# Exploring the Landrace Rice (*Oryza sativa* L) for Salinity Tolerant and Agronomic Traits

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**ABSTRACT**

Rice genotype tolerant to salinity at seedling stage with high yield potential is very desirable to overcome the saline soil problem in a coastal area in the early monsoon. A total of one hundred and fifty four rice accessions were evaluated for salinity tolerance at the seedling stage at 12 dSm$^{-1}$ in screen house using Yoshida salinized solution for sixteen days. A total of 47 accessions having survived seedling were transferred to media non-saline and grown until harvesting to have some seed for evaluation of agronomic traits and resistant to $X_{oo}$ pathotype IV in the following season. There were eight accessions showed tolerant to salinity at 12 dSm$^{-1}$ which were Talun Bagang (1783), Talun Bintik (1786), Padi Sawah Ngale (1790), Ketan (4578), Nongkong Jambu (4588), Pulut Kemuken (4598), and Gadis Kuning (4591). There were two accessions, i.e. Pulut Kuning (3321) and Serepet Tinggi (3928) showed resistant to Xoo pathotype IV under field screen condition. Three accessions, Nongkong Jambu (4588), Pulut Kemuken (4598), Gadis Kuning (4591), had a good agronomic trait which were very potential to be used as a parent to improve high yielding tolerant to salinity.

## Introduction

Soil salinity is one of the major abiotic stresses to crop production and is expected to increase due to global climate changes and as a consequence of many irrigation practices. An average of 2 000 hm$^2$ irrigated land across 75 countries has been degraded by salt every year (Reddy *et al*., 2017). In Indonesia, degradation of land due to salt water intrusion affected the paddy field in the coast of Sumatera, Java, and West Nusatenggara (Rejekningrum *et al*., 2011). About 43% rice irrigated area is in the coastal area of Java, the effect of salinity on rice production in Indonesia can be as high as 50% of the rice fields along the north coastal area of Java (Cahyoargo *et al*., 2015).

Rice is considered sensitive to salinity, particularly during early vegetative and later at reproductive stages. Nonetheless, it is one of the few crops that can thrive on salt-affected soils because of its ability to grow well in standing water that can help leach salts from topsoil and is, therefore, recommended as an entry crop for desalinization of salt-affected lands (Ismail *et al*., 2007; Singh and Flowers, 2016).

Rice productivity at salt affected area is very low, <1.5 t/ha but can reasonably be raised by at least 2 t/ha. It has a salinity threshold of 3 dS/m, with a 12% reduction in yield per dS/m, beyond this threshold. Therefore, rice yields can be reduced by up to 50% when grown under moderate (6 dS/m) salinity levels (Shannon *et al*., 1998; Sankar *et al*., 2011). The crop yield reduction in salt soils can be overcome by soil reclamation or by improving salt tolerance in target crops.

Improving the salt tolerance of crop requires access to new genetic diversity (Munns and James, 2003). Local rice varieties or landrace rice is very valuable germplasm carried a potential gene for several abiotic stresses including salinity tolerance gene. Platten *et al*. (2013) found the wide variability of salinity tolerance existed in rice. Breeders have long made use of the high level of salinity tolerance in landraces like Nona Bokra and Pokkali (Thomson *et al*., 2010). To increase the genetic diversity among high yielding rice tolerant to salinity, other gene resources need to be explored.

The Indonesian Center for Rice Research had more than 5000 rice accessions, however, only less than 500 accessions had been evaluated for salinity tolerance (Yunani, 2018). There is probably a wide range of salinity tolerance in ICRR germplasm collection but it has not been underutilized.

Agronomic traits determined the grain yield ((Li *et al*., 1997; Zeng *et al*., 2017). The agronomic traits of the parents determined the phenotypic of the progenies and finally affected the farmer acceptance of the improved varieties. Bacterial leaf blight (BLB) is the main disease affected rice yield. In Indonesia, the yield losses in the wet season can reach up to 28-36%, (Sudir and Yuliani, 2016; Yuliani *et al*., 2017, Suparyono *et al*., 2004) and in Japan the yield losses up to 50% (Qi and Mew, 1989).

The choice of parents for the potential of crosses was a key success to broaden a variability in particular traits (Janwan *et al.,* 2013). For further utilization in breeding program, the thorough knowledge of the existing genetic variation for a potential target traits was required (Wani and Khan, 2006).

