Population genomics and pathotypic evaluation of the bacterial leaf blight pathogen of rice reveals rapid evolutionary dynamics of a plant pathogen

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Abstract

Xanthomonas oryzae pv. *oryzae* (*Xoo*) causes bacterial blight disease, which reduces crop yield by up to 50% in rice production. Despite its substantial threat on food production worldwide, knowledge about its population structure, virulence diversity and the relationship between them is limited. We used whole-genome sequencing to explore the diversity and evolution of *Xoo* during the past 30 years in the main rice-planting areas of China. Six separate lineages were revealed by phylogenomic analysis, with CX-5 and CX-6 predominating in the population for decades. The recent sporadic outbreaks were respectively caused by *Xoo* derived from these lineages especially the two major ones. The lineage and sub-lineage distribution of isolates strongly correlated to their geographical origin, which was found to be mainly determined by the planting of the two major rice subspecies, *indica* and *japonica*. Large-scale virulence testing was conducted to evaluate the diversity of pathogenicity for *Xoo.* We found rapid virulence dynamics against rice, and its determinant factors including genetic background of *Xoo*, rice resistance genes and the planting environment of rice. Genetic background was investigated deeply by comparative genomics, which indicates that transposition events contributing the most to evolution of the *Xoo* genome and the rapid diversification of virulence. This study provided a good model to understand the evolution and dynamics of plant pathogens in the context of interaction with their hosts which are influenced by both geographical conditions and farming practices.

Introduction

Population genomics is a powerful tool for understanding the formation and evolution of bacterial pathogens of human and some important domestic animals [1-6]. It has also provided new strategies for the detection, prevention and control of important human pathogens [7,8]. Though plant pathogens are serious threat to food security worldwide, their evolution and dynamics at population scale in the content of modern agricultural practice have not been well understood. A few studies have used genome-based methods to study their population structure, which have provided some new insights into evolution and transmission of some important crop pathogens [9-13].

Xanthomonas oryzae pv. *oryzae* (*Xoo*) causes bacterial blight (BB) disease of rice (*Oryza sativa*) and has been considered one of the top 10 plant bacterial pathogens based on its scientific/economic importance [14]. It is a notorious destructive pathogen which can cause a considerable reduction in rice production in both temperate and tropical regions, especially in Asia [15]. On average, BB can lead to 20%~30% overall yield reduction with some severe cases even causing up to 50% loss [16]. As the world's largest producer and consumer of rice, China has suffered serious BB outbreaks and huge food loss since the 1930s, with the most serious damage occurring between 1950s and 1980s [17,18]. Breeding resistant rice cultivars has been proved to be the most effective method for controlling this pathogen. Since 1980s, BB-resistant rice varieties have been widely planted in China and have significantly reduced the loss caused by BB [19-21]. However, sporadic outbreaks still occurred in different areas of China after

the year of 2000 [22,23]. It is unknown whether the recent outbreaks relate to changes in the genetics and virulence of the past *Xoo* populations in China, allowing it to overcome host defenses. Only few studies focused on population structure of *Xoo* from limited rice-planting areas of China were conducted by DNA fingerprinting methods [24-26]. So very little is known about the evolution, spread and dynamics of *Xoo* involved in different outbreaks during the past decades around the whole country in the context of rapid development of modern agricultural science and technology.

The virulence differentiation among different strains of *Xoo* which was evaluated by defining different races or pathotypes, was frequently studied to infer its virulence dynamics and diversity against rice host. Strains of the same race share a common pathogenic phenotype in a set of tested host cultivars. Rice lines carrying different resistance genes (R genes) determine the race of *Xoo*. Up to now, tens of races of *Xoo* were identified in different rice-planting locations worldwide [22,27-31]. The pathotypes of *Xoo* showed huge diversity among different locations and rapid dynamics along the isolated times. It was predicted that many factors including genetic background of *Xoo* contributing to its virulence diversity [28-30]. However, many crucial details were overlooked due to the intrinsic low sensitivity and stability of the typing method.

Different resistant cultivars carrying bacterial blight R genes have been planted in the different Asian countries for decades, including China [32,33]. Resistance from R genes carried by rice cultivars plays a crucial role of determining *Xoo* evolution during its interaction with rice hosts. Recent study has showed that R gene *Xa4* has largely

86 affected the race composition of *Xoo* from the Philippines [33]. The pathogenic
87 diversity of *Xoo* in China has been studied previously [22,23,25,26]. Race compositions
88 showed huge diversity and rapid dynamics among different areas along different time
89 points [34,35]. However, it is unknown the genetic basis for the pathogenicity diversity
90 and dynamics of *Xoo* from China, which means the relationship between genetic
91 population and the ability *Xoo* to overcome different R genes has not been studied.
92 Moreover, the evolutionary force behind the genetic and pathogenic diversity and
93 dynamics is also not clear.
94 In this study, a comprehensive population genomic study was combined with a large-
95 scale determination of virulence of *Xoo* that have been isolated in the past 30 years in
96 China, to obtain a framework of genetic dynamics and virulence diversity over time
97 and space. Results from this work shed new light on the evolution of this important
98 plant pathogen.
99

100 **Results**

101 **Population structure of *Xoo* from China**

102 To understand the population structure of *Xoo* in China, genomes of the 247 strains
103 were sequenced, including 237 isolated from China, 4 and 6 representative ones from
104 Japan and Philippines, respectively (Table S1). Reads were mapped to the genome of
105 PXO99^{A}; SNPs were called by Samtools. After the prophage region, repeat sequences
106 and recombinant regions being filtered, the core SNPs contained 5,271 variable sites.
107 Two methods were used to outline the population structure based on the core SNPs,

108 Maximum likelihood (ML) phylogenetic analysis and a tree-independent hierarchical
109 Bayesian clustering (BAPS).
110 All *Xoo* from China were clustered into 6 lineages (CX-1 to CX-6), with more than 70%
111 members belonging to CX-5 and CX-6 (Figure 1). The three major rice production areas,
112 including South China, Yangtze Valley area and North China, were displayed in Figure
113 2a. We annotated the lineage-specific tree with the time and space information of the
114 *Xoo* strains isolated (Figure 1, 2b and 2c). CX-1 and CX-2 were most frequently
115 represented in South China at the year of 2003, of which most isolates were from
116 Yunnan province. The *Xoo* belonging to CX-3 were isolated from North China in 1984,
117 2003 and 2014, with most of which from Northeast China in 2014. CX-5 and CX-6
118 were nationally distributed during the past 30 years; while most members in CX-5 were
119 dominant in South China and Yangtze Valley, and CX-6 were more frequently from
120 North China and Yangtze Valley.
121 **Phylogeography of CX-5 and CX-6**
122 To investigate more details about evolution and dynamics of *Xoo* in China, we focused
123 on the two major lineages, CX-5 and CX-6. Phylogenetic analysis based on core SNP
124 alignment (Figures 3a and 3b) and pairwise SNP distance between isolates were
125 performed for both lineages (Figure 3c). Both CX-5 and CX-6 can be divided into 5
126 sub-lineages, respectively (Figure 3a and 3b).
127 CX-5.1, CX-5.2, and CX-5.5 were restricted in South China, while CX-5.3 and CX-5.4
128 can be found in some places of both South China and Yangtze Valley (Figure 3a). When
129 time was considered, we found that all these sub-lineages persisted at these places for

130 at least 30 years. Then we focused on the recent sporadic outbreaks being attributed to
131 this lineage. Besides clustered together on the tree, we considered epidemiologically
132 and genomically linked outbreak with an average SNP pairwise distance less than 5. In
133 2003, the outbreak in Hunan province of Yangtze Valley was caused by CX-5.4 (HN-
134 2003), while the outbreak in Guangdong province of South China ascribed to two sub-
135 population of CX-5.5 (GD-2003-1 and GD-2003-2). One cluster of CX-5.3 caused
136 outbreak in Guangxi province of South China in 2014 (GX-2014).
137 Seven out of 10 isolates in CX-6.1 were from different places of South China. This sub-
138 lineage was the most diverse one in CX-6 with long branches. In CX-6.2, *Xoo* from
139 North China and Yangtze Valley were dominant on different branches, respectively.
140 CX-6.3 and CX-6.4 were most frequently found in Yangtze Valley, while *Xoo* from
141 North China predominated in CX-6.5. Similar as CX-5, all these sub-lineages have
142 contributed to BB on rice for a long time in China. These sub-lineages totally caused at
143 less 4 local outbreaks in 2003 and 2014. In 2003, the local outbreaks in two provinces
144 of Yangtze Valley (Henan, and Jiangsu) were caused by CX-6.2 (HeN-2003) and CX-
145 6.4 (JS-2003), respectively, while the one in North China (Jilin province) was ascribed
146 to CX-6.5 (JL-2003). Outbreak in Jiangsu province (Yangtze Valley) in 2014 was
147 caused by one sub-population of CX-6.4 (JS-2014).

148 **Chinese *Xoo* diversity and dynamics in the context of rice host**

149 There are two major domesticated rice subspecies *Oryza sativa japonica* and *indica*
150 existing in different areas of China; and most of rice varieties were derived from these
151 two subspecies [34,35]. Rice planted in high-altitude areas of Yunnan province from

South China and North China belong to subspecies *japonica* (http://www.ricedata.cn). *Xoo* isolated from these areas were respectively clustered into 3 lineages, CX-1, CX-2, CX-3 and one sub-lineage of CX-6 (CX-6.5) (Figure 1 and Figure 3b). Lineage CX-5 mainly contained *Xoo* isolated from rice in South China where subspecies *indica* is planted (Figure 3a). CX-6 also contained *Xoo* isolated from rice of *japonica* from Yangtze Valley (CX-6.4), *indica* from Yangtze Valley (CX-6.3) and some *indica* from South China (CX-6.1), indicating the widest adaptation to different rice varieties of *Xoo* in this lineage (Figure 3b). In CX-6.2, old *Xoo* were dominant on indica, while recent isolated ones were almost from *japonica*, suggesting a putative host switch in this sub-lineage.

Taken together, the distribution of Chinese *Xoo* lineages appeared to be impaired by biogeography and rapid dynamics over isolated time, which may be mainly due to the distribution and dynamics of the two major subspecies of rice, *japonica* and *indica*.

