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Salinity Effect at Seedling and Flowering Stages of Some Rice Lines and Varieties (*Oryza sativa* L.)

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Abstract: This study used 10 rice lines and varieties, including the salt tolerant variety (Pokkali) and salt susceptible variety (IR29) as control. Experiment for screening rice salinity tolerance at seedling stage was conducted in hydroponic system using five different salinity concentrations (0, 4, 6, 8 and 10 dS/m). Flowering stage was evaluated at the salinity level of 8 dS/m. The experiment was designed in randomized completely block (RCBD) with three replications for each line/variety under non-saline and saline condition. The results showed that the growth of rice lines/varieties was retarded severely with increasing salinity levels, and almost all of leaves were drying and dead completely (score 7 and 9). Plants of IR29 and OM7347 were dead at the electrical conductivity (EC) = 10 dS/m, whereas Pokkali (the tolerant variety) and IR93350 were evaluated at score 5. Salinity stress caused the reduction of overall vigor of rice lines/varieties especially in the pollen germination, fertilization and grain yield. Rice lines/varieties were classified to salinity tolerance levels as following: IR5040, IR93350, IR86385-8D-1-2-B and Pokkali (tolerant); OM8104, IR93340 and IR93343 (moderate susceptible); OM7347, IR87832-303-1-B and IR29 (susceptible).

Key words: Tolerant salinity, pollen, seedling stage, flowering stage, screening for salinity tolerance.

1. Introduction

Rice is one of the plants sensitive to soil salinity, and the limited salinity that allows the rice to normally grow and develop is less than 2 dS/m. Rice yield decreases more than 50%, if salinity is too severe (> 10 dS/m) [1]. A lot of research results about the effect of salinity on rice yields show that productivity losses depend greatly on the characteristics of rice variety. Therefore, in research and selection of salinity tolerant varieties suitable with the ecological conditions of each region, each salinity is an urgent issue raised. However, the growth and development stages of rice are affected by different levels of salinity and they must be divided into several stages for full research of the salinity tolerance mechanism. Many studies have documented that rice is resistant to salinity at the germination stage and then becomes very sensitive during seedling stage (2-3 leaves) [2, 3]. Although rice tolerates salinity throughout the vegetative growth and ripening, but it is sensitive to salinity during pollinating and fertilizing period [4].

According to Singh et al. [3], seedling and flowering stages are two highly susceptible stages of salinity. The seedling stage is the first stage of growth during which salinity has a great influence on rice life, plan height, number of tillers/plant, dry mass of straw and roots, root length and growth time. At the flowering stage, the salinization affects primary branch and spikelet formation, pollinating and germination of the pollen, reduce the number of effective spikelets/panicle and increase unfilled grain ratio. Salinity also reduces panicle length, grains/panicle and 1,000 grain weight, resulting in reduced productivity [5, 6].

One of the highly-effective measures for rice production in saline soils is to select salinity tolerance rice varieties suitable for the salinity conditions of each ecological region. This is a strategic direction, inexpensive and very practical. It is also a socially and economically acceptable measure. This measure is

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intended to evaluate the adaptability and tolerance of crop to the maximum level of salinity. The first important step in the selection of saline tolerant rice variety is the screening in the greenhouse. It helps shorten the time and cost savings for the selection process before growing in the field, and simultaneously, takes as a scientific basis to select the primary materials for breeding.

2. Materials and Methods

There were total of 10 rice lines/varieties used in this study, including OM7347 and OM8104 collected from Cuu Long Rice Research Institute, IR93340, IR93343, IR5040, IR93350, IR87832-303-1-B and IR86385-8D-1-2-B from International Rice Research Institute, as well as two control varieties IR29 (standard salinity susceptible variety) from International Rice Research Institute and Pokkali (standard saline tolerance) from NIAS Genebank (Japan), as shown in Table 1.

2.1 Experiment 1: Screening Rice for Salinity Tolerance at Seedling Stage

The experiment was conducted with five treatments of different salinity concentrations (0, 4, 6, 8 and 10 dS/m NaCl). Each level of salinity was arranged for rice varieties in sequential order.