The research was aimed to identify rice land races tolerant to salinity at seedling stage and to evaluate the agronomic traits for further utilization in breeding program.

## Materials and Methods

The research consisted of two activities which were (1). Screening for salinity tolerance at seedling stage and (2). Evaluation of salinity tolerance accessions for agronomic traits and resistance to bacterial leaf blight ($X_{oo}$) pathotype IV.

### Screening for salinity tolerance at the seedling stage

The activity was done in screen house of the Indonesian Center for Rice Research (ICRR) from September to October 2017. A total of 154 accessions was evaluated for salinity tolerance at the seedling stage at 12 dSm$^{-1}$ for 16 days (Gregorio *et al*., 1997). The research was arranged in strip check design with three replications using Pokhali as resistant check and IR 29 as susceptible check. The survival seedling were transferred to the field for seed propagation for further evaluation.

### Evaluation of agronomic traits and resistance to $X_{oo}$ pathotype IV

The research was done at the ICRR research station on Mei to August 2018. The research was arranged in augmented design using four blocks and four check variety (Inpari 34, Ciherang, Sidenuk, Inpara 6 and Mekongga. Each genotype was planted in 3.75 m$^2$ size plot with 25 cm x 25 cm planting space. The data for yield and yield component (plant height, productive tiller number, day to 100% of plant flowering, filled grain number, unfilled grain number, total grain number, fertility, and panicle weight were collected followed Standard Evaluation System for Rice (SES) (IRRI, 2014).

Evaluation for BLB pathotype IV at the generative stage was done using clipping methods at 55 days after transplanting. This pathotype was the dominant and most virulence in Indonesia (Sudir *et al*., 2009). To prepare the inoculum, the isolate of $X_{oo}$ was stored with glycerol at a constant of -20 °C. $X_{oo}$ isolate was regrown on nutrient agar at 27-30 °C for 48 hours. Each bacterial colony was suspended in sterilized, distilled water and adjusted to concentrations of 109 cfu mL$^{-1}$ (OD=0.3 at 600 nm) by spectrophotometer. Six plants were selected from each rice accession tested then inoculated with the $X_{oo}$ pathotype containing a bacterial suspension of $10^9$ cfu/m. The inoculation was done according to the method of Kauffman (Kauffman *et al*., 1973). The BLB infection was evaluated at two weeks after the inoculation and scored followed Standard System Evaluation for Rice (SES) (IRRI, 2014).

The variance analysis was done using PB tools, and multivariate analysis to reveal the similarity of accessions was done using Star Nebula.

## Results and Discussion

### Salinity tolerance of rice germplasm at the seedling stage

Among 154 accessions, there were eight accessions

showed tolerance to salinity at the seedling stage (score 3), and 39 accessions showed moderate tolerance (score 5) and others were susceptible (score 7-9). The eight tolerance accessions were Talun Bagang (1783), Talun Bintik (1786), Padi Sawah Ngale (1790), Ketan (4578), Nongkong Jambu (4588), Pulut Kemuken (4598), and Gadis Kuning (4591). The survival seedling of all 47 accessions for



A                                 B
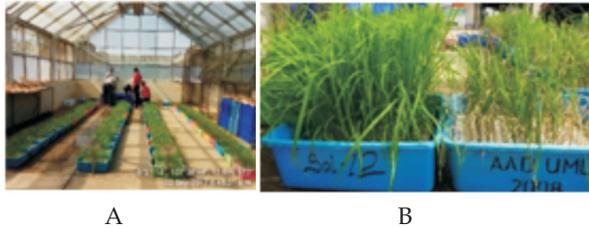
**Fig. 1.** **A:** Ten days after application of salinized solution ICRR screen house, **B**: Performance of seedling under normal condition (left side) vs saline condition (right side) at 7-10 days after salinization.

both tolerance and moderate tolerance accessions were transferred to the field for seed propagation at ICRR research station for further agronomic traits evaluation. The list of survival seedling accession presented in Table 1.