Diversity of *Xoo* from different counties of Asia

To place *Xoo* from China into a global context, phylogenomic analysis was performed on the core genome SNPs of 109 *Xoo* genomes available in Genbank (including 100 from India, 8 from the Philippines and 1 from Japan), and the 247 sequenced ones in this study. Phylogenetic analysis revealed a similar topological structure of Asian *Xoo* to that of Chinese *Xoo*, with small differences in some branches (Figure S1). Eleven genetic lineages were identified for the *Xoo* population. *Xoo* from India and the Philippines were respectively assigned into PX-A to PX-C and IX-I to IX-V according to previous studies [36,37]. Eight lineages displayed strict geographic distribution

features, most of them being specific to one or at least a limited amount of rice-planting areas. Besides the three Chinese lineages (CX-1, CX-2 and CX-3), PX-B and PX-C were mainly represented in the Philippines, and IX-I to IX-III were from India. CX-4 contained old *Xoo* from China and some recently isolated ones from India belonging to IX-V. For CX-5 and CX-6, besides both represented the major *Xoo* from China, they also contained a few *Xoo* from Japan, India and Philippines, with PX-A of Philippines being nested within the former one and IX-IV from India being encompassed among the members of the last one, indicating frequently transmission of these 2 lineages not only among different places of China but also among different Asian countries.

Rapid virulence dynamics of *Xoo* strains isolated in China during the past 30 years

As describe above, the population structure *Xoo* maintains relative stability during the past 30 years. We next inferred the pathogenic diversity and dynamics of these *Xoo* strains. Based on the interactions between *Xoo* strains and rice lines, most of the tested *Xoo* were classified into 9 pathogenic races (Table S1and Figure 4a). The top two races with most members, R5 and R8, contain 26% and 18% of the isolates, respectively.

The three major rice-planting regions had different race compositions (Figure 4b). Yangtze River Valley mainly contained *Xoo* from races R5 and R8, with the respective percentages of the total population being 50% and 30%, respectively. *Xoo* isolates from North China mostly belongs to R7 and R5, together accounts for about two third of the total population. Five of the nine *Xoo* races were well represented in the *Xoo* population from South China, with each of them account for more than 10% of the total population. The virulence dynamics of *Xoo* during the last 30 years was inferred by analyzing race

196 distribution in China at different period (Figure 4c). Strains isolated before the year of
197 2000 were mainly of the races of R5, R4, R8 and R1, while most *Xoo* isolated in 2003–
198 2004 were R8, R7, R5 and R6; and about 45% of the isolates in 2014 were R5.
199 We also found different lineages have different race compositions, indicating genetic
200 background playing important roles in the interaction between *Xoo* and rice hosts
201 (Figure 4d). Compared to other lineages, the CX-6 *Xoo* isolates fall mostly within the
202 races of R5, R8 and R4, while have reduced representation in R1, R2 and R6. Moreover,
203 the rapid dynamics of pathogenic features can be inferred by focusing on the races of
204 *Xoo* isolated from the same places at the same time and belonging to the same lineage
205 (Figure 1). For example, most *Xoo* from Yunnan, one province of South China, in 2003
206 were clustered in CX-1 and CX-2. The 19 isolates in CX-1 represented 8 of the 9 races
207 described above, and the 14 strains in CX-2 belonged to 6 races. Even *Xoo* isolates from
208 the same outbreak were allocated into different races, with 16 *Xoo* in JL-2003 belonging
209 to 3 different races and 10 isolates in GD-2003-2 representing 2 races.
210 **Resistance gene of rice contributes to the interaction between *Xoo* and rice**
211 As each of the near-isogenic rice lines used in this study contains one known resistance
212 (R) gene, we studied the interaction between *Xoo* and rice host by focusing on the
213 capacity of *Xoo* to overcome the resistances conferred by these genes (Figure 1, Tables
214 1 and 2). When the time of isolation was considered, we found that *Xoo* from different
215 times have different capacity to overcome different R genes (Table 1). R gene *xa5*
216 showed the most effective resistance against *Xoo* all the time. At different periods, more
217 than 90% *Xoo* could not infect the rice line IRBB5, which contained gene *xa5*. The

218 resistance mediated by R gene *Xa3* was effective during the period of 1990 to 2004.
219 However, rice containing this R gene only showed resistance against 5% of *Xoo* in 2014.
220 Rice cultivars carrying R gene *Xa4* are resistant to more than 60% of *Xoo* isolated from
221 1970s to 2000 and 2014. However, they are only resistant to about 30% of the isolates
222 recovered in 2003 and 2004.
223 To determine whether the *Xoo* genetic background contributes to the interaction
224 between *Xoo* and rice lines, we studied the resistance capacity of R genes against *Xoo*
225 from different lineages (Table 2). We found that resistance provided by R genes against
226 *Xoo* are indeed depended on the genetic background of *Xoo*. R genes *Xa2* and *Xa14*
227 effectively confer resistance against *Xoo* from Lineages CX-1, CX-2 and CX-3,
228 preventing infection by more than 70% of *Xoo* strains from these lineages. Similarly, R
229 gene *Xa3* and *Xa4* showed potential resistance against *Xoo* from CX-1 and CX-2, and
230 CX-2 and CX-3, respectively. No R gene except *xa5* conferred resistance to more than
231 50% of *Xoo* strains from CX-5 and CX-6, which is consistent with the situation that
232 CX-5 and CX-6 had much more extensive distribution across different areas of China
233 during the past decades, comparing to the other Chinese *Xoo* lineages. Among the sub-
234 lineages of CX-5 and CX-6, *Xa2* was only effective against *Xoo* of CX-6.5, with
235 effectiveness of 90%, while *Xa4* could confer the resistance against more than 60% *Xoo*
236 from these two lineages except for CX-5.1 and CX-6.5; *Xa14* showed potential medium
237 level of resistance against *Xoo* from CX-6.1 and CX-6.5 (Table S2).

238 **Genome dynamics of *Xoo* is mainly determined by transposition events**

239 To study the genomic evolution contributing to the rapid dynamics of virulence, several

240 *Xoo* isolates from different lineages were chosen to complete genome for comparative
241 genomics. All proteins of the 22 *Xoo* with complete genomes were clustered into 7542
242 groups, with 3513 belonging to core genes. The pan genes plot showed that Asian *Xoo*
243 has an open pan genome (**Figure S2**).
244 It was reported that *Xoo* genome contains numerous insert sequence elements, so we
245 firstly studied the distribution of transposase genes to understand the dynamics of *Xoo*
246 genomes. Each *Xoo* genome contained more than 800 transposase genes, taking up
247 above 16% of the total genes, with most of them from IS5, IS3, IS1595, IS701, IS30
248 and IS630 (**Figure 5 and Table S3**). In the core gene sets, 8%, (266 of 3543) were
249 predicted to encode transposases. However, 71% (1251 of 1771) and 62% (942 of 1523)
250 of the distributed gene and unique gene families were transposases (**Figure S3**). It
251 suggests that transposon elements contribute the most to the diversity of *Xoo* genomes.
252 Based on the analysis of gene presence/absence analysis, the core genome of *Xoo* can
253 be split into about 200 conserved blocks by less conserved genome fragments (Figure
254 5). Among the blocks, there were genome segments with more sequence diversity, and
255 most transposases were in these regions.
256 As a rice pathogen, the host selection was reported to play crucial roles on the evolution
257 of *Xoo*. Type III effectors (T3Es) were found to be crucial to the pathogenicity of
258 *Xanthomas* spp. on plant hosts. Among them, transcription activator-like effectors
259 (TALEs) (Avirulence protein AvrBs3) are the most important in *Xoo* during the
260 interaction with rice. TALEs function as transcriptional factors activate expression of
261 target genes in the host in a sequence specific manner, resulting in enhanced bacterial

growth and development of disease symptom (Bogdanove et al 2010). Most TALEs from *Xoo* activate the expression of *SWEET* genes in rice to promote susceptibility [32]. Significant variations in the number of TALEs was observed among the 22 *Xoo* with complete genomes, ranging from 11 to 21 (**Table S4**). Half of the TALE families (21 out of 42) were only predicted in one or two isolates. And some families only distributed or lost in some special lineages. *Xoo* from the same lineage had some common and different families of TALEs. Even *Xoo* isolated in the same geographical location at the same time with the same phylogenetic relationship, JL28 and JL33, contain different compositions of TALE families. This suggests that interaction among rice and *Xoo* can determine rapid diversity of TALE effectors. When locations of TALE genes were identified, we found that almost all of them were in the variable regions where enrich in transposase genes (Figure 5). Conversely, other T3Es were very conserved, almost all of them located in the conserved genome blocks with less transposons (Figure 5).

Discussion

Most *Xoo* clusters identified by traditional molecular typing methods from several Asian countries showed strict geographical distribution [28-30,38]. Previous report focusing on *Xoo* from India through population genomics defined 5 lineages, with each of them showing a restricted-region distribution [37]. In this study, based on population genomics, we found that there is a general correspondence between the areas of *Xoo* isolated and most of Asian lineages and some sub-lineages in CX-5 and CX-6 (Figures 1, 3 and S2). Moreover, Asian *Xoo* was reported to be very different from those of

Africa and North America regions [39,40]. This suggests that the geographical factors as affected by climate, soil types, or the choice of different rice cultivars and varieties may influence on the diversity of *Xoo*. Though thousands of rice cultivars have been planted in different areas of China, almost all of them were derived from the two subspecies, *indica* and *japonica*, or hybrid varieties between them (http://www.ricedata.cn). Information of the *Xoo* host subspecies in China indicates that variety of rice was one of the major factors forces on the formation and dynamics of Chinese *Xoo* population. Rice varieties planted in different counties and different areas of a country are determined by geographical environment, climate factors and local agricultural policy [41-43]. Taken together, geography-specific rice cultivars in different countries or different areas in the same country contribute the most to the genetic diversity of *Xoo* infecting them.