The screening rice for salinity tolerance of the seedling stage followed the IRRI protocol [7] in the Yoshida medium [8] with the addition of NaCl at different concentrations. Salinize the nutrient solution by adding NaCl while stirring up to the desired electrical conductivity (EC) (2.40, 3.51, 4.68 and 5.58 g NaCl/L nutrient solution give the EC of 4, 6, 8 and 10 dS/m, respectively). Fill up the trays with this solution high enough to touch the nylon net bottom of the styrofoam. The effective culture solution needed per tray was about 12 L. However, it was practical to prepare more than the exact amount in case of spillage. The experiment was done in the greenhouse.

Monitoring indicators included root length, fresh weight and dry weight of the plant and evaluation of salinity tolerance (Table 2). Monitoring and evaluation of these indicators were conducted at 21 d after saline treatment.

2.2 Experiment 2: Analysis of Pollen Germination and Individual Yield

The experiment was designed according to randomized completely block design (RCBD) with three replications in greenhouse where covered by plastic nylon surround, with average temperature about 35 °C inside.

Rice plant was grown in normal water from germination until to panicle initiation. After the first flag leaf appeared, the rice crops were transferred in the saline medium (EC = 8 dS/m). At 8:00 am to 10:00 am every day, the solution was examined to keep EC = 8 dS/m and pH = 5 ± 0.5. The EC of the solution will change significantly everyday by

Table 1 Name and origin of 10 rice lines/varieties used in the study.

No.	Name of lines/varieties	Origin
1	OM7347	Cuu Long Rice Research Institute
2	OM8104	Cuu Long Rice Research Institute
3	IR93340	International Rice Research Institute
4	IR93343	International Rice Research Institute
5	IR5040	International Rice Research Institute
6	IR93350	International Rice Research Institute
7	IR87832-303-1-B	International Rice Research Institute
8	IR86385-8D-1-2-B	International Rice Research Institute
9	IR29	International Rice Research Institute
10	Pokkali	National Institute of Agrobiological Sciences (NIAS) Genebank (Japan)

Table 2 Modified standard evaluation score (SES) of visual salt injury at seedling stage.

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

Table 3 Evaluation of tolerant salinity on some lines/varieties (score).

Line/variety	Score of tolerant salinity					Survival time (d)
	0 dS/m	4 dS/m	6 dS/m	8 dS/m	10 dS/m	
OM7347	1	3	5	7	9	15
OM8104	1	1	3	5	7	21
IR93340	1	3	3	5	7	21
IR93343	1	1	3	5	7	21
IR5040	1	3	3	5	7	21
IR93350	1	1	3	5	5	21
IR87832-303-1-B	1	1	3	5	7	21
IR86385-8D-1-2-B	1	3	3	5	5	21
IR29	1	3	7	9	9	10
Pokkali	1	1	3	3	5	21

fluctuate of temperature, while errors in EC reading from the meter could be easily detected because pre-measured amount of NaCl should register the expected EC level. Maintaining the pH of the culture solution is very critical. Any error in the pH may create unnecessary stress to the plants due to toxicity and/or deficiency of nutrients. After 10 d in salinized solution, the appearance of the panicle in the flag leaf cover was checked.

For measurements of pollen viability, 6-7 unopened spikelets in reproductive stage were collected randomly with three replications, and then stored in refrigerator in 70% ethanol until analyzed. While analyzing, anthers from the stored spikelets were removed and crushed thoroughly so as to release all the pollens. Pollens were stained with a drop of 1% potassium iodide (KI) solution. The samples were covered with the cover-slip and viewed under light microscope. Fertile and unfertile pollens were counted at three different areas per slide. Pollen viability (%) was calculated by dividing number of fertile pollen grains to total number of pollen grains [9].

Yield components were measured as number of filled grains/panicle, number of unfilled grain/panicle and individual yield (g/plant) collected under two conditions of water (control) and saline treatment.

2.3 Data Analysis

Comparison of differences in indicators among experimental varieties was done by one-way analysis of variance (one-way ANOVA) on Statistix 10.0.

3. Results and Discussion

3.1 Screening at Early Seedling Stage

The results of 10 rice lines/varieties after 21 d in salinized solution at different of salinity levels are presented in Table 3.