**Resistance to BLB and agronomic traits performance**

The result of screening to bacterial leaf blight resistance showed that the check variety Mekongga, Ciherang and Inpari 34 were highly susceptible (score 7) while both Inpara 6 and Sidenuk were susceptible (score 5) to $X_{oo}$ pathotype IV. There were two accessions showed moderate resistance (score 3) which were entry #15 Pulut Kuning (3321) and #19 Serepet Tinggi (3928). A total of 31 accessions showed susceptible (score 5) and the others were highly susceptible (score 7) (Table 1).

$X_{oo}$ pathotype IV is the most virulence pathotype in Indonesia (Sudir, 2009). Until now, only several

**Table 1.** The Score of salinity tolerance and BLB resistance in tested accessions

| Entry # | Designation | Salinity tolerance | BLB resistance | Entry # | Designation | Salinity tolerance | BLB resistance |
|---|---|---|---|---|---|---|---|
| 1 | Ketan Bujuk(1561) | 5 | 5 | 29 | Pulut Lompet(4592) | 5 | 5 |
| 2 | Ketan Abang(1562) | 5 | 7 | 30 | Pulut Lewok(4595) | 5 | 5 |
| 3 | Sawah Kanyut II(1569) | 5 | 7 | **31** | **Pulut Kemuken(4598)** | **3** | **7** |
| 4 | Boneng(1577) | 5 | 5 | 32 | Jalu Briwit(4599) | 5 | 5 |
| 5 | Talun Bagang(1783) | **3** | 5 | 33 | Muntai(4604) | 5 | 7 |
| 6 | Talun Bintik(1786) | **3** | 5 | 34 | Pulut Kutai(4616) | 5 | 7 |
| 7 | Padi Sawah Ngale(1790) | **3** | 7 | 35 | Padi Lima Bulan(4618) | 5 | 5 |
| 8 | Padi Pandan(1852) | 5 | 7 | 36 | Loneng(4762) | 5 | 7 |
| 9 | Padi Cina(1864) | 5 | 5 | 37 | Pulut Saru(4772) | 5 | 5 |
| 10 | Tampai(2188) | 5 | 5 | 38 | Hitam(4780) | 5 | 7 |
| 11 | Palao Bantanan(2738) | 5 | 5 | 39 | Kujam Cina(4945) | 5 | 5 |
| 12 | Jambai(2835) | 5 | 5 | 40 | Bonti(4948) | 5 | 7 |
| 13 | Padi Jambai(2842) | 5 | 5 | 41 | Si Rendah(6158) | 5 | 5 |
| 14 | Krawang(3125) | 5 | 7 | 42 | Payak(7865) | 5 | 5 |
| 15 | Pulut Kuning(3321) | **5** | **3** | 43 | Tranggulang(7909) | 5 | 5 |
| 16 | Padi Hungkai(3339) | 5 | 5 | 44 | Si Rendah Hitam Ekor(7979) | 5 | 5 |
| 17 | Padi Sibur(3741) | 5 | 5 | 45 | Widas | 5 | 5 |
| 18 | Si Bujang Banu(3743) | 5 | 5 | 46 | Cisadane | 5 | 5 |
| 19 | Serepet Tinggi(3928) | **5** | **3** | 47 | Krueng Aceh | 5 | 7 |
| 20 | Serepet Rendah(3929) | 5 | 5 | | Check variety | | |
| 21 | Raden Kuning(3934) | 5 | 5 | 48 | Ciherang | - | 6 |
| 22 | Padi Putih(3942) | 5 | 5 | 49 | Mekongga | - | 6 |
| 23 | Pontianak(4202) | **3** | 5 | 50 | Sidenuk | - | 5 |
| 24 | Ketan(4578) | **3** | 7 | 51 | Inpari 34 | - | 6 |
| 25 | Keriting(4581) | 5 | 5 | 52 | Inpara 6 | - | 5 |
| 26 | Nongkong Jambu(4588) | **3** | 5 | | Min | 3 | 3 |
| 27 | Merayang Kuning(4590) | 5 | 5 | | Max | 5 | 7 |
| 28 | Gadis Kuning(4591) | **3** | 5 | | Mean | 4 | 5 |

Remarks: Class for
-salinity tolerance: score 3 (tolerance), score 5 (moderate tolerance)
-BLB resistance: 3 (moderately resistance), 5 (susceptible), 7 (highly susceptible)

high yielding rice varieties resistant to $X_{oo}$ pathotype IV such as Inpari 24, Angke, Conde, Inpari 17, Kalimas, Inpara 8, and Pandan wangi (Nafisah *et al*., 2019). Tasliah *et al*. (2013) reported the effective gene to control BLB resistance in Indonesia was gene xa5, Xa7 and Xa21. From the parentage background, the existing resistance varieties may confer these effective genes. The resistant entries found in this study might carry the different gene to be utilized in a breeding program that needs to be further confirmed.