Development resistance for novel cultivars of crops and vegetables, is proved to be the most effective strategy fighting against bacterial and fungi pathogens [44-47]. But the durability of plant resistance can be neutralized by evolutionary changes in pathogen populations. Knowledge about pathogen population evolution and dynamics under the force from the resistant plant are crucial for further cultivar development [48]. Though resistance breeding of rice have effectively prevented the unprecedented outbreak of BB since 1990s, sporadic outbreaks occurred in different areas of China after the year 2000 [49]. Genomic epidemiology analysis indicates all *Xoo* strains causing the recent epidemic outbreaks except those in CX-1 were derived from the ones leading to heavy losses in the history. On the other hand, only the minor lineage CX-4 was not

306 represented by the recent outbreaks, so the diversity of the *Xoo* population was not
307 reduced due to wide planting of BB-resistant rice varieties. For the current practice of
308 rice production, the major BB threat in the three rice-planting areas of China is still
309 mainly from CX-5 and CX-6 as they were present in history, which call for different
310 detection and prevention strategies for different areas and rice varieties.
311 The virulence diversity of *Xoo* in China against rice was well investigated during the
312 past 30 years, however, the relationships between population genetic structure and
313 pathotype of *Xoo* were not explored well [22-24,26,49]. This study shows that races of
314 *Xoo* from South China were more diverse than those from North China and Yangtze
315 Valley areas. In line with this, phylogenetic analysis revealed that *Xoo* from South
316 China are more diverse compared to other places of China (Figure 1). Some rice
317 growing regions in South China, particularly in the Yunnan province, have three
318 harvests per year and use different rice cultivars for each harvest [50,51]. The greater
319 genetic and virulence diversity of *Xoo* thus could be caused by a greater diversity of
320 rice cultivars in use. Our result also showed that there were diverse races in the same
321 lineage, for example, 85% *Xoo* from lineage CX-6 could be assigned to 8 races (Figure
322 4d), indicating rapid virulence dynamics in the same lineage. Moreover, the isolates
323 from the same local outbreak even belonged to different races. Although *Xoo* strains
324 isolated in China were consistently assigned to one of the 6 Chinese lineages, the
325 dominating population of *Xoo* races in specific areas displayed rapid dynamics and high
326 level of diversity during the past 30 years (Tables 1 and 2). Rapid virulence dynamics
327 with relative stability of the standing genetic variability of *Xoo* population indicates fast

328 adaptation to host during interaction for this plant pathogen.

329 In China, since the 1980s, *indica* hybrid rice (especially Shanyou 63) had been planted
330 on a large scale [52]. Later, bacterial blight resistant rice varieties, mainly the *india* rice
331 carrying *Xa4* and *japonica* rice carrying *Xa3*, were grown on a large scale for a long
332 time [31,52]. Soon after these rice varieties widely used for rice production, the
333 effectiveness of these R genes conferring resistance against infection causing by *Xoo*
334 was significantly compromised (Table 1). Actually, the resistance abilities of these two
335 R genes were also overcome frequently by *Xoo* population in other Asian countries
336 [30,38,53]. R gene *xa5* showed very good resistance (above 95%) to *Xoo* from all
337 lineages. Previously, isolates of *Xoo* having compatibility with *xa5* were only reported
338 from Indian Lineages IX-I and IX-III, and some Philipps and Korea isolates [27,54,55].
339 The only 2 Chinese *Xoo* from Yunnan province (YN24 and YN04-5) belonging to IX-I
340 and IX-III showed good infection ability to rice with *xa5*, suggesting putative
341 transmission of *Xoo* from India to South China. Besides, several isolates from CX-5
342 and CX-6, especially those obtained recently, can overcome the resistance contributed
343 by *xa5*, indicating different origins of these resistance compatibilities (Tables 1 and 2).
344 Since rice variants with gene *xa5* were less used in China, the development of its
345 compatibility could not be due to a direct selection pressure of this R gene.

346 It was reported recently that different *Xoo* population from the Philippines evolved
347 different mechanisms to adapt to the R gene *Xa4* [22]. Chinese *Xoo* Isolates from
348 different lineages and sub-lineages showed distinct capabilities to fight against the same
349 R gene. Overall, lineages and sub-lineages with stricter geographical distribution can

350 be more easily defeated by R genes than the wider distributed ones (Tables 2 and S2).
351 The underlying genetic determinations of these differences call for advanced
352 comparative genomics and wet experiments. However, there is no lineage or sub-
353 lineage with all isolates incompatible with one of the tested R genes, which means that
354 *Xoo* with different genetic backgrounds can adapt to different rice hosts with variable
355 resistances. This suggests that the introduction of new R genes could reduce the loss
356 causing by *Xoo* but can't change the genetic diversity of the *Xoo* population, as no
357 lineage or sub-lineage can be eliminated thoroughly. This is partially explained by that
358 R genes usually restrict the pathogens to the initial infection site, but have no killing
359 abilities [56].
360 Most of the R genes cloned up to now were reported to mediate resistance associated
361 with the TALEs during the interaction between rice and *Xoo* [57,58]. The TALE
362 proteins are the most import effectors used by *Xoo* to interact with rice, enforcing
363 virulence, proliferation, and dissemination against rice [59]. All the TALE genes were
364 found in the several variable regions where enriched in transposase genes belonging to
365 families IS3, IS5, IS30 and IS701, respectively. We also find that other T3Es didn't
366 have much diversity. It is predicted that transposons containing these kinds of
367 transposons contribute to the rapid dynamics of virulence of *Xoo* isolates mainly
368 through affecting the compositions and structure of TALEs. Similar situation was
369 reported that Tn3-like transposon was proved to play a major role of the spread of
370 pathogenicity determinants in *Xanthomonas* spp. [60]. Actually, transposon-rich
371 genome regions were reported to play crucial roles in rapid evolution of effectors after

host changes in the Irish potato famine pathogen *Phytophthora infestans* [61].

Conclusion

The introduction of several resistance genes into rice tremendously reduced the damage of this notorious plant pathogen; only sporadic events were reported after 2000s [19,21-23]. However, this study found that the genome evolution and virulence dynamics of *Xoo* are extraordinary rapid, indicating its ability to overcome the resistance conferred by the current resistance genes and cause the potential for large-scale outbreaks in the future. It is therefore prudent to continue surveillance of disease outbreaks caused by this plant pathogen, and to develop novel strategies for its control. The rice varieties and resistance genes were predicted to be crucial to the evolution and dynamics of *Xoo*. The subspecies of *Oryza sativa* used for rice production and resistance gene introduction into the rice planted were mainly decided by human. The results from the population genomics and large-scale virulence tests provided new insight into how plant pathogens evolve and spread in the context of human agricultural activities. Resistance genes from rice can be used to fight against *Xoo*, but *Xoo* can rapid develop strategies to overcome these resistances. Transposon elements were proved to play a crucial role in the evolution progress during the interaction between pathogen and it rice host. The interaction between plant pathogen and host was reported to conform to zig-zag-zig model [62], this study presented a well-documented example for co-evolution of a pathogen and its host.

394 **Materials and Methods**

395 **Genome sequencing**

396 *Xoo* strains from 3 countries were used in this study (Table S1). The major strains were
397 isolated from China. And the represented *Xoo* strains for 4 different races from Japan
398 and 7 different races from Philippines were also sequenced.

399 The bacterial DNA of overnight cultures (30 °C) was extracted and purified using the
400 Easy-DNA kit (Invitrogen, USA) following the manufacturer's protocol. Total DNA
401 was sequenced by with Illumina HiSeq 2000, 2500, or 4000 to produce pair-end reads
402 with lengths of 100, 125 and 150 bp. For each *Xoo*, raw reads were assessed with the
403 FastQC tool (https://github.com/s-andrews/FastQC) and quality filtered using
404 Trimmomatic (https://github.com/timflutre/trimmomatic). The filtered reads were
405 error-corrected by library with Quake to produce clean reads [63]. Most of the analysis
406 were conducted with the PGCGAP pipeline developed by our bioinformatic team [64];
407 and more details are described as below.

408 **Mapping and SNP calling**

409 To study the population structure of *Xoo* from China, reads from each strain sequenced
410 was mapped onto the reference genome PXO99^{A}, which is used as a model strain for
411 studying the interaction between *Xoo* and rice host and is the first *Xoo* with complete
412 genome sequence [65], using BWA [66]. Variant detection was performed using
413 Samtools mpileup (https://github.com/samtools/samtools) and filtered with a minimum
414 mapping quality of 30. SNP was excluded if its coverage was less than 10% or more
415 than 200% of the average coverage, if it was not supported by at least 5 reads on each

416 strand. Phage regions and repetitive sequences of the PXO99^{A} genome were predicted
417 by PHASTER (http://phaster.ca) and RepeatScout (https://bix.ucsd.edu/repeatscout),
418 respectively. Snippy (https://github.com/tseemann/snippy) was used to generate the
419 whole genome alignment of all studied *Xoo*, and then the recombination was detected
420 by Gubbins (https://sanger-pathogens.github.io/gubbins) on the core genome alignment
421 after prophage and repeat regions were filtered. SNPs located within phage regions,
422 repetitive sequences or recombinant regions were excluded.

423 **Assembly and whole genome alignment**

424 To study the population structure of Asian *Xoo*, genomes from different locations were
425 downloaded from the Genbank as follows: 100 from India, 9 from Philippines, 1 from
426 South Korea and 1 from Japan [36,37]. We performed genome assembly from all the
427 strains sequenced in this study. For each strain, the pair-end clean reads were assembled
428 using Spades [67]. After a few evaluations, different sets of k-mer values were chosen
429 for Spades according to different read lengths. The final assemblies were obtained by
430 filtering out contigs with few reads supported or with lengths lower than 200 bp (Table
431 S1). Whole genome alignment was carried out on all the genomes obtained from public
432 database and assembled here by Parsnp using the genome of PXO99^{A} as reference [68].
433 Core SNPs were extracted from the core genome alignment by snp-sites
434 (https://github.com/sanger-pathogens/snp-sites). Recombination of the core genome
435 alignment was inferred by Gubbins. The final SNPs were obtained by filtering out the
436 prophage region and repeat sequences mentioned above, and the recombined regions
437 predicted here.

438 **Phylogenetic analysis**

439 The SNPs-based maximum likelihood (ML) phylogenies were built by RAxML with
440 the generalized time-reversible model and a Gamma distribution to model site-specific
441 rate variation [69]. Bootstrap support values were calculated from 500 replicates. *Xoo*
442 population structure of China was defined with the hierBAPS module of the BAPS
443 software, which delineates the population structure by nested clustering [70]. Three
444 independent iterations with upper population sizes of 5, 10, and 15 were used to obtain
445 optimal clustering of the population. Phylogenetic trees were annotated and visualized
446 by iTOL (https://itol.embl.de/).

