to an analysis of amino acid pattern that evaluated by means of principal component analysis (PCA). Diversity between rice genotypes in terms of amino acid composition was determined by multivariate analysis [22]. The PAST software was used to determine PCA and cluster analysis. The resulting matrix of Euclidian distance was used to produce the cluster dendrogram [23].

3. Results and Discussion

BB disease severity. Disease occurrence in the rice field during the heading stage was noted and the disease in the field generally was found from moderate to susceptible reactions. Leaves of rice genotypes that infected by Xoo bacterium showed disease symptom in the beginning of maximum tillering stage. In the severe Xoo - rice infection, symptom of Xoo can be distinguished appeared earlier, with typical necrosis and water soaking symptoms, followed by leaf blight damage where leaves plant became grayish at later stage.

The BB disease severity was ranging from 2.3% to 75%. The severity of BB disease on rice leaves genotypes of BP 4110-2d-33 (*Xa-8*), and BP 3688e-23 (*Xa-8*) were lower than that of BP 3688e-22 (*Xa-8*), as well as TN-1 (*Xa-14*) cultivar as a control treatment (Table 1). Bustaman *et al*. [24], pointed out that the reaction of IR BB21 (*Xa-21*) was susceptible to some Indonesian isolates. Code and Angke genotypes (containing resistant genes of IR BB7 (*Xa-7*) and IR BB5 (*xa-5)* backcrosses with IR64 containing (*Xa4*) were showed resistant to BB [9]. In the current breeding program, more traditional tropical japonica germplasm and most advanced IR BB lines having pyramiding BB gene were employed as a donor parent, those efforts is done to broaden the genetic background of BB resistant gene in the future of high yielding varieties. In the green house study for BB disease reaction, showed that the severity caused by BB ranged from 27.5 to 87.5%. The lowest severity was observed on genotype Widas, while the highest severity was observed on Memberamo. It was identified that those three genotypes used in this study showed resistant and susceptible to BB. Those indicated that the BB resistant gene carried by BP 4110-2d-33 (*Xa-4, xa-5*) and BP 3688e-23 (*xa-8*) were stable, whilst BP 3688e-22 (*xa-8*) was less effective to inhibit BB disease severity. Previous study indicated that the isogenic lines reactions were vary greatly in disease severity from season to season, and from site to site. Result of two years trials in Sukamandi, Subang, Cianjur (West Java), one year study in Batang (West Java) and one season study at Ngale (East Java) showed that despite the presence of high virulence Xoo races in the different test site, the IR BB7 containing *Xa-7* R gene was the most resistant isogenic lines, therefore it is very valuable donor for breeding BB resistant [10].

Amino acid content. Study on rice resistance mechanisms against Xoo has been conducted on various aspects both morphological and biochemical [25]. It was reported that inoculated rice plant had higher lesion length than that of uninoculated plant; The susceptible plant was reported to have an increment of free amino acid and total phenol content; however; it was showed less sugar reduction [4]. The nutritional environment inside the host plant seems to be an important factor in determining resistance. When the bacterial cells enter the plant, they soon begin to multiply in both resistant and susceptible cultivars, but growth becomes static in resistant cultivars and no lesions or only very small ones result [26]. Total amino acids accumulations in rice plants infected by Xoo pathogen showed lower content on the control treatment TN-1 than that on other treatments. Seventeenth amino acids were determined in the analysis of relative proportion of amino acids composition on four genotypes (Figure 1). The BB disease severity was ranging from 2.34% to 75%; whilst total amino acid was ranging from 1.95% to 4.22%. Previous study indicated BB infected rice showed levels of N and P which were relatively not much different, but the protein content and K on genotypes IR BB5 and IR BB7 were slightly higher than other genotypes. In the present study evaluation of rice resistance against Xoo was based upon a relationship between amino acid content with BB disease severity on rice leaves after Xoo infection. The BB severity of BP 3688e-23 and BP 3688e-22 was 9.37% and 75%, respectively but these genotypes had almost the same total amino acid content. Correlation analysis using the data presented in Table 1, showed that the relationships between amino acid level and disease severity is not significant (r= 0.628; P= 0.297). Weibull *et al.*, [27] stated that plant age and growth condition may influence the total amino acid content. Carbohydrates as a result of photosynthesis play an important role in plant metabolism where this compound will be changed into lipid and amino acid forms. Protein synthesized that was obtained from amino acid translation may influenced by level of Nitrogen (N) content on plant; however too much N content favour pathogen development thus increase rice disease severity [28]. Nitrogen as the main compound of plants will store in the form of protein; and the high level of threonine in the free amino acid pool, suggest that the protein being synthesized contain a high level threonine. Percentage of total N in leaves of citrus plants grown in inoculated soil was significantly less than in leaves of uninoculated plants [29]. Young *et al.* [30] reported that the content of free amino acids was three to ten times greater in roots of vesicular arbuscular mycorrhiza (VAM)-infected than that of non-infected