**Yield component**

The descriptive statistics for the 47 accessions was presented in Table 2.

The relatedness of yield and yield component could be revealed using correlation analysis. Indirect selection could be applied for the strong positive correlation among of yield and yield component. Correlation analysis can reveal the relatedness of yield and yield component which could be applied in indirect selection for the yield trait. The correlation analysis found a strong positive correlation (r=>0.5 in plant height (PH) with day to flowering (DTF), productive tiller number (PTN) with both fertility (FER) and panicle weight (PW), the day to flowering (DTF) with unfilled grain number (UFG), total grain number (TG), and panicle weight (PW), Filled grain number (FGN) with both total grain number (TG) and fertility, unfilled grain number (UFG) with total grain number (TG), fertility (FER) with panicle weight, thousand- grain weight (TGW) with panicle weight (PW). While a strong negative correlation was found in plant height with productive tiller number (PT). Productive tiller number PT) with both day to flowering (DTF) and unfilled grain number (UFG). DTF with fertility (FER), thousand-grain weight (TGW) and panicle weight (PW). Un-filled grain number (UFG) with both fertility (FER) and panicle weight (PW).

Based on this study, the panicle weight or grain yield per hill had a strong correlation with productive tiller number, fertility and a thousand- grain weight and strong negative correlation with day to flowering and unfilled grain number. This result collaborates with the finding of (Akter *et al*., 2018) for a positive correlation in grain yield with productive tiller number and thousand-grain weight in Jhum rice genotypes, grain yield with filled spikelet per panicle in hybrid rice growth in moderate-yielding region grain yield with filled grain number per panicle, 1000-grain-weight and fertility in rice varieties released in China (Li *et al*., 2019) grain yield with productive tiller number, spikelet fertility and spikelet per panicle and in Myanmar rice germplasm (Htwe *et al*., 2019) grain yield with productive tiller number, grain total number, filled grain number and thousand-grain weight in local upland rice (Kadida *et al*., 2017).

The high negative correlation between panicle weight with the day to flowering (DTF) probably because the entries with longer maturity was exposed with higherair temperature and less water irrigation. The water irrigation was very limited after some earlier entries harvested. The micro climate might affect the grain filling and reduced the panicle weight. Therefore some entries with longer duration had more unfilled gran number, less thousand grain weight and lower grain yield.

To reveal the phenotypic similarity among tested accessions in agronomic traits, the principle analysis was used. The first principle analysis explained 49% of the variance, and two principle analysis explained 71% of the variance (Table 4). Therefore, two principle analysis can be used to determine the phenotypic variance among accessions.

**Table 2.** Descriptive statistics for the tested accessions

| Variable | Min | Max | Mean | Stdev |
|---|---|---|---|---|
| Plant height (PH) | 93 | 174 | 127.63 | 25.27 |
| Productive tiller number (PT) | 8 | 20 | 14.29 | 3.27 |
| days to flowering (DTF) | 76 | 110 | 91.16 | 11.53 |
| Filled grain number (FG) | 43.1 | 139.3 | 86.3 | 21.04 |
| Unfilled grain number (UFG) | 16 | 105 | 46.04 | 20.03 |
| Total grain number (TG) | 87.4 | 199.5 | 132.14 | 27.54 |
| Fertility (FERT-%) | 0.39 | 0.85 | 0.66 | 0.12 |
| Thousand grain weight (TGW) | 15.39 | 31.27 | 24.17 | 3.31 |
| Panicle weight (PW) | 27.14 | 145.05 | 85.06 | 28.13 |
| Score of Bacterial leaf blight (BLB) | 2.93 | 6.93 | 5.38 | 1 |