447 **Comparative genomics of compete genomes**

448 Ten isolates were selected for sequencing by long-read sequencing platform based on
449 their positions on the phylogenetic tree and the races they represented. High quality
450 total DNA of each isolate was sequenced by the PacBio RS II sequencing platform
451 using P6-C4 chemistry to a mean fold coverage of 150. De novo assembly was
452 conducted by HGAP3 [71].

453 For more comprehensive analysis of the genomic dynamics of *Xoo*, complete *Xoo*
454 genomes available on Genbank were included. All the genomes were annotated by
455 Prokka [72]. The TAL effectors of each genome were identified and classified by
456 AnnoTALE [73]. Pan and core genome analysis was conducted by the tool Roary [74],
457 based the gff format file constructed by Prokka. The inflation value of 2 was used for
458 the MCL clustering.

459 **Virulence evaluation of *Xoo* strains**

460 Virulence of *Xoo* was assesses by inoculating 6 near-isogenic rice lines, each carrying
461 a specific R gene, IRBB2 (*Xa2*), IRBB3 (*Xa3*), IRBB4 (*Xa4*), IRBB5 (*xa5*), IRBB14
462 (*Xa14*) and IR24 (*Xa18*). IR24 was used as a susceptible check. Seeds of all lines in
463 this study were obtained from the China National Rice Research Institute (CNRRI).
464 Three weeks after sowing on a seedbed, seedlings were uprooted and transplanted into
465 the field. The management of the rice plants in the field was proceeded as usual.
466 Bacterial strain was cultivated on NA plates at 28 °C for 48 h. Then the bacterial
467 colonies were suspended in sterile ddH_2O with concentration adjusted to 3×10^8 cfu/ml
468 before inoculation. The leaf-clipping method was used to test the virulence of all the
469 *Xoo* strains [75]. Fifteen top fully expanded leaves per rice plant were inoculated with
470 each strain. The lesions length was measured after 21 days post inoculation. The ratio
471 of the lesion length compared to the whole leaf length was calculated as described
472 previously [76]. When the ratio of the rice line was lower than 1/4, the rice line was
473 classified as resistant (R); when the ratios were between 1/4 and 1, the rice line was
474 rated as susceptible (S). The lesion length 14 days following inoculation was also
475 measured and used to compare with the data obtained 7 days later.

476 **Data access**

477 Sequence data generated for this study have been submitted to the NCBI Sequence Read
478 Archive under BioProject accession PRJNA350904.

479

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Conflict of Interest

The authors declare no conflict of interest.

Reference

1. Didelot X, Walker AS, Peto TE, Crook DW, Wilson DJ (2016) Within-host evolution of bacterial pathogens. Nat Rev Microbiol 14: 150-162.
2. Baker S, Thomson N, Weill FX, Holt KE (2018) Genomic insights into the emergence and spread of antimicrobial-resistant bacterial pathogens. Science 360: 733-738.
3. Feasey NA, Hadfield J, Keddy KH, Dallman TJ, Jacobs J, et al. (2017) Distinct *Salmonella Enteritidis* lineages associated with enterocolitis in high-income settings and invasive disease in low-income settings. Nat Genet 49: 1211-1217.
4. Okoro CK, Kingsley RA, Connor TR, Harris SR, Parry CM, et al. (2012) Intracontinental spread of human invasive *Salmonella Typhimurium* pathovariants in sub-Saharan Africa. Nat Genet 44: 1215-1221.
5. Weill FX, Domman D, Njamkepo E, Tarr C, Rauzier J, et al. (2017) Genomic history of the seventh pandemic of cholera in Africa. Science 358: 785-789.
6. Coll F, Phelan J, Hill-Cawthorne GA, Nair MB, Mallard K, et al. (2018) Genome-wide analysis of multi- and extensively drug-resistant *Mycobacterium tuberculosis*. Nat Genet 50: 307-316.
7. Quainoo S, Coolen JPM, van Hijum S, Huynen MA, Melchers WJG, et al. (2017) Whole-Genome Sequencing of Bacterial Pathogens: the Future of Nosocomial Outbreak Analysis. Clin Microbiol Rev 30: 1015-1063.
8. Gardy JL, Loman NJ (2018) Towards a genomics-informed, real-time, global pathogen surveillance system. Nat Rev Genet 19: 9-20.
9. Zhong Z, Chen M, Lin L, Han Y, Bao J, et al. (2018) Population genomic analysis of the rice blast fungus reveals specific events associated with expansion of three main clades. ISME J 12: 1867-1878.
10. Baltrus DA, McCann HC, Guttman DS (2017) Evolution, genomics and epidemiology of Pseudomonas syringae: Challenges in Bacterial Molecular Plant Pathology. Mol Plant Pathol 18: 152-168.
11. Brader G, Compant S, Vescio K, Mitter B, Trognitz F, et al. (2017) Ecology and Genomic Insights into Plant-Pathogenic and Plant-Nonpathogenic Endophytes. Annu Rev Phytopathol 55: 61-83.
12. Hartmann FE, Croll D (2017) Distinct Trajectories of Massive Recent Gene Gains and Losses in Populations of a Microbial Eukaryotic Pathogen. Mol Biol Evol 34: 2808-2822.
13. Hartmann FE, Sanchez-Vallet A, McDonald BA, Croll D (2017) A fungal wheat pathogen evolved

host specialization by extensive chromosomal rearrangements. ISME J 11: 1189-1204.
14. Mansfield J, Genin S, Magori S, Citovsky V, Sriariyanum M, et al. (2012) Top 10 plant pathogenic bacteria in molecular plant pathology. Mol Plant Pathol 13: 614-629.
15. Mew T, Alvarez A, Leach J, Swings J (1993) Focus on bacterial blight of rice. Plant Dis 77: 5-13.
16. New T Current status and future prospects of research on bacterial blight of rice. Annu Rev Phytopathol: 359-382.
17. Fang Z (1963) Rice Bacterial Blight: Jiangsu People Press.
18. Zhang Q (2009) Genetics and Improvement of Bacterial Blight Resistance of Hybrid Rice in China. Rice Science 16: 83-92.
19. Huang N, Angeles ER, Domingo J, Magpantay G, Singh S, et al. (1999) Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. Theor Appl Genet 95: 313-321.
20. Kottapalli KR, Narasu ML, Jena KK (2010) Effective strategy for pyramiding three bacterial blight resistance genes into fine grain rice cultivar, Samba Mahsuri, using sequence tagged site markers. Biotechnol Lett 32: 989-996.
21. Pradhan SK, Nayak DK, Mohanty S, Behera L, Barik SR, et al. (2015) Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deepwater rice variety, Jalmagna. Rice (N Y) 8: 51.
22. Liu H, Yang W, Hu B, Liu F (2007) Virulence analysis and race classification of *Xanthomonas oryzae* pv. *oryzae* in China. J Phytopathol 155: 129-134.
23. Chen XL, Yu L, Gao LL, Jiang T, Li QY, et al. (2012) Elevational Variation in Diversity of *Xanthomonas oryzae* pv. *oryzae* in South-West China. J Phytopathol 160: 261-268.
24. Hua J, Zhang Y, Qian W, He CZ (2007) Avirulence gene and insertion element-based RFLP as well as RAPD markers reveal high levels of genomic polymorphism in the rice pathogen *Xanthomonas oryzae* pv. *oryzae*. Syst Appl Microbiol 30: 587-599.
25. Chen X, Wei S, Yan Q, Huang F, Ma Z, et al. (2019) Virulence and DNA fingerprinting analysis of *Xanthomonas oryzae* pv. *oryzae* identify a new pathotype in Guangxi, South China. J Basic Microbiol 59: 1082-1091.
26. Li G, Song CF, Pang XM, Yang Y, Wang JS (2009) Analysis of Pathotypic and Genotypic Diversity of *Xanthomonas oryzae* pv. *oryzae* in China. J Phytopathol 157: 208-218.
27. Jeung JU, Heu SG, Shin MS, Vera Cruz CM, Jena KK (2006) Dynamics of *Xanthomonas oryzae* pv. *oryzae* Populations in Korea and Their Relationship to Known Bacterial Blight Resistance Genes. Phytopathol 96: 867-875.
28. Adhikari TB, Mew TW, Leach JE (1999) Genotypic and Pathotypic Diversity in *Xanthomonas oryzae* pv. *oryzae* in Nepal. Phytopathol 89: 687-694.
29. Mishra D, Vishnupriya MR, Anil MG, Konda K, Raj Y, et al. (2013) Pathotype and genetic diversity amongst Indian isolates of *Xanthomonas oryzae* pv. *oryzae*. PLoS One 8: e81996.
30. Nelson RJ, Baraoidan MR, Cruz CM, Yap IV, Leach JE, et al. (1994) Relationship between Phylogeny and Pathotype for the Bacterial Blight Pathogen of Rice. Appl Environ Microbiol 60: 3275-3283.
31. Xu Z, Sun Q, Liu F, Chen Z, Hu B, et al. (2004) Race monitoring of rice bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) in China. Zhongguo Shuidao Kexue 18: 469-473.
32. Jiang N, Yan J, Liang Y, Shi Y, He Z, et al. (2020) Resistance Genes and their Interactions with Bacterial Blight/Leaf Streak Pathogens (*Xanthomonas oryzae*) in Rice (*Oryza sativa* L.)—an Updated Review. Rice 13: 1-12.