Almost varieties had good growth at 0 dS/m. At the salinity level of 4 dS/m, some of varieties remained development, but others in performance with leaves rolling (score 3), such as OM7347, IR93340, IR5040, IR86385-8D-1-2-B and IR29. When increasing in concentration of NaCl up to 6 dS/m, the performance of almost varieties were clearly injury of leaf, leaf rolling and leaf drying on the tip (score 3), especially IR29 stopped completely, almost leaves were dry and some of shoots were dead (score 7). At 8 dS/m, the sensitive variety IR29 was dead completely, while some of shoots of OM7347 dead and the seedling plant not grew (score 7). Others lines/varieties were at score 5, except the control variety Pokkali was score 3. The OM7347 and IR29 varieties were completely dead at EC = 10 dS/m, and the survival time was only 15 d and 10 d, respectively, under saline condition. Other varieties had some of shoots dead and growth stopped (score 7), excepting for IR93350 (score 5) and IR86385-8D-1-2-B with similar tolerance salinity with control variety Pokkali. However, the survival time in saline condition was 21 d (Table 3).

Root length was decreased with increasing salt stress (Fig. 1). In all salinity treatments, root length of Pokkali and IR29 was significant difference as compared to other lines/varieties. Root length of OM7347 was the lowest at EC = 8 dS/m and dead at EC = 10 dS/m, similar to sensitive salinity of IR29. Therefore, the gradual decrease in root length with the increase of salinity might be due to more inhibitory effect of NaCl to root growth than that of shoot growth [10].

About 80%-90% dry matter weight of plant is product of photosynthesis process, while others absorb minerals nutrition from the soil [11]. Dry matter weight of all the rice lines/varieties declined with increasing salt concentrations (Fig. 2). Different lines/varieties were pronounced in this character. OM7347 was dead at 10 dS/m salt concentration. The lowest dry weight was found in IR29 and it was dead at 8 dS/m. At all salinity levels varieties, Pokkali produced significantly high dry weight as compared to other lines/varieties. IR5040 had the lowest dry weight in comparison to others at all salinity levels.

As a results, root length and dry matter weight were decreased with increasing salinity levels, as well as in the performance including leaf rolling and leaf drying at seedling stage (Figs. 1 and 2, Table 3). Higher concentration of NaCl showed strong inhibition of parameters. This result is in suitable with previous ones [12, 13]. The reduction in seedling fresh and dry weight is due to decreasing water uptake by seedlings in salt presence [14]. Plant growth inhibition is a common response to salinity and plant growth is one of the most important agriculture indices of salt stress tolerance [15].

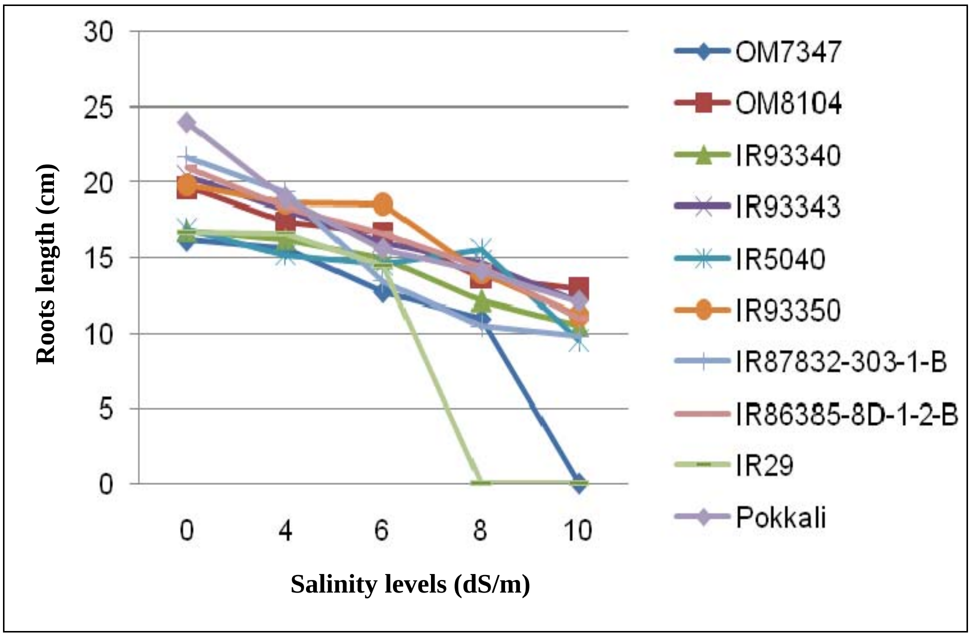


Fig. 1 Effect of salinity levels on roots length (cm) of different rice lines/varieties after 21 d in salinized solution.

