http://dx.doi.org/10.22161/ijeab/2.6.50 ISSN: 2456-1878

Phenotypic Screening of Drought-Tolerant Lines for Brown Planthopper, Blast and Phytic Acid Content Assay of Rice (*Oryza sativa* L.)

Pham Thi Thu Ha[1*], Nguyen Thi Lang[2], Dang Minh Tam[1], PhamThi Kim Vang[1], Ramin Rayee[3]

[1]Cuu Long Delta Rice Research Institute, Can Tho, Vietnam
[2]High Agricultural Technology Research Institute for Mekong Delta Vietnam (HATRI)
Agriculture Faculty, Takhar University, Afghanistan

Abstract—*Advanced drought tolerant lines were analysed for blast disease, brown planthopper (BPH), and phytic acid content. Thirsty lines of BC2F4 derived from OMCS2000/ IR75499-73-1 were used to screen for BPH and blast resistance. Three good resistant lines were screened against blast (45, 54, and 310) under greenhouse condition. As eight lines were identified to be resistant to BPH. The results further reveal that BC2F4-45 was the best line resistant to both BPH and blast disease. These lines will be useful in reducing grain phytic acid and improving the nutritional value of rice grain. Based on an assay for high phosphate germination stage of rice, the lowest content was found in the I5 variety (line 45). Hence, this line provides the urgent objective for breeders in cultivars of these crops to genetically enhance a healthy and functional diet. These characters will then need to be incorporated into high yield under drought stress with others such as disease and insect resistance.*

Keywords—Brown planthopper, blast, phytic acid content, screening, phenotype, drought-tolerant, rice

I. INTRODUCTION

Rice is a supreme commodity to mankind an important staple food for more than half of the world population, may provide 60-70 % body calorie intake to the consumers. Vietnam is one of the world's richest agricultural regions and is the second-largest (after Thailand) exporter worldwide and the world's seventh-largest consumer of rice. Rice production in India as well as in Vietnam must be doubled by 2025 to meet the requirement of the increasing population. This demand can be met only by enhancing the production and productivity of rice[1, 2].

A recent estimate on climate change predicts the water deficit to deteriorate further in years to come [3]and the intensity and frequency of drought are predicted to become worse [4]. Among biotic stresses, the disease has considered being the most devastating worldwide in rice, blast by *Pyriculariagrisea*. Similarly, groups of insects, brown planthopper [BPH], *Nilaparvatalugens*), has been the most damaging pest[5]. Brown planthopper is the most dangerous insect pest for rice and it causes severe yield losses by direct feeding and viral transmission of serious diseases. At high population density, hopper burn or complete drying of the plants is observed. From 2005 to 2006, more than 485000 ha of rice in the southern Vietnam was severely affected by viral diseases seemingly spread by BPH, resulting in the loss of 828000 tons of rice valued at US$120 million.

During water stress conditions or severity of drought, a major biotic stress- rice blast disease, caused by the filamentous ascomycete fungus *Magnaportheoryzae* (anamorph *Pyriculariagrisea*.) becomes a serious threat to rice production and leads to significant yield loss, as high as 70-80 % during an epidemic [7,8]. In Vietnam, this disease occurs particularly in a year with the long-wet season and causes the yield loss of up to 20%. Therefore, development of durable blast resistant varieties has been recognized as desirable means of disease management [9]. Thus drought-tolerant lines promoted at the advanced stage should possess tolerance of blast.

Besides, the major storage compound of phosphorus in plants tissue is phytic acid, (inositol hexakisphosphate)[10]. This compound can soak up irons and in foods and animal system and it decreases the absorption capacity of minerals like zinc, manganese, copper, molybdenum, calcium, magnesium, iron as well as protein [11]. Phosphorus content in phytic acid is also controlling inorganic phosphate concentration in developing seeds and seedling [12]. Loreti et al. [13] showed that during germination, phytates are broken down and release phosphorous, minerals, and myo-inositol which promotes rice germination and seedling stages. The low phytic acid trait addresses an urgent goal for the genetic improvement of rice because of anemia syndrome in rice. These characters will then need to be incorporated into high yield under drought stress with others such as disease and insect resistance. Therefore, this study was conducted based on the traits released behind major-effect drought-yield, to understand how the lines being interacted in stable tolerance to biological stress such as pests, diseases and improve promising nutritional drought tolerant lines.

II. MATERIALS AND METHODS

1. Plant materials

Thirty lines of BC_2F_4 from OMCS2000/ IR75499-73-1-B were screened for drought tolerance using phenotyping and molecular markers by Ha et al. [14]. These lines will be screened for BPH and blast resistance before they are introduced to farmers.

2. Screening for brown planthopper resistance

The seeds were presoaked and sown in rows in 60 x 45 x 10 cm seed boxes along with resistant and susceptible checks. A total of 10 seedlings per row were maintained per line with. There were three replications for each line and these were infected at 12 - 14 d old with the 2nd to 3rd instar hopper 4-6 nymphs per seedling. Seeds of susceptible check TN1 were sown in two border rows and in half of the middle row. Approximately one week after infestation hopper burn 'symptom' was observed. When more than 90% of susceptible check shows wilting, the plants were scored individually based on the scoring system proposed by the International Rice Research Institute [15] and each seedling was scored as 0 = no visible damage, 1 = partial yellowing of the first leaf, 3 = first and second leaves partially yellowing, 5 = pronounced yellowing or some stunting, 7 = mostly wilted plant but still alive, 9 = the plant completely wilted or dead.