33. Quibod IL, Atieza-Grande G, Oreiro EG, Palmos D, Nguyen MH, et al. (2020) The Green Revolution shaped the population structure of the rice pathogen *Xanthomonas oryzae* pv. *oryzae*. ISME J 14: 492-505.
34. Huang X, Kurata N, Wei X, Wang ZX, Wang A, et al. (2012) A map of rice genome variation reveals the origin of cultivated rice. Nature 490: 497-501.
35. Kovach MJ, Sweeney MT, McCouch SR (2007) New insights into the history of rice domestication. Trends Genet 23: 578-587.
36. Quibod IL, Perez-Quintero A, Booher NJ, Dossa GS, Grande G, et al. (2016) Effector Diversification Contributes to *Xanthomonas oryzae* pv. *oryzae* Phenotypic Adaptation in a Semi-Isolated Environment. Sci Rep 6: 34137.
37. Midha S, Bansal K, Kumar S, Girija AM, Mishra D, et al. (2017) Population genomic insights into variation and evolution of *Xanthomonas oryzae* pv. *oryzae*. Sci Rep 7: 40694.
38. Adhikari TB, Cruz C, Zhang Q, Nelson RJ, Skinner DZ, et al. (1995) Genetic Diversity of *Xanthomonas oryzae* pv. *oryzae* in Asia. Appl Environ Microbiol 61: 966-971.
39. Triplett LR, Hamilton JP, Buell CR, Tisserat NA, Verdier V, et al. (2011) Genomic analysis of *Xanthomonas oryzae* isolates from rice grown in the United States reveals substantial divergence from known X. oryzae pathovars. Appl Environ Microbiol 77: 3930-3937.
40. Gonzalez C, Szurek B, Manceau C, Mathieu T, Sere Y, et al. (2007) Molecular and pathotypic characterization of new *Xanthomonas oryzae* strains from West Africa. Mol Plant Microbe Interact 20: 534-546.
41. Stuecker MF, Tigchelaar M, Kantar MB (2018) Climate variability impacts on rice production in the Philippines. Plos One 13: e0201426.
42. Lu ZJ, Song Q, Liu KB, Wu WB, Liu YX, et al. (2017) Rice cultivation changes and its relationships with geographical factors in Heilongjiang Province, China. J Integr Agr 16: 2274-2282.
43. Wang Y, Xue Y, Li J (2005) Towards molecular breeding and improvement of rice in China. Trends Plant Sci 10: 610-614.
44. Hu J, Xiao C, He YQ (2016) Recent progress on the genetics and molecular breeding of brown planthopper resistance in rice. Rice (N Y) 9(1): 30.
45. Miedaner T, Korzun V (2012) Marker-Assisted Selection for Disease Resistance in Wheat and Barley Breeding. Phytopathol 102: 560-566.
46. Mesterhazy A, Lemmens M, Reid LM (2012) Breeding for resistance to ear rots caused by *Fusarium* spp. in maize - a review. Plant Breeding 131: 1-19.
47. Tiwari JK, Siddappa S, Singh BP, Kaushik SK, Chakrabarti SK, et al. (2013) Molecular markers for late blight resistance breeding of potato: an update. Plant Breeding 132: 237-245.
48. McDonald BA, Linde C (2002) Pathogen population genetics, evolutionary potential, and durable resistance. Annu Rev Phytopathol 40: 349-379.
49. Yang SQ, Liu SY, Zhao S, Yu YH, Li RB, et al. (2013) Molecular and pathogenic characterization of new *Xanthomonas oryzae* pv. *oryzae* strains from the coastline region of Fangchenggang city in China. World J Microbiol Biotechnol 29: 713-720.
50. Tu M, Lu BR, Zhu Y, Wang Y (2007) Abundant within-varietal genetic diversity in rice germplasm from Yunnan Province of China revealed by SSR fingerprints. Biochem Genet 45: 789-801.
51. Xiong Z ZS, Wang Y, Ford-Lloyd BV, Tu M, Jin X, Wu Y, Yan H, Yang X, Liu P, Lu BR (2010) Differentiation and distribution of *indica* and *japonica* rice varieties along the altitude gradients in Yunnan Province of China as revealed by InDel molecular markers. Genet Resour Crop Evol 57: 891-

902.
52. Chen S, Lin XH, Xu CG, Zhang QF (2000) Improvement of bacterial blight resistance of 'Minghui 63', an elite restorer line of hybrid rice, by molecular marker-assisted selection. Crop Science 40: 239-244.
53. Leach JE, Rhoads ML, Vera Cruz CM, White FF, Mew TW, et al. (1992) Assessment of genetic diversity and population structure of *Xanthomonas oryzae* pv. *oryzae* with a repetitive DNA element. Appl Environ Microbiol 58: 2188-2195.
54. Carpenter SCD, Mishra P, Ghoshal C, Dash PK, Wang L, et al. (2018) A Strain of an Emerging Indian *Xanthomonas oryzae* pv. *oryzae* Pathotype Defeats the Rice Bacterial Blight Resistance Gene xa13 Without Inducing a Clade III SWEET Gene and Is Nearly Identical to a Recent Thai Isolate. Front Microbiol 9: 2703.
55. Huang S, Antony G, Li T, Liu B, Obasa K, et al. (2016) The broadly effective recessive resistance gene xa5 of rice is a virulence effector-dependent quantitative trait for bacterial blight. Plant J 86: 186-194.
56. Shen Y, Ronald P (2002) Molecular determinants of disease and resistance in interactions of *Xanthomonas oryzae* pv. *oryzae* and rice. Microbes Infect 4: 1361-1367.
57. White FF, Yang B (2009) Host and Pathogen Factors Controlling the Rice-*Xanthomonas oryzae* Interaction. Plant Physiology 150: 1677-1686.
58. Ji Z, Wang C, Zhao K (2018) Rice Routes of Countering *Xanthomonas oryzae*. Int J Mol Sci 19: 3008.
59. Boch J, Bonas U (2010) Xanthomonas AvrBs3 family-type III effectors: discovery and function. Annu Rev Phytopathol 48: 419-436.
60. Ferreira RM, de Oliveira AC, Moreira LM, Belasque J, Jr., Gourbeyre E, et al. (2015) A TALE of transposition: Tn3-like transposons play a major role in the spread of pathogenicity determinants of *Xanthomonas citri* and other xanthomonads. mBio 6: e02505-02514.
61. Raffaele S, Farrer RA, Cano LM, Studholme DJ, MacLean D, et al. (2010) Genome evolution following host jumps in the Irish potato famine pathogen lineage. Science 330: 1540-1543.
62. Jones JD, Dangl JL (2006) The plant immune system. Nature 444: 323-329.
63. Kelley DR, Schatz MC, Salzberg SL (2010) Quake: quality-aware detection and correction of sequencing errors. Genome Biol 11: R116.
64. Liu H, Xin B, Zheng J, Zhong H, Yu Y, et al. (2020) Build a Bioinformatics Analysis Platform and Apply it to Routine Analysis of Microbial Genomics and Comparative Genomics , 27 January 2020, PROTOCOL (Version 1) available at Protocol Exchange. [+https://doiorg/1021203/rs221224/v1+].
65. Salzberg SL, Sommer DD, Schatz MC, Phillippy AM, Rabinowicz PD, et al. (2008) Genome sequence and rapid evolution of the rice pathogen *Xanthomonas oryzae* pv. *oryzae* PXO99A. BMC Genomics 9: 204.
66. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25: 1754-1760.
67. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, et al. (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19: 455-477.
68. Treangen TJ, Ondov BD, Koren S, Phillippy AM (2014) The Harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. Genome Biol 15: 524.
69. Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312-1313.

70. Cheng L, Connor TR, Siren J, Aanensen DM, Corander J (2013) Hierarchical and spatially explicit clustering of DNA sequences with BAPS software. Mol Biol Evol 30: 1224-1228.
71. Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, et al. (2013) Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 10: 563-569.
72. Seemann T (2014) Prokka: rapid prokaryotic genome annotation. Bioinformatics 30: 2068-2069.
73. Grau J, Reschke M, Erkes A, Streubel J, Morgan RD, et al. (2016) AnnoTALE: bioinformatics tools for identification, annotation, and nomenclature of TALEs from Xanthomonas genomic sequences. Sci Rep 6: 21077.
74. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, et al. (2015) Roary: rapid large-scale prokaryote pan genome analysis. Bioinformatics 31: 3691-3693.
75. Kauffman H, Reddy A, Hsieh S, Merca S (1973) An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. Plant Dis Rep 57: 737-741.
76. Fang C, Xu Z, Guo C, Yin S, Wu S, et al. (1990) Studies on pathotypes of *Xanthomonas campestris* pv. *oryzae* in China. Acta Phytopathol Sin 20: 81-88.