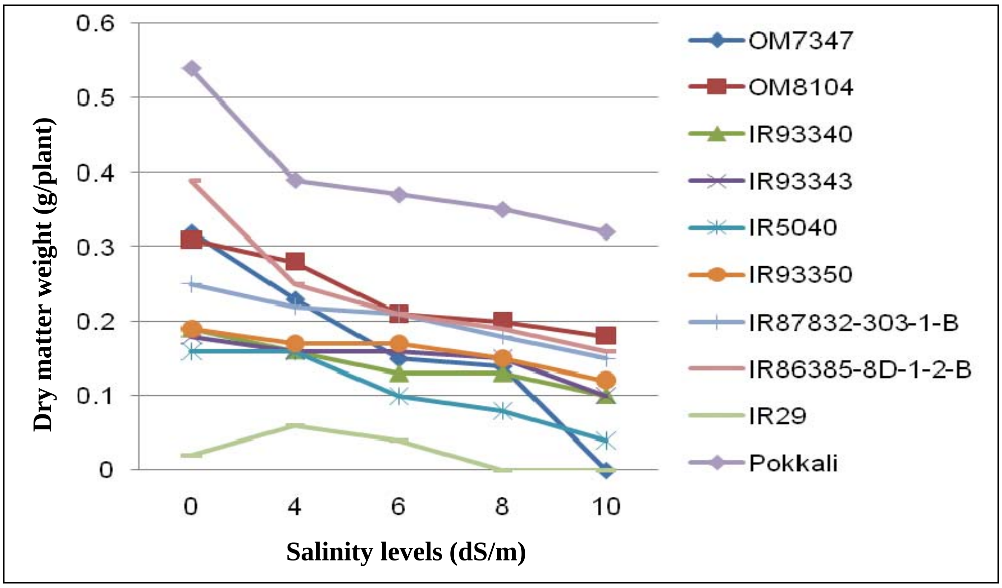


Fig. 2 Effect of salinity levels on dry matter weight (g/plant) of different rice lines/varieties after 21 d in salinized solution.

3.2 At Reproductive Stage

Reproductive stage is one of the most sensitive growth stages under the saline condition [16, 17]. This is the most important stage as far as grain yields is concerned, because the successful fertilization at this stage is ultimately translated to grain yield. The experiment 2 was conducted at booting/heading stage for 20 d from the day of "flag leaf appearance" to define fertilization rate under saline condition at 8 dS/m, which makes foundation of assessment of effect of saline on pollen germination, number of unfilled spikelets, number of filled spikelets and grain yield in both normal (control) and salinized conditions (saline).

Under saline condition (EC = 8 dS/m) within 10 d until the panicle appeared, the pollen germination was clearly decreased in comparison to non-stress conditions. All varieties showed inconsistency on salt tolerant over increasing salt concentration. Pokkali and IR5040 were of the highest pollen viability in both environment conditions (Fig. 3). Four of the 10 lines/varieties were evaluated as salt tolerant (T), namely IR5040, IR93350, IR86385-8D-1-2-B and Pokkali. Whereas, the pollen fertilization rate of IR29 (sensitive variety) under saline condition decreased two times (51.07%) as compared to non-saline condition (Fig. 3), and the similar was for IR87832-303-1-B and OM7347 (decease 52.38% and 32.05%, respectively), so they were classified as susceptible (S). The other three varieties (OM8104, IR93340 and IR93343) were moderate susceptible (MS) (Table 4).