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**Table 3.** Correlation matrix for agronomic and BLB resistance

| Var | PH | PT | DTF | FG | UFG | TG | FERT | TGW | PW | BLB |
|-----|----|----|-----|----|-----|----|------|-----|----|-----|
| PT | | 1 | **-0.80** | 0.10 | **-0.59** | -0.36 | **0.54** | 0.47 | **0.66** | 0.40 |
| DTF | | | 1 | 0.05 | **0.63** | **0.50** | **-0.54** | **-0.60** | **-0.72** | -0.40 |
| FG | | | | 1 | -0.11 | **0.68** | **0.55** | -0.13 | 0.38 | -0.08 |
| UFG | | | | | 1 | **0.65** | **-0.86** | -0.35 | **-0.69** | -0.26 |
| TG | | | | | | 1 | -0.22 | -0.35 | -0.22 | -0.25 |
| FERT | | | | | | | 1 | 0.28 | **0.79** | 0.16 |
| TGW | | | | | | | | 1 | **0.51** | 0.13 |
| PW | | | | | | | | | 1 | 0.27 |

Remarks: PH=plant height, PT=productive tiller number, DTF=day to flowering, FG=filled grain number per panicle, UFG=unfilled grain number per panicle, TG=total grain number, FER=panicle fertility, TGW=thousand-grain weight, PW=panicle weight.

**Table 4.** Principle of component analysis

| Statistics | PC1 | PC2 | PC3 |
|------------|-----|-----|-----|
| Standard deviation | 2.21 | 1.47 | 0.96 |
| Proportion of Variance | 0.49 | 0.22 | 0.09 |
| Cumulative Proportion | 0.49 | 0.71 | 0.80 |
| EigenValues | 4.90 | 2.17 | 0.93 |

The Eigen values of PC1 correlated with increase in plant height (PH), day to flowering (DTF), unfilled grain number (UF), total grain number (TG) and resistant to bacterial leaf blight (BLB) (decreased in score of BLB), and decreased in productive tiller number (PT), fertility (FERT), thousand grain weight (TGW)and panicle weight (PW). While the eigen values in PC2 correlated with increased in plant height, filled grain number, total grain number (TG), and panicle weight (PW) (Table 5).

The vector magnitude of biplot PC1 and PC2 showed the degree of variability. The variability of filled grain number (FG) is the highest, followed by total grain number (TG), panicle weight (PW), fertility (FERT), and day to flowering (DTF), and the lowest was resistant to bacterial leaf blight. The dispersion of the accessions in the biplot showed the morphological divergence among the accessions. The accessions which were very closed to each other indicated highly morphological similarity. Entry # 47, 34, 2, 38, 39, 30, 22, 44, 46, 22, 44 closely related. They had same character in term of resistant to $X_{oo}$ pathotype IV and thousand-grain weight. Entry #49 and 50 had similarity in the productive tiller number and panicle weight.

The biplot PC1 and PC2 (Graph 1) showed all check varieties used in this research was adjacent to each other located in Quadrant III excepted entry # 49 and #50 which were at Quadrant IV. The entry# 49

and 50 had more productive tiller number and higher panicle weight, while the other check had better thousand grain number. The entry #21 (Raden Kuning) had the highest grain-filled number, followed by entry #29 (Pulut Lompet), entry # 42 (Payak (7865), and #26 Nongkong Jambu (4588).

**Table 5.** Eigen value for agronomic traits

| Statistics | PC1 | PC2 | PC3 |
|------------|-----|-----|-----|
| Variables | PC1 | PC2 | PC3 |
| PH (cm) | **0.32** | **0.32** | 0.00 |
| PT | **-0.37** | 0.05 | **0.25** |
| DTF (das) | **0.41** | 0.05 | -0.15 |
| FG | 0.00 | **0.67** | 0.09 |
| UFG | **0.39** | -0.09 | **0.33** |
| TG | 0.28 | **0.44** | **0.31** |
| FERT (%) | -0.33 | **0.39** | **-0.25** |
| TGW (g) | **-0.28** | -0.09 | -0.04 |
| PW (g) | **-0.37** | **0.27** | 0.00 |
| BLB | **-0.20** | -0.09 | **0.80** |