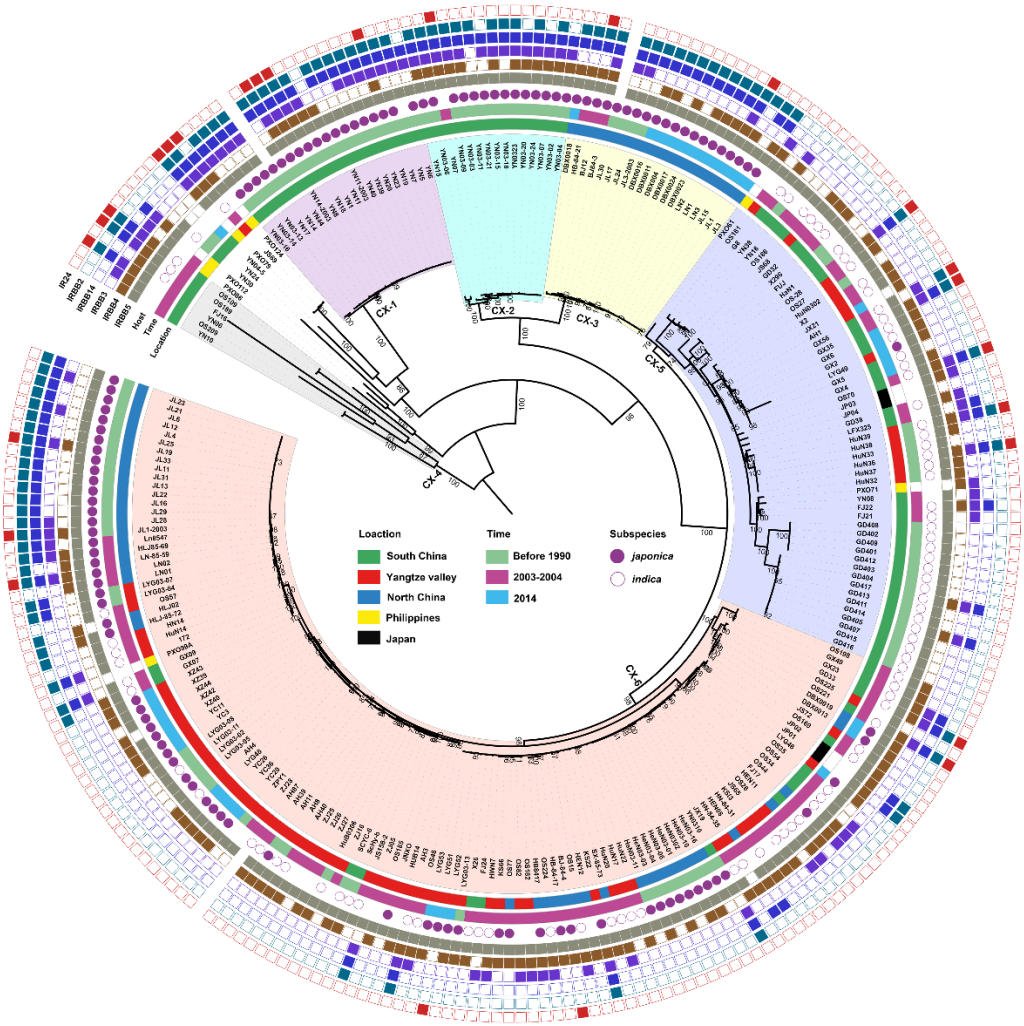


Figure 1 Population structure of *X. oryzae* pv. *oryzae*. The maximum likelihood tree was inferred by RAxML with the generalized time-reversible model and a Gamma distribution to model site-specific rate variation based on the core SNPs of all the *Xoo* genome sequences. The six lineages were decorated with different colors. From inner to outer, the first circle refers to major rice planting areas, the second one describes isolated times of these strains and the third one represents the rice subspecies which the *Xoo* strain isolates. The outmost 6 squares represent the virulence analysis of these *Xoo* against 6 different rice near-isogenic lines with one known resistance gene in each line, with solid square for resistance and hollow one for sensitivity. Bootstrap support values were calculated from 500 replicates, and only values of > 70% were labelled.

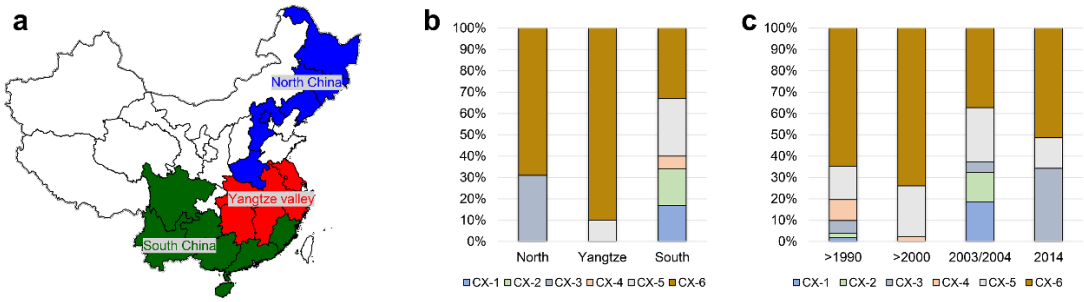


Figure 2 Distribution of the 6 major Chinese *X. oryzae* pv. *oryzae* lineages. Pathotypes of *Xoo* were determined on the following 13 near-isogenic lines, each carrying a specific R gene, IRBB2 (*Xa2*), IRBB3 (*Xa3*), IRBB4 (*Xa4*), IRBB5 (*xa5*), IRBB14 (*Xa14*). IR24 was used as a susceptible check. (a) The map of the 3 major rice producing areas in China. (b) Distribution of the 6 *Xoo* lineages among the 3 rice planting areas. (c) Dynamics of the 6 *Xoo* lineages during the past 30 years.

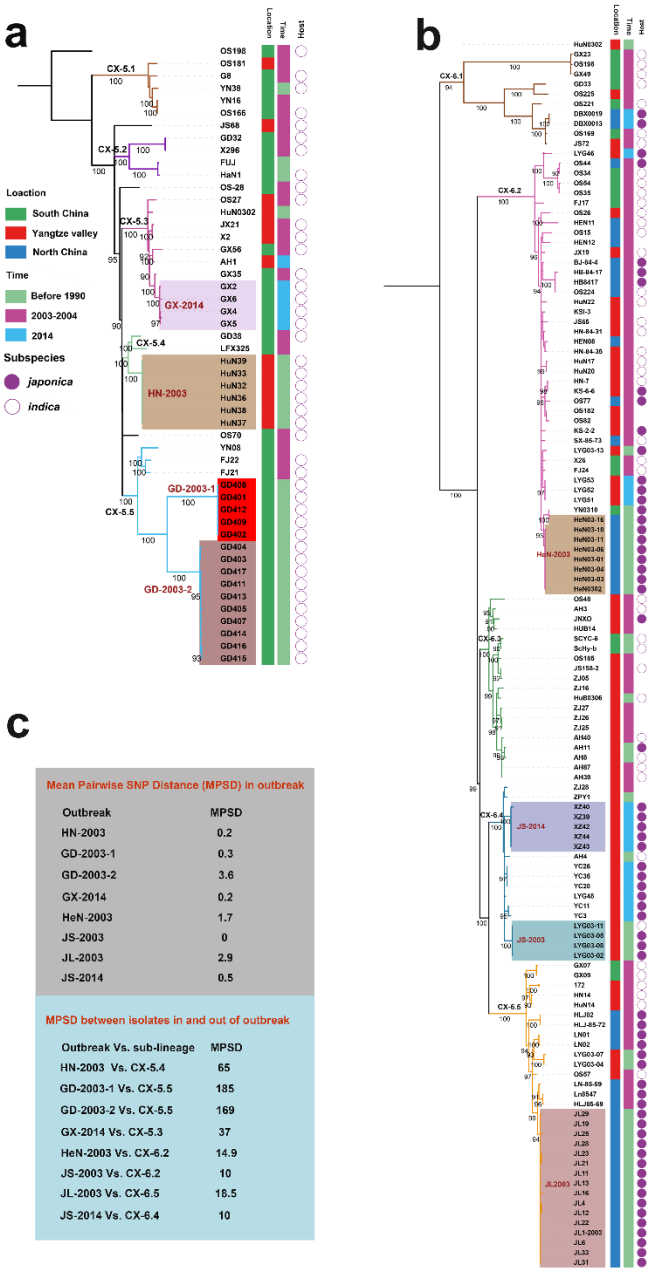


Figure 3 Phylogeography of CX-5 and CX-6. Maximum likelihood phylogeny of Lineage CX-5 (a) and CX-6 (b). Different sub-lineages were displayed by different branch colors. Bootstrap support values were calculated from 500 replicates. The recent outbreaks are represented by different backgrounds. Isolated information of *Xoo* including location, time and rice subspecies were shown in three colored strips. (c) Mean Pairwise SNP Distances (MPSDs) of *Xoo* from the outbreak, and between one in an outbreak and the other one out of the outbreak but in the same sub-lineage were displayed by two different boxes beside the tree.

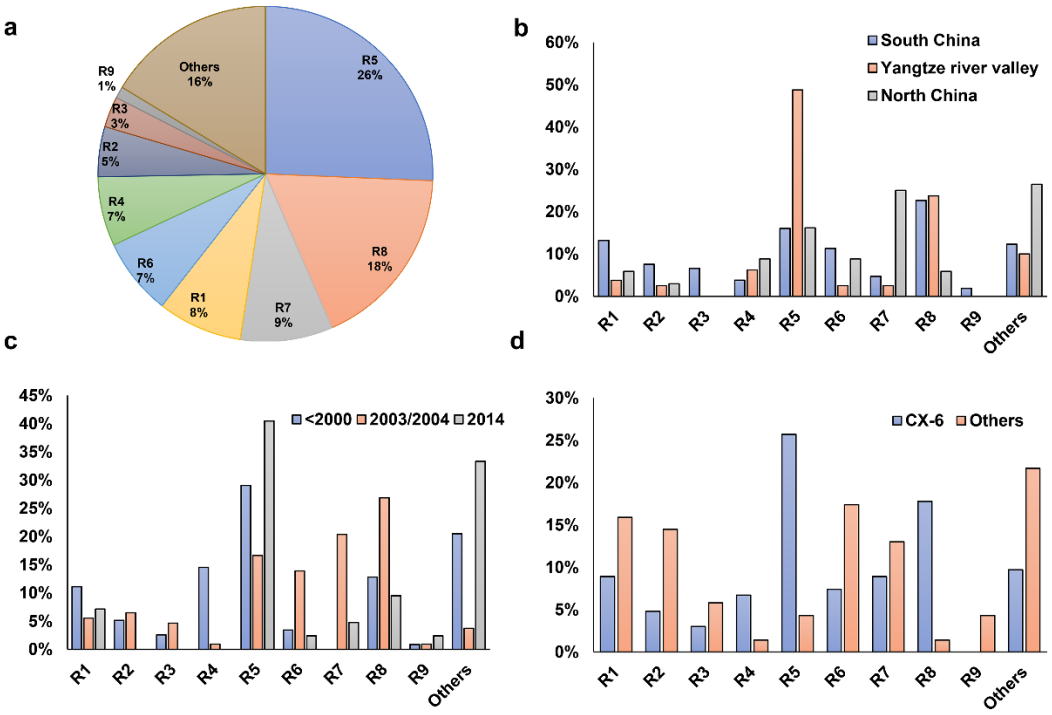


Figure 4 Virulence analysis and race classification of *X. oryzae* pv. *oryzae* China. Pathogenicity profile of each race corresponding to was shown in Table S1. (a) Proportions of the 9 *Xoo* races. (b) Distribution of the 9 races among the 3 major rice planting areas in China. (c) Dynamics of the 9 *Xoo* races during the past 40 years. (d) Distribution of the 9 races in Lineage CX-6 and others.