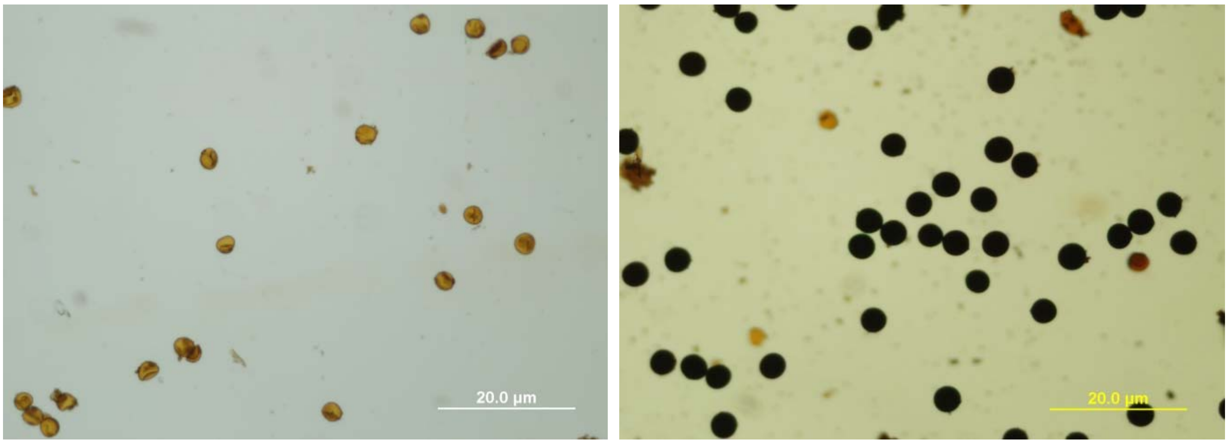
Rice panicles consist of primary branches, secondary branches differentiated from primary branches and flower primordia that develop into spikelets on these branches [18]. There is only one flower structure in each spikelet that will develop into a rice kernel after fertilization and filling [19]. The loss of potential spikelets is due to degeneration of primary and secondary branches and flower mordia.

The yeild components of tiller number per plant, spikelet number per plant, fertility and kernel weight were believed to have their own critical development periods which can affect the final grain yield. Salinity stress caused the reduction of overall vigor especially in the pollen viability and fertilization [20, 21]. Under saline condition during the boot stage, their pollen viability was drastically affected. This resulted into poor fertilization and consequent poor seed setting. Growth of plants due to osmotic stress forced by a high concentration of salts in the root zone significantly decreased the grain yield. The number of filled grain per panicle in saline condition was remarkably decreased

Table 4 Effect of saline on pollen germination and their classification to salinity tolerance.

Line/variety	Pollen germination (%)		Decrease (%)	Classification to salinity tolerance
	Control	Saline		
OM7347	85.16^{cd}	53.11^{ef}	32.05	S
OM8104	90.31^{bc}	69.73^{b-e}	20.58	MS
IR93340	84.65^{d}	61.41^{def}	23.24	MS
IR93343	90.60^{b}	64.59^{cde}	26.01	MS
IR5040	96.38^{a}	91.26^{a}	5.12	T
IR93350	92.48^{ab}	83.89^{abc}	8.59	T
IR87832-303-1-B	93.06^{ab}	40.68^{fg}	52.38	S
IR86385-8D-1-2-B	91.96^{ab}	82.90^{a-d}	9.06	T
IR29	81.63^{d}	30.56^{g}	51.07	S
Pokkali	94.30^{ab}	87.83^{ab}	6.47	T
F test	1.41	0.07	-	-
CV (%)	3.49	17.87	-	-
$LSD_{0.05}$	5.40	21.48	-	-

T: tolerant; MS: moderate susceptible; S: susceptible.
$^{a-g}$ Values in the same column with the same letter indicate no significant difference at $\alpha = 0.05$.



IR29 Pokkali

Fig. 3 Observation of pollen viability at EC = 8 dS/m under light microscope at 20×.
The picture was only used to illustrate observation of pollen viability at EC = 8 dS/m under light microscope at 20× between susceptible variety and tolerant variety.

in all of varieties. The salt sensitivity variety reduced over 50% of the number filled grain, such as OM7347 (60.69% reduction), OM8104 (58.80% reduction), IR93340 (58.78% reduction), IR93343 (57.74% reduction) and IR29 (68.88% reduction). By contrast, the number of filled grain of Pokkali (tolerant variety) was the highest in comparison with others varieties, and there was only the reduction of 11.88% in salt treatment. Under saline condition, as against decrease in the number of filled grain, the number of unfilled grain increased, specifically OM7347 increased 57.95%, IR87832-303-1-B 48.55% and IR29 34.46%, respectively.