Table 1. Relationships between Total Amino Acid Content and Degree of BB Disease Severity on Rice Genotypes

Genotypes	Resistant gene	Total amino acid content (%)	BB disease severity (%)
BP 4110-2d-33	*Xa- 4, xa-5*	4.22	2.34
BP 3688e-23	*xa-8*	3.24	9.37
BP 3688e-22	*xa-8*	3.41	75
TN-1	*Xa-14*	1.95	75

Remarks: Coefficient correlation (r) = 0.628; P = 0.297

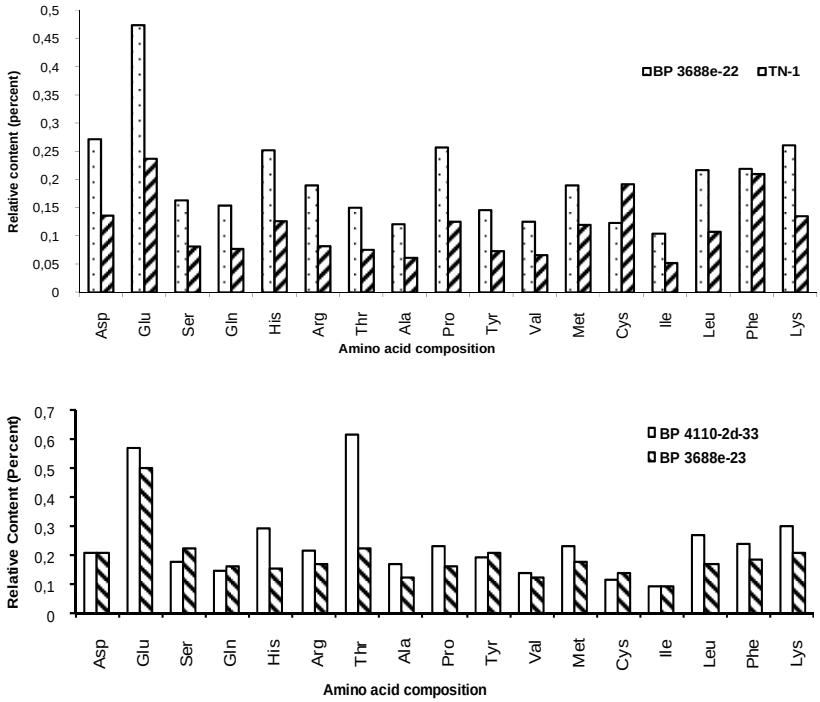

Figure 1. The Relative Proportions of Amino Acid Composition (%) on Rice Genotypes Showing Different Reaction to Xoo Pathogen. Amino Acid Abreviation: asp = aspartic acid; glu = glutamic acid; ser = serine; gln = glycine; his = histidine; arg = arginine; thr = threonine; ala = alanine; pro = proline; tyr = tyrosine; val = valine; met = methionine; cys = cystine; ile = isoleucyne; leu = leucine; phe = phenyl alanine; lys = lysine

Table 2. Composition Structures of Amino Acid Residue (%) on Four Rice Genotypes Infected by Xoo

Structure	Genotypes				
	BP4110-2d-33	BP3688e-23	BP3688e-22	TN-1	Mean $\pm$ SD
Hydrophobic	1.37	1.04	1.23	0.74	0.82 $\pm$ 0.41
Neutral	1.11	0.79	0.58	0.42	0.72 $\pm$ 0.29
Acidic	0.78	0.71	0.74	0.37	0.65 $\pm$ 0.18
Basic	0.95	0.69	0.85	0.42	0.72 $\pm$ 0.23

corn plants. It was reported that the composition of amino acids might be involved in the resistance of *Brassica* plant to insect *Lipaphis erysimi* causing nutritional deficiencies [31].