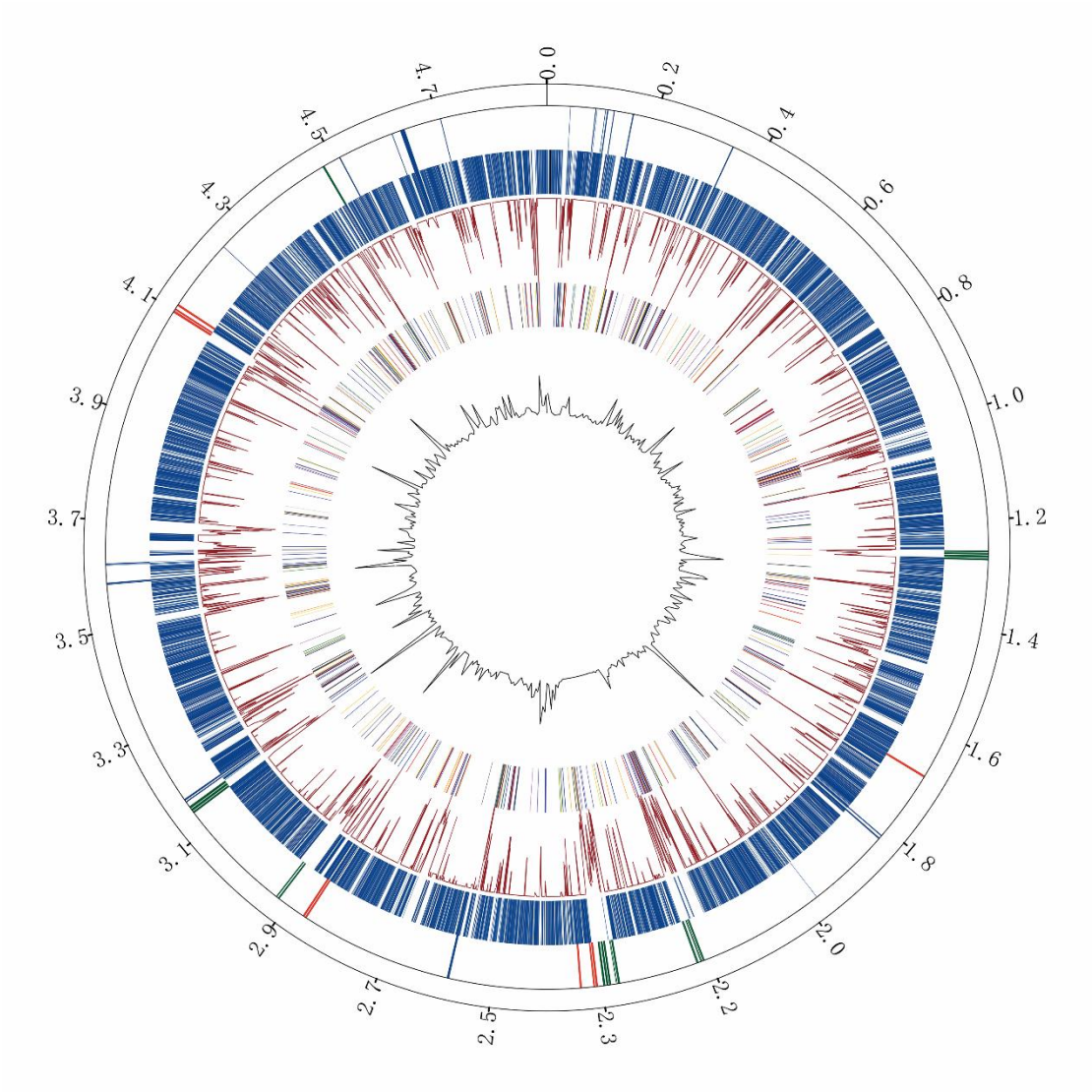


Figure 4 Core and pan genome of *Xoo*. The results showed here is from the comparative analysis of the 22 *Xoo* complete genomes, using OS198 belonging to Lineage CX-6 as reference. From the inner to the outmost, (1) Circle 1, the black break line, SNPs per 10kbp; (2) Circle 2, the colorized lines, different families of transposase genes (magenta for IS3, blue for IS5, yellow for IS30, green for IS630, red for IS701, orange for IS1595, black for others); (3) Circle 3, the red break line, numbers of genome having that gene family (ranging 1 to 22); (4) Circle 4, the dark blue lines, positions of core gene; (5) Circle 5, the colorful lines, presences of type III effector genes, with green for TALEs in OS198, red for TALEs in other Xoo genomes, blue for other T3Es; (6) Circle 6, scales according to genome of OS198.

Table 1 Resistance of different rice lines to *Xoo* strains isolated at different times. Shown is the percentage of *Xoo* strains isolated at different periods that are unable to cause disease.

Time (# of strains)	**IRBB2 (*Xa2*)**	**IRBB3 (*Xa3*)**	**IRBB4 (*Xa4*)**	**IRBB5 (*xa5*)**	**IRBB14 (*Xa14*)**	**IR24 (*Xa18**)**
<1990 (35)	37	0	97	94	25	20
<2000 (69)	25	58	64	100	28	12
2003/2004 (97)	49	35	34	100	56	7
2014 (42)	36	5	79	90	33	5

*: Recurrent parent *Xa18*

718 **Table 2 Resistance of different rice lines to *Xoo* strains of different lineages.** Shown

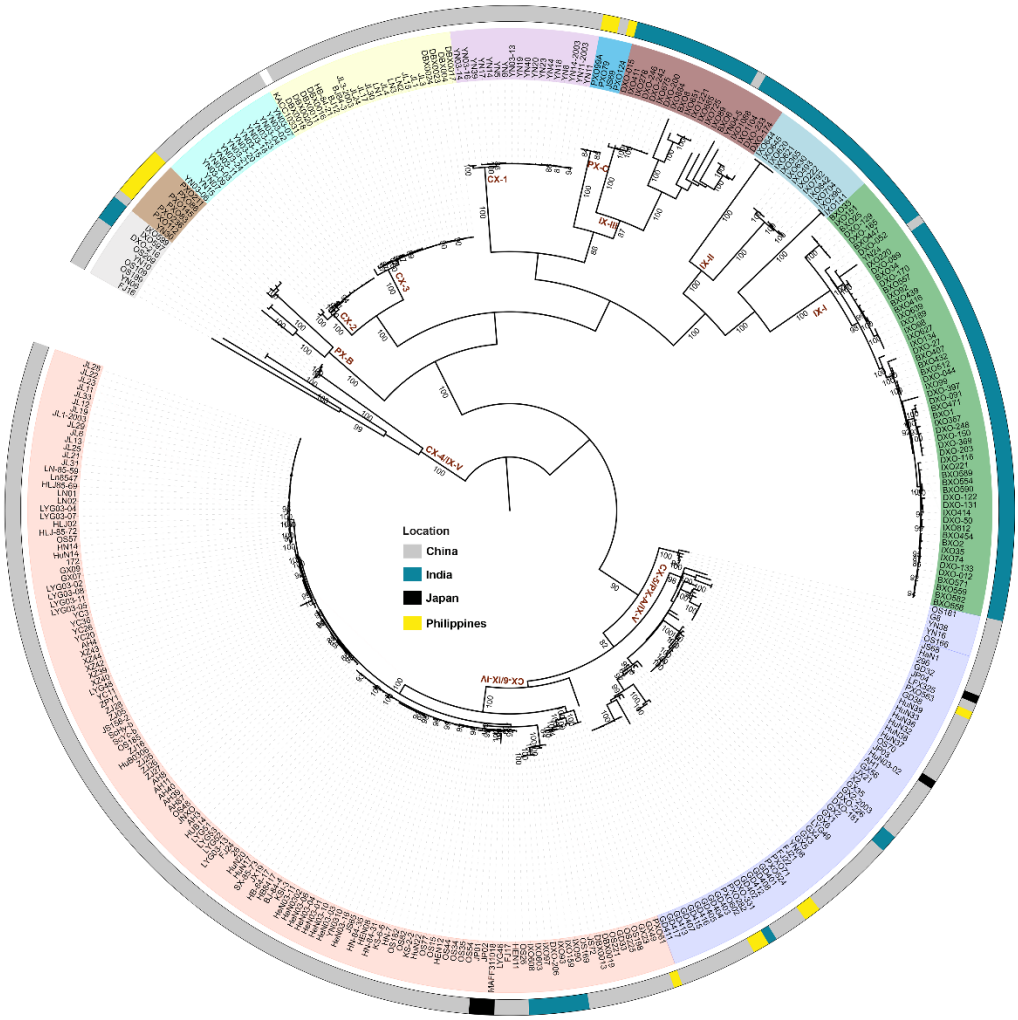
719 is the percentage of *Xoo* strains of different lineages that are unable to cause disease.

Lineage (# of strains)	**IRBB2 (*Xa2*)**	**IRBB3 (*Xa3*)**	**IRBB4 (*Xa4*)**	**IRBB5 (*xa5*)**	**IRBB14 (*Xa14*)**	**IR24 (*Xa18**)**
CX-1 (19)	89	79	42	100	95	21
CX-2 (15)	73	93	80	100	100	7
CX-3 (20)	100	20	70	100	99	10
CX-5 (53)	11	32	47	96	17	11
CX-6 (123)	29	25	62	98	28	6

720 *: Recurrent parent *Xa18.* CX-4 was not included because of few isolates it contained.