Almost all of the varieties reduced their pollen viability due to salinity stress, resulting into poor grain yield, such as OM7347 (1.32 g/plant), IR87832-303-1-B (1.11 g/plant) and IR29 (0.70 g/plant) at EC = 8 dS/m. Pokkali has the highest grain yield in both control (7.56 g/plant) and saline conditions (5.23 g/plant). Next, there was non significantly statistical difference of IR86385-8D-1-2-B (5.02 g/plant) and IR5040 (4.69 g/plant), in comparison

Table 5 Effect of salinity on yield components to grain yield.

Line/variety	No. of filled grain			No. of unfilled grain			Grain yield per plant (g/plant)	
	Control	Saline	Decrease (%)	Control	Saline	Increase (%)	Control	Saline
OM7347	55.52[e]	21.70[ef]	60.69	21.48[bc]	51.08[a]	57.95	4.50[bc]	1.32[de]
OM8104	74.60[bcd]	30.74[d]	58.80	20.67[bcd]	45.21[ab]	54.28	6.20[ab]	3.49[bc]
IR93340	62.63[de]	25.82[def]	58.78	18.15[bcd]	35.53[bc]	48.92	5.38[bc]	2.39[cd]
IR93343	66.77[cde]	28.22[de]	57.74	23.60[b]	30.78[cd]	23.33	4.63[bc]	2.51[cd]
IR5040	65.56[cde]	42.29[c]	34.50	15.44[cde]	25.30[cd]	38.97	6.01[ab]	4.69[ab]
IR93350	88.46[a]	51.04[b]	49.30	14.90[de]	25.90[cd]	42.47	5.38[bc]	3.29[c]
IR87832-303-1-B	78.40[abc]	23.37[def]	29.91	23.39[b]	45.46[ab]	48.55	4.99[bc]	1.11[e]
IR86385-8D-1-2-B	80.80[ab]	45.06[bc]	44.23	18.49[bcd]	21.24[d]	12.95	7.33[a]	5.02[a]
IR29	62.62[de]	19.49[f]	68.88	34.16[a]	52.12[a]	34.46	4.03[c]	0.70[e]
Pokkali	80.28[ab]	70.74[a]	11.88	15.94[cde]	26.12[cd]	38.98	7.56[a]	5.23[a]
F test	0.68	0.21	-	0.29	6.78	-	0.02	14.90
CV (%)	10.59	13.72	-	18.86	19.94	-	19.26	24.89
$LSD_{0.05}$	13.00	8.44	-	6.31	11.60	-	1.85	1.27

[a-f] Values in the same column with the same letter indicate no significant difference at $\alpha = 0.05$.

with Pokkali (Table 5). The changes in grain weight and sterility were significantly correlated with changes in the panicle sodium concentration at several panicle development stages [22]. This results were also in agreement with Singh et al. [17] and Babu [23], who indicated that germination, active tillering and maturity are considered to be less sensitive to salinity than seedling stage, early reproductive stage, pollination and seed information.

4. Conclusions

Increased salt concentration will reduce the growth process of rice plants, reflecting through the following morphological characteristics: leaf rolling, tip leaf drying, plant height, root length and biomass of seedling rice stage. The seedling plans will die if the salinity is too high, especially discontinuing growth at 8 dS/m or more. Depending on the tolerant salinity, the rice lines/varieties have a longer survival time in saline condition.

Salinity has a severe effect on the flowering stage, causing pollen unviable and decreasing the germination rate and fertilization ability, finally leading to a decrease in rice grain yield. The pollen germination rate with decrease by ≥ 30% in these salinity sensitive lines/varieties will result in loss of yield > 50%, if salinity is too severe.

By the study of salt tolerance of the 10 lines/varieties, OM7347, IR87832-303-1-B and IR29 were classified as susceptible; OM8104, IR93340 and IR93343 as moderate susceptible; IR5040, IR93350, IR86385-8D-1-2-B and Pokkali as strong tolerance.

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