Based upon this study, rice genotype BP 4110-2d-33 (backcross between Ciherang/Angke) was suggested that this genotypes inherited the resistance gene from its donor parent Angke that contain resistant gene *Xa-4, xa-5*. In general, the contents of amino acids on TN-1 (*Xa-14*) were less than in rice genotypes BP 4110-2d-33, BP 3688e-23, and BP 3688e-22 (Figure 1). Acidic amino acid such as glutamic acid levels was higher than those of other amino acid content. The structure of amino acid was hydrophobic with the ratio between hydrophobic : neutral : acidic and base was 3.27 : 2.9 : 2 : 2.9, respectively (Table 2). Threonine was also showed somewhat higher on rice line's BP 4110-2d-33. No amino acid decreased in genotype BP 4110-2d-33 that showed lower severity to BB, however; in genotypes such as BP 3688e-23, and BP 3688e-22 (two sister lines derived from cingri/memberamo//widas///IR BB8) more amino acid levels were decline although these advance lines showing IR BB8 (*Xa-8*) background. The total amino acid in cultivar's TN-1 was accounted for about one third to about a half of total amino acid than those of genotypes BP 4110-2d-33, BP 3688e-23, and BP 3688e-22. The decrease or complete disappearances of certain amino acids may either be due to the utilization by pathogen enzymatic degradation or have been utilized by host plant for the defense mechanism. In different study, Kurlovich *et al.* [32] reported that genotypes resistant to *Fusarium* wilt display the tendency to increase the level of methionine amino acid.

A multivariate analysis to evaluate rice genotypes was used based on variable of amino acid content. PCA using matrix correlation resulted in four PC, Eigen

value and percentage of total variation (Table 3). PCA enables the identification of linier combinations on dependent variables with maximum variance which is uncorrelated to each other [22]. In addition, multivariate analysis using PCA could employed species cluster [33]. The first four PCs axes are shown in Table 3 that accounted for a total variance of 73.06%, 16.92%, 6.58% and 3.43%, respectively. Eigen value indicated suitability value to evaluate PC. According to Karson [34], Eigen value < 1 means that variable was not significant. Jollife method used Eigen value limit of > 0.7 to evaluate PC [35]. Based on this method only one PC showed Eigen value >0.7. As seen in the table, the PCA of first component was statistically significant. PC-1 explained total variance of 73% which was dominated by glu-amino acid with Eigen-value = 2.90 (Table 3). The pattern of variation for PCA is shown in Table. 4, with the highest score was obtained in glu-content. Two dimensional ordination of four rice genotypes based on PC axes was presented in biplot as shown in Figure 2, whereas the dendrogram analysis of the resulting matrix of Euclidian distances was presented in Figure 3. Based on these figures, the three rice genotypes are spread mostly in the same quadrant. TN-1 was more distant from three other rice genotypes based on PCA score plot. The results obtained from cluster analysis are consistent with those of PCA.

The results of this study demonstrated that TN-1 (*Xa14*) as the susceptible cultivar accumulates less amino acid than other rice genotypes, particularly serine, threonine and tyrosine. The decreased of amino acid levels in TN-1 was subjected to various complex factors such as deficiency of minor elements required by plants for its metabolism due to low essential amino acid. The decrease is also may associated with a rise in proteolytic activity during pathogen infection as supported by Campbell *et al.*, [36] that bacterial infection may affect lyses of cell walls or pectin degradation on central lamella due to proteolytic enzyme produced by bacteria.

As to the amount of essential amino acid, the resistant genotypes can serve as a source of well-balanced amino acid protein [19]. It was predicted that shifting of varieties composition planted in the fields may cause shifting of races population [37]. In this experiment, the dominant bacterial Xoo race occurrence in the field was presumably occupied by race IV, since no resistant rice genotypes observed in the surroundings experiment area which influenced by the composition of genotypes planted in the field. According to Hifni and Kardin [11] Xoo formed new strains/races in spacious time.