721

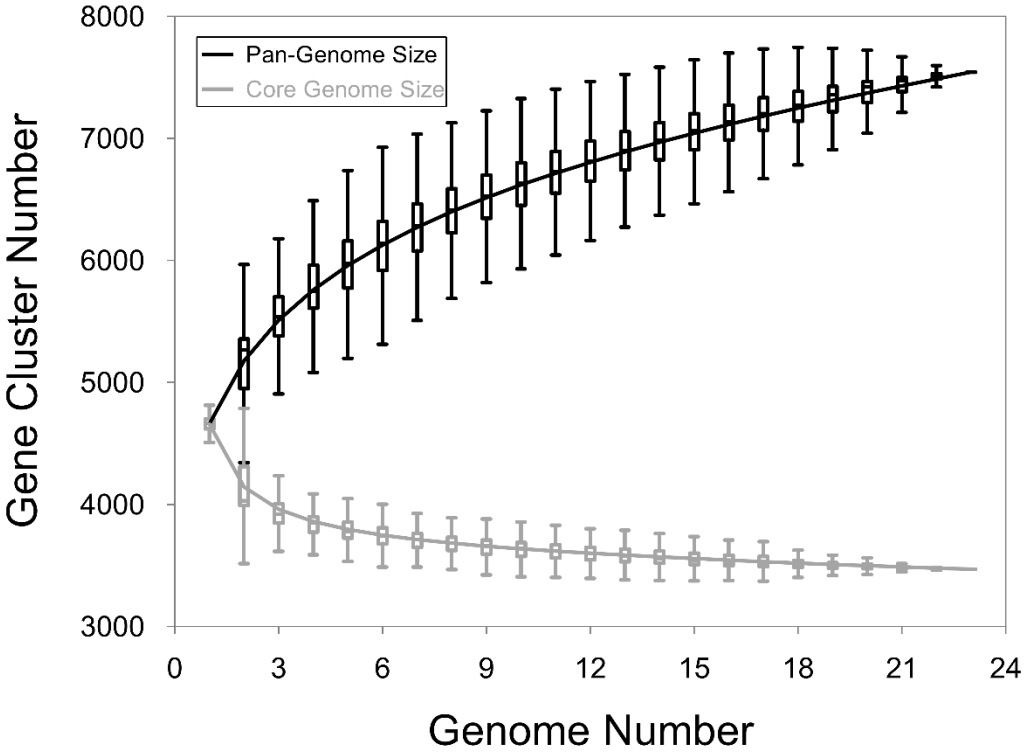
722 **Supplementary Information**



723

724 **Figure S1 Population structure of Asian *Xanthomonas oryzae* pv. *oryzae*.** For phylogenetic
725 analysis, parsnp was used to call SNPs, and RAxML was used to build the ML tree. Background
726 colors of nodes represent different lineages. The outmost circle refers to different countries where
727 Xoo were isolated.

728



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730 **Figure S2 Plot of Pan and core genes from the 22 complete *Xoo* genomes.**

731

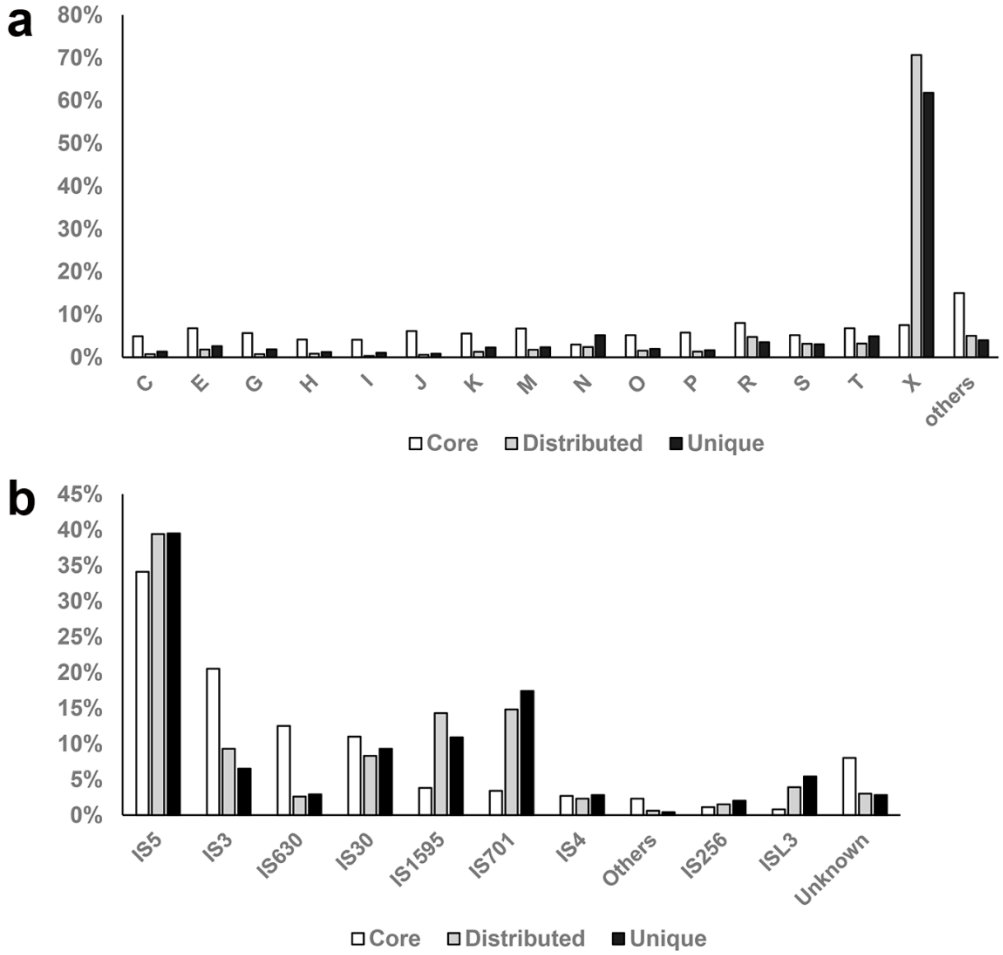

Figure S3 Function analysis of pan genes. (a) COG function of different types of gene families. Core, genes shared by all the 22 genomes; distributed, gene shared by 2 ~ 21 genomes; unique, genes unique to each genome. (b) Classification of transposase genes. COG categories are energy production and conversion (C), amino acid metabolism and transport (E), carbohydrate metabolism and transport (G), coenzyme metabolism (H), lipid metabolism (I), translation (J), transcription (K), cell wall/membrane/envelop biogenesis (M), cell motility (N), posttranslational modification, protein turnover, and chaperone functions (O), inorganic ion transport and metabolism (P), general functional prediction only (R), function unknown (S), signal transduction (T), Mobilome: prophages, transposons (X).

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744 **Table S1 *Xoo* isolates used in this study.**

745

Table S2 Resistance of different rice lines to *Xoo* strains of different sub-lineages of CX-5 and CX-6. Shown is the percentage of *Xoo* strains of different lineages that are unable to cause disease.

Lineage (# of strains)	**IRBB2 (*Xa2*)**	**IRBB3 (*Xa3*)**	**IRBB4 (*Xa4*)**	**IRBB5 (*xa5*)**	**IRBB14 (*Xa14*)**	**IR24 (*Xa18**)**
CX-5.1 (5)	0	20	20	100	0	0
CX-5.3 (12)	25	25	67	92	17	17
CX-5.4 (8)	25	50	75	100	28	25
CX-5.5 (18)	0	28	28	100	28	0
CX-6.1(10)	30	30	90	100	50	20
CX-6.2 (47)	13	38	79	98	23	4
CX-6.3 (18)	10	17	68	100	11	0
CX-6.4 (18)	0	0	61	94	0	0
CX-6.5(31)	90	32	29	97	74	10

*: Recurrent parent *Xa18.* CX-5.2 was not included because of few isolates it contained.

752
753 **Table S3 Transposase content of *Xoo* with complete genomes.**
754
755 **Table S4 Distribution of TAL among different *Xoo* with complete genomes**
756
757

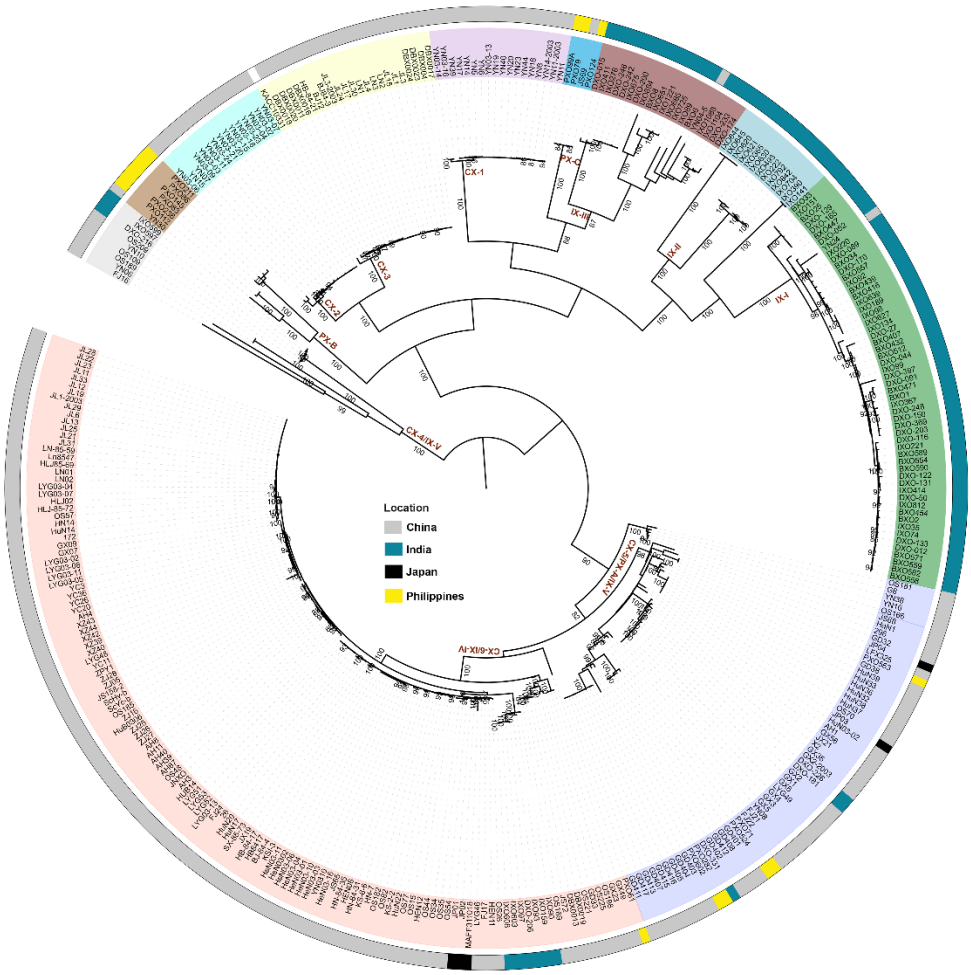

Figure S1 Population structure of Asian *Xanthomonads oryzae* pv. *oryzae*. For phylogenetic analysis, parsnp was used to call SNPs, and RAxML was used to build the ML tree. Background colors of nodes represent different lineages. The outmost circle refers to different countries where *Xoo* were isolated.

Table S2 Resistance of different rice lines to *Xoo* strains of different sub-lineages of CX-5 and CX-6. Shown is the percentage of *Xoo* strains of different lineages that are unable to cause disease.

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