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Screening of traditional scented rice (*Joha*) of Assam for presence of Badh2, blast and brown spot diseases

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ABSTRACT

The traditional scented rice (Joha) varieties are very popular among the people of Assam. The traditional scented rice though carries a premium price throughout the world, is a low yielder. However, the traditional Joha varieties are tolerant to many disease and pest, indicating the joha germplasm may be a source for several disease/pest resistant genes. However not much work has been carried out to identify the factors responsible for disease/pest tolerance in these lines. Hence, an attempt was made in 39 Joha germplasms to identify blast and brown spot resistant/tolerant germplasms at Regional Agricultural Research Station, Assam Agricultural University, Titabar. In most of the aromatic lines the aroma is due to the function of the Badh2 gene. Therefore, an attempt was made to study the action of Badh2 gene in these germplasms with the help of SNP analysis. The present study reveals the associated between Badh2 gene fragrance in most of the Joha germplasms. However in some aromatic Joha germplasms the fragrance is not correlated with the action of Badh2, and it may be due to some other factors. This finding needs further studies to identify the factors contributing to the fragrance in Joha rice.

Key words: *Blast, brown spot, Badh2, SNP*

INTRODUCTION

Rice is the principle food grain crop of the Assam occupying 2.51 million hectares which accounts for more than 80% of the total cultivated area of the state. The total rice production of Assam is estimated to be around 5.20 million tones. However, the production of rice is constrained by various fungal, bacterial and viral diseases. Assam is be considered as the hotspot of rice genetic resources as compared to the other parts of the country.

Among different categories of rice, aromatic rice is higly valued and demands for special attention. It is a unique class and falls under Sali rice, traditionally known as '*Joha*' rice. The rice is indeed a special heirloom grain of Assam, known for its sweet scented aroma, superfine kernel, good cooking qualities and excellent taste. The unique taste and aroma of the rice is mostly due to the presence of a chemical compound 2-acetyl-1-pyrroline (2AP). Moreover, it also contains proteins, phenolic compounds, carbohydrates, flavanoids and volatile oils. Besides the rice has a tremendous health benefit against cardiovascular diseases, cancer, inflammation, allergy, aging, etc. The specialty of *Joha* rice is it is only grown in the North-East region of India and it is completely different from the famous Basmati rice.

The challenge in *Joha* rice cultivation is it is prone to diseases. The high wind and rain are believed to aid in rapid dispersal of the pathogen. Blast disease of rice, caused by the fungus *Magnaporthe oryzae* is one of the most destructive diseases which can affect all above ground part of a rice plant; leaf, collar, node, neck, panicles and leaf sheaths, besides infecting the roots (Sharma et al., 2012; Sesma and Osbourn, 2004). It is prevalent in the areas with low soil moisture, frequent and prolonged periods of rainfall and low temperature during the daytime. Blast pathogen infects rice during all the growth stages. The typical symptoms include white to gray-green lesions on leaves initially with dark green borders. Later the lesions turn elliptical and whitish to gray in color having red to brownish border. Blast lesions are usually slender and pointed at each end, can enlarge to kill the entire leaves. Leaf blast can kill rice plants at seedling stage and in mature plants it cause yield losses in case of severe infection. To date, around 100 blast resistance genes have been identified, and many of them have been cloned and characterized, such as *Pb1, Pia, Pib, Pid2, Pid3, Pik, Pik-h/Pi54, Pik-m, Pik-p, Pish, Pit, Pita, Piz-t,Pi56,Pi63, Pi5, Pi9, Pi21, Pi25, Pi36,Pi1, Pi2/Piz5* and *PiCO39* (Ramadevi et al., 2015; Devanna et al., 2014; Devanna et al., 2017; Kumari et al., 2018). Studied on the genetic diversity for blast resistance gene *Pi54* in landraces and wild *Oryza* species recorded that alleles derived from wild species had more divergence than the landraces and these alleles can be use potentially in blast resistance breeding programme. (Kumari et al., 2013; Ramkumar et al., 2016).