Table 3. Percentage of Total Variation Accounted for the First Four Principle Component Axes of the Ordination Rice Genotypes

PC axes	Eigen-value	Percentage of total variation
PC-1	2.90	73.06
PC-2	0.67	16.92
PC-3	0.26	6.58
PC-4	0.13	3.43

Table 4. Factor Scores of the Variables Associated with the First Four Principle Component Axes of the Ordination Rice Genotypes

Code*	PC-1	PC-2	PC-3	PC-4
asp	0.61	0.52	-0.41	-0.28
glu	5.63	-0.07	-0.53	0.33
Ser	-0.51	-0.56	-0.26	0.54
gln	-1.10	-0.05	-0.36	0.13
his	0.30	0.09	0.20	-0.72
arg	-0.57	-0.25	-0.29	-0.12
thr	0.74	-2.40	1.16	0.09
ala	-1.58	-0.33	-0.10	0.05
pro	0.22	0.34	-0.13	-0.58
tyr	-0.76	-0.40	-0.42	0.51
val	-1.63	-0.11	-0.18	0.05
met	-0.18	0.16	0.11	-0.004
cys	-0.56	1.57	0.90	0.57
ile	-2.19	-0.06	-0.25	0.01
leu	-0.03	-0.14	0.03	-0.33
phe	0.82	1.31	0.84	0.07
lys	0.79	0.09	-0.01	-0.33

Notes: Amino acid abreviation: asp = aspartic acid; glu = glutamic acid; ser = serine; gln = glycine; his = histidine; arg = arginine; thr = threonine; ala = alanine; pro = proline; tyr = tyrosine; val = valine; met = methionine; cys = cystine; ile = isoleucyne, leu = leucine; phe = phenyl alanine; lys = lysine

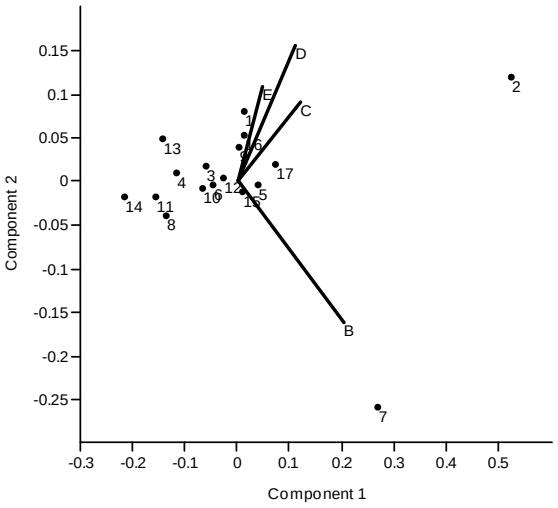

Figure 2. Biplot of the Score of the First Two Principal Components Based on Amino Acid Data from the Infected Rice Genotypes with Xoo. B = BP 4110-2d-33, C = BP 3688e-23; D = BP 3688e-22; E = TN-1

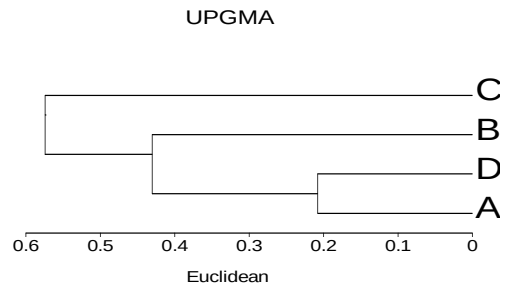

Figure 3. Dendrogram Analysis of Rice Genotypes Infected by Xoo based Upon their Amino Acid Content Using Unweighted Pair-group Method Averaging (UPGMA). A = BP 4110-2d-33, B = TN-1;C = BP 3688e-22; D = BP 3688e-23

Pathogen-host analysis has enabled to incorporate appropriate genes in improved genotypes with resistance against Xoo populations [38]. Since the Xoo interacts with the host in a gene-for-gene manner, it would be useful to characterize the Xoo race that infected its host such as the resistance gene possessed by BP 3688e-23. Characterization on such genotypes is being determined.

4. Conclusion

It was observed that rice genotypes BP 3688e-23, BP 3688e-22 showed less BB disease severity than that of TN-1 causing severity of 2.3% and 9.3%, respectively. Genotypes BP 4110-2d-33, BP 3688e-23, and BP 3688e-22 showed in tight cluster based on amino acid content; however amino acids were not found to be related to the level of BB resistance. Additional studies are needed to further determine the protein levels that affect degree of resistance in rice plants infected by Xoo pathogen as well as resistance gene possessed by BP 3688e-23.

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