Remark: das: days after sowing
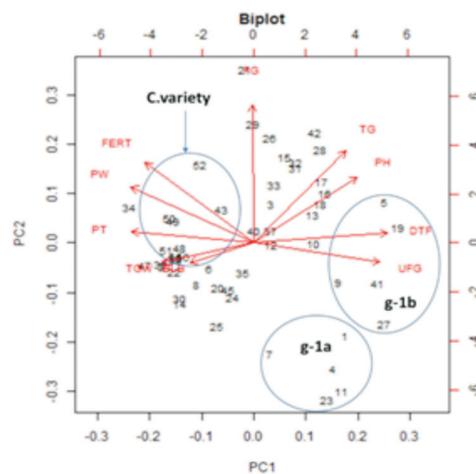


**Fig. 2.** Biplot for PCA1 and PCA 2

Based on the agronomic traits, the accession tolerant to salinity (entry # 5, 6, 7, 23, 24, 26, 28 and 31) were very dispersed. The entry #23 and #7 was adjacent, however, the genetic distance was very far from other tolerant entries. Entry # 26, 28, 31 were very closed to each other. These entries had high filled grain number and total grain number. Entry #5 had a longer maturity and higher plant height, while entry #6 more close to check varieties. While entry #24 closed to #45 (Widas) and #20 (Serepet rendah). Both entry #19 and #15 which were resistant to BLB had a longer maturity and higher plant.

The dendrogram showed the tested accessions could be divided into two groups (Graph 2). Entry number 1, 4, 5, 7, 9, 11, 19, 23, 27, and 41 belonged to group 1, while others (2, 3, 6, 8, 10, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 24, 25, 26, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 belonged to group 2. Cophenetic correlation coefficient of the dendrogram was 0.75 showed the fitness ofthe dendrogram for clustering the accessions.

Biplot PC1 and PC2 also showed that entries belonged to group II had better agronomic trait compared to group I. Entries belonged to group I had more unfilled grain number (UFG), less panicle weight, longer day to flowering (DTF), less panicle weight (PW) and less productive tiller number.

Based on the biplot 1 and 2 and dendrogram, accession resistant to $X_{oo}$ pathotype IV (entry #15) were very potential to use as a donor parent to make cross combination with the recurrent parents of popular varieties compared to entry #19 since it had better agronomic trait (more filled grain number and total grain number), and entry #19 had disadvantage in longer duration.



A          B

**Fig. 2. A:** The phenotypic of panicle trait in #2 (Ketan Abang), #34 Pulut Kutai, #49 (Mekongga) and #38 (Hitam); **B:** Thephenotypic similarity of #49 Mekongga (C31) and #30 Pulut lewok (C121)

The accessions tolerant to salinity belong two different groups. Entry #5, 7, 19 and 23 belong to
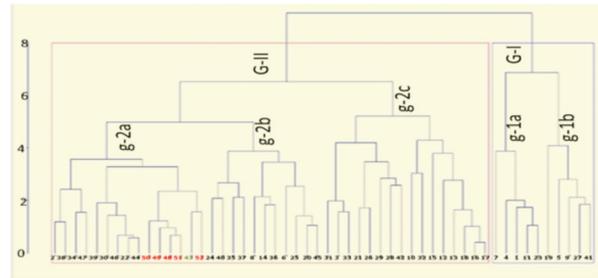


**Fig. 3.** Dendrogram the phenotypic similarity among accessions based on the agronomic traits

group I, while entry #6, 24, 26, 28 and 31 belonged to group II (Graph 2). Entry #31 had advantage over other salinity tolerantentriesbelong to group II. Entry # 31 had more total grain (TG) and filled grain number (FG), therefore,entry #31was a potential parent to increase salinity tolerance with a good agronomic trait.

## Conclusion

a.  There were eight accessions tolerant to salinity at seedling stage which were entry #5 Talun Bagang (1783), #6 Talun Bintik (1786), #7 Padi Sawah Ngale (1790), #23 Pontianak (4202), #24 Ketan (4578), #26 Nongkong Jambu (4588), #28 Pulut Kemuken (4598), #31 (Gadis Kuning (4591) at 12 dSm-1.

b.  Entry #31 a potential parent to used in salinity tolerance varietal improvement.

c.  The entry #26 Nongkong Jambu (4588), #28 Pulut Kemuken (4598), #31 Gadis Kuning (4591) superior in filled grain number and total grain number.

d.  Two entriesshowed resistant to $X_{oo}$ pathotype IV under field screening which was entry #15 Pulut Kuning (3321) and #19 Serepet Tinggi (3928) while entry #15 had better agronomic trait and potential as a donor parent.

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