Blast lesions can be confused with brown spot lesions in rice. Therefore all the traditional *Joha* varieties were also screened for brown spot disease along with screening of blast disease. Brown Spot is also one of the most common and destructive diseases of rice plant, caused by the fungus *Cochliobolus miyabeanus*, infecting coleoptiles, leaves, leaf sheath, panicle and spikelets. Numerous big spots can be observed on the leaves of the diseased plant which can kill the whole leaf. The spots are initially small, circular and dark brown to purple-brown in color. Fully developed spots are circular to oval, light brown to gray in color with reddish brown margin. When this fungus infects the seeds, unfilled grains and or spotted or discolored seeds are formed. Heavily infected seeds can results in seedling blight leading to 10-58% seedling mortality, also affects the quality and the number of grains per panicle, reduces the kernel weight.

The susceptibility of the rice plant to the brown spot disease increases with age. In the early stage of development of the rice plant, only minute spots were formed and this was not affected by soil nutrition (Sato, 1964-65). Deficiency and excess of nitrogen both increase brown spot development. The disease occurs at the temperature range of 16-36 ^{o}C at a higher relative humidity of over 89 percent. Moreover, free water on the leaf surface also favors infection process (Chattopadhyay et al., 1960)

Through conventional and molecular breeding many blast-resistant varieties have been developed. Conventional breeding for disease resistance is tedious, time consuming and mostly dependent on environment as compare to molecular breeding particularly marker assisted selection, which is easier, highly efficient and precise(Sharma et al., 2012; Miah et al., 2013; Kumari et al., 2017).

MATERIALS AND METHODS

Plant material

To carry out the experiment 39 *joha* germplasms were collected from different parts of Assam and cultivated at Regional Agricultural Research Station (RARS), Titabar, Assam, India.

Blast screening

For screening blast, the uniform blast nursery was used. rice line Mahsuri was used as the susceptible check and Bahadur was used as resistant check. In the blast nursery, which was raised in three replications, the susceptible check Mahsuri was planted in every third row and the resistant check, Bahadur was planted after every ten entries. The testing rows were 50 cm long and raised 10 cm apart. The susceptible check, Mahsuri was also planted around the tested lines in three rows at a distance of 10 cm apart. The blast nursery was raised during March 2015 and October 2015. All the favourable conditions were created to ensure the incidence of blast disease.

The prevalent blast races in RARS, Titabar, Assam are IF 3 (Anonymous, 1994), IB 32 (Anonymous, 2010), IH (Anonymous, 2011). The inoculum of these races was applied artificially on the testing lines. Disease incidence was recorded adopting standard evaluation system, IRRI, 1996 (Anonymous, 1996) for rice.

Further, the *Joha* germplasms were sent to Intertek India Private limited, Hyderabad to screen for three blast resistant genes namely, *Pi-ta* on chromosome 12, *Pi9* on chromosome 6 and *Pi54* on chromosome 11 with the help of SNP marker. The table below shows the genetics behind blast resistance and the allelic responses of the resistant genes (Table 1).

Table 1. Genetics behind blast resistance and the allelic responses of the resistant genes.

Genes	Chromosome	Physical position(Mb)	allele.1	allele.2	Allele 1 trait	Allele 2 trait
Pi-ta	12	10.6	A	C	Susceptible	Resistant
Pi9	6	10.4	CGATGGTTTC	-	Susceptible	Resistant
Pi54	11	25.3	C	T	Susceptible	Resistant

Brown spot screening

For screening Brown Spot disease, rice line Prafulla was used as susceptible check and Satyaranjan was used as resistant check. In the blast-screening nursery, along with blast susceptible check Mahsuri, the susceptible check for brown spot Prafulla was planted and along with blast resistant check Bahadur, the brown spot resistant check Satyaranjan was planted.

The inoculum of this race was applied artificially on the testing lines. The disease was recorded adopting standard Evaluation System for rice (Anonymous, 1996).

Screening for BADH2

Besides blast and brown spot screening, we have also sent the 39 *Joha* germplasms to Intertek, Hyderabad to screen for aroma with the help of SNP markers. The gene responsible for aroma in rice is *Badh2* present on chromosome 8, encoding a protein betaine aldehyde dehydrogenase (BADH2). A full length BADH2 protein encoded by a dominant *Badh2* allele (AAAAGATTATGGC) inhibits the synthesis of 2-acetyl-1-pyroline (2AP), a potent flavor component in rice fragrance. Whereas, a recessive *Badh2* allele (TATAT) encodes a partial, non-functional BADH2 protein which induces the synthesis of 2AP and hence the aroma (Prathepha, 2009). The result obtained from SNP analysis is presented in Table 2. Genetics behind aroma is as represented below

Further, the organoleptic evaluation has also been carried out for these *Joha* germplasms to measure the aroma intensity and the result obtained is represented in Table 5.

RESULTS AND DISCUSSION

The susceptible check Mahsuri was severely infected by the blast disease indicating high incidence of the infection in the experimental plots. Out of 39 genotypes under the study, 25 exhibited resistant reaction, 1 moderately resistant, 12 moderately susceptible and only one traditional *Joha* variety exhibited susceptible reaction to blast (Table 3).

However from the SNP analysis of these 39 *joha* germplasm for 3 blast resistance (R) genes (*Pi-ta*, *Pi9* and *Pi54*), it was observed that only Bhungri *Joha* showed the presence of two blast resistance genes (*Pita* and *Pi54*), nine germplasms *viz*., Bokul *Joha*, Bhungri *Joha*, *Joha*, Krishna *Joha*, Borsal *Joha*, *Joha*bora, Khorika *Joha*, Kola *Joha* I and Monipuri

Table 2. The result obtained from SNP analysis

Gene	Chromosome	Physical position(Mb)	allele.1	allele.2	Allele 1 trait	Allele 2 trait
BADH2	8	20.4	AAAAGATTATGGC	TATAT	No. fragrance	Fragrance

Table 3. Reaction of *Joha* varieties for blast.

Disease reaction	Rice lines
Resistant	Monipuri *Joha*, Kola*Joha* I, Khorika*Joha*, *Joha*bora, Basabhog, Bengali*Joha*, Bhaboli*Joha*, Bokul*Joha*, Boga*Joha*, Bhugri*Joha*, , Cheniguti *Joha*, Chufon *Joha*, Goalporia *Joha* I, *Joha*, Kali*Joha*, Kon*Joha* I, Kon*Joha* II, Kon*Joha* III, Kopow*Joha*, Krishna*Joha*, Kotari*Joha*, Nepali *Joha*, Rampal *Joha*, Tulsibhog, KDML
Moderately resistant	Tulsi *Joha*
Moderately susceptible	Bogi*Joha*, Boga Manikimadhuri *Joha*, Boga Tulsi*Joha*, Bosal *Joha*, Goalporia *Joha* II, Kunkuni *Joha*, Kon Bogi*Joha*, Kola*Joha* II, Kaljira, Kamini*Joha*, Manikimadhuri *Joha*,, Ronga *Joha* II
Susceptible	Govindabhog

Table 4. Reaction of *Joha* varieties for brown spot.

Disease reaction	Rice lines
Moderately resistant	Bhaboli*Joha*, Bokul*Joha*, Boga*Joha*, Bhugri*Joha*, Cheniguti *Joha*, Kali*Joha*, Kon*Joha* I, Nepali *Joha*, KDML
Moderately susceptible	Basabhog, Bengali*Joha*, Bogi*Joha*, Boga Manikimadhuri *Joha*, Boga Tulsi*Joha*, Bosal *Joha*, Chufon *Joha*, Goalporia *Joha* I, Goalporia *Joha* II, Govindabhog, *Joha*, *Joha*bora, Kola*Joha* II, Kaljira, Khorika*Joha*, Kamini*Joha*, Manikimadhuri *Joha*, Monipuri *Joha*, Ronga *Joha* II, Tulsibhog
Susceptible	Rampal *Joha*
Highly susceptible	Tulsi *Joha*

Table5. The result obtained from SNP analysis for screening of *Badh2* along with organoleptic evaluation for aroma intensity.

Sl no	Names	BADH2	Aroma intensity
1.	Badsahbhog	fragrance	++++
2.	Bengali*Joha*	No fragrance	+++
3.	Bhaboli*Joha*	fragrance	++
4.	Bokul*Joha*	No fragrance	++++
5.	Boga*Joha*	fragrance	++
6.	Bhungri*Joha*	No fragrance	+++
7.	Cheniguti *Joha*	fragrance	++
8.	Chufon *Joha*	fragrance	++
9.	Goalporia *Joha* I	fragrance	++
10.	*Joha*	fragrance	++