3. Evaluation of blast resistance

Seeds were soaked for 1 day and sown in a 15 x 30 x 4-cm plastic tray containing sieved topsoil media. The rice plants were inoculated with blast pathotype spore suspension (1 x 10^5 spores/mL) 21 days after. Plants were incubated in a dark dew chamber for 24 h at 25°-28°C. After 24 h, the plants were returned to the greenhouse with a controlled water sprinkler to maintain the humidity around the plants. Disease reactions were recorded as the number of plants infected by a pathotype observed after 7 days of inoculation with the blast spores. Five infected leaves were recorded for each replication.

4. Phytic acid content assay

Seeds of rice varieties (0.05 g) were grind to a fine powder, mixed in 2 ml of 0.4 M HCl and incubated at 4 °C for overnight. The solution was mixed and 100 µl of the mixture was transferred to a cuvette. A volume of 1 ml was maintained by adding 900 µl distilled water. After that, 1ml of Chen' reagent ((6N H_2SO_4: 2.5% ammonium molybdate: 10% ascorbic acid: distilled water (1:1:1:2)) were added to a cuvette, covered with parafilm and mixed well by inversion. A blank was used as control having 1ml Chen' reagent and 1ml water [16]. The samples were then incubated at 37 °C for 1.5 hours. The absorbance of the reaction was measured at 820nm. The phytic acid content was determined using the known molarities of phosphate standard curve in triplication of 1mM KH_2PO_4 ranging from 25, 50, 100, 150, to 200 µl. Fig 1 showed the standard curve of phosphate for the Microtiter Plate PI assay followed by Chen's method.

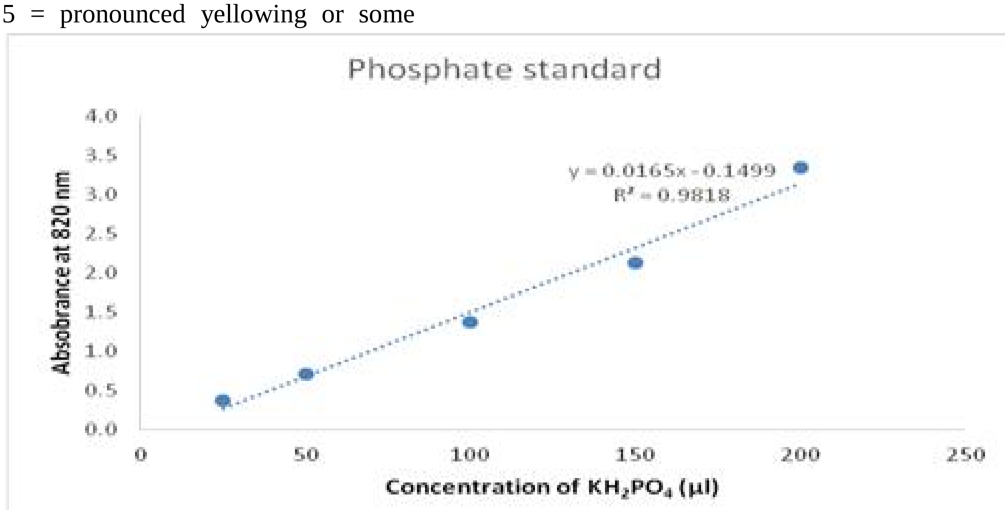

Fig. 1: Standard of phosphate

Table 1: The list of lines/varieties in this research.

Codes	Varieties/lines
I8	F7 (OM6162/Swanasub1)
I34	BC2F4-54
I5	BC2F4-45
I49	F7 (IR75499-29-2-B/IR64 Sub1)

5. Statistical analysis

All experiments and data provided in this paper were repeated three times. Statistical analysis was carried out by using Minitab software. The data are presented as the means ± the standard deviation. Comparisons with $P < 0.01$ were considered significantly different.

III. RESULTS AND DISCUSSION

1. Screening for blast and Brown planthopper resistance

Development of the disease resistance or stress-tolerant plants is an important objective in rice breeding programs because the production of rice can be constantly affected by several major abiotic and biotic stresses. The phenotypic evaluation showed clear distinction between resistant and susceptible typesand clearly revealing moderately resistant types as well.

The isolate 2(U61-i0-k101-z05-ta102) of*Pyriculariagrisea* was isolated using the method described by Hayashi et al. [17] in this study. The Table 6.4 shows the reaction of BC_2F_4 lines derived from OMCS2000/ IR75499-73-1-B to brown planthopper and blast resistance. Two of resistant checks had the best level of 3 for BPH and a level of 3 for blast.

For blast, IR 24 which was a susceptible check variety and had a score of 9 which indicate susceptibility. Most lines had level 5, which is at moderately susceptible level. One line (BC_2F_4-310) had level of 1 and three lines (BC_2F_4-45, BC_2F_4-54 had level of 3. These lines were resistant to blast disease. One line was highly susceptible, level of 7. This result showed that one of these lines was better than the resistant check but the reminded of the lines were better than the susceptible check and parent varieties.

Among all the insect pests, brown planthopper, is one of the most destructive pests of rice causing severe yield losses [18](Sai Harini et al., 2013). The screening of lines/varieties resistant to BPH is an important experiment because new varieties should be tested before they are introduced to farmers. For brown planthopper, most of the lines had levels in the arrange 1-7. Three lines had the level of 1 and five had the level of 3. These lines are resistant to BPH. Three lines were highly susceptible, scores of 7. The nineteen lines had level 5, which is at moderately susceptible level. This result showed that three of these varieties were better than the resistant check (BC_2F_4-89, BC_2F_4-45, and BC_2F_4-95). Though many chemicals were recommended for the control of this pest [18], due to its feeding behavior at the base of the plant, the farmers are unable to control this pest effectively. Thus, farmers resort to blanket application of insecticides which often disrupts the ecological balance of rice ecosystem due to which this pest has already developed resistance against many insecticides in different Asian countries [19, 20]. The use of genetic resistance is the most effective measure for BPH management. Cultivation of resistant varieties is an economical, efficient and environmentally sound strategy for population management of insect-pests.

Table.2: Reaction of BC_2F_4 lines derived from OMCS2000/ IR75499-73-1-B against brown planthopper and blastresistance.

N_0	Name of variety	BPH (level)	Reaction	Blast (level)	Reaction
Susceptible	TN1	9	S	-	-
Resistance	PtB33	3	R	-	-
Susceptible	IR24	-	-	9	S
Resistance	Tetep	-	-	3	R
P1	OMCS2000	5	MS	5	MS
P2	IR75499-73-1-B	3	R	5	MS
1	BC_2F_4-17	5	MS	5	MS
2	BC_2F_4-25	5	MS	5	MS
3	BC_2F_4-45	1	R	3	R
4	BC_2F_4-54	5	MS	3	R

N_0	Name of variety	BPH (level)	Reaction	Blast (level)	Reaction
5	BC_2F_4-56	5	MS	5	MS
6	BC_2F_4-68	5	MS	5	MS
7	$BC_2F_4$77	5	MS	5	MS
8	BC_2F_4-79	3	R	5	MS
9	BC_2F_4-89	1	R	5	MS
10	BC_2F_4-95	1	R	5	MS
11	BC_2F_4-99	7	S	5	MS
12	BC_2F_4-100	5	MS	5	MS
13	BC_2F_4-105	3	R	5	MS
14	BC_2F_4-112	7	S	5	MS
15	BC_2F_4-120	3	R	3	R
16	BC_2F_4-123	7	S	5	MS
17	BC_2F_4-130	5	MS	7	S
18	BC_2F_4-145	5	MS	5	MS
19	BC_2F_4-152	3	R	5	MS
20	BC_2F_4-155	5	MS	5	MS
21	BC_2F_4-158	3	R	5	MS
22	BC_2F_4-175	5	MS	5	MS
23	BC_2F_4-179	5	MS	5	MS
24	BC_2F_4-200	5	MS	5	MS
25	BC_2F_4-211	5	MS	5	MS
26	BC_2F_4-256	5	MS	5	MS
27	BC_2F_4-358	5	MS	5	MS
28	$BC_2F_4$278	5	MS	5	MS
29	BC_2F_4-289	5	MS	5	MS
30	BC_2F_4-310	5	MS	1	R

R: Resistance; S: Susceptible; MS: Medium Susceptible

2. Phytic acid content

Study of low phytic acid content in riceis important to improve promising nutritional lines.The present study revealed that highest content of phytic acid was observed in the I49 variety with 38.701 a ± 0.093, followed by I34 variety (33.610 ± 0.153). Besides that, the lowest content was found in the I5 variety (25.630 d ± 0.182) (Table 3). According to Khattak et al. [21] and Beleia,[22] phytates play an important part in mineral metabolism and mayreduce the availability of Fe, Zn, Ca, Mg, Cu, Mn, and Mo as well as protein. Therefore, low-phytic acid rice has higher bioavailable Zn^{2+} and Fe^{3+}, and this means that the low phytic acid content could serve theprinciple objective for breeding by improving nutritional value.

Table.3: The phytic acid content in the drought tolerant lines.

Varieties	Phytic acid content (µg/mL)
I8	30.721 c ± 0.061
I34	33.610 b ± 0.153
I5	25.630 d ± 0.182
I49	38.701 a ± 0.093

IV. CONCLUSION

The screening of varieties resistant to BPH and blast is an important experiment because new varieties should be tested before they are introduced to farmers. Advanced drought- tolerantlines indicate that BC_2F_4-45 was the best line resistant to both BPH and blast disease and had low phytic acid content. This variety will able to provide disease control at essentially no cost to the farmers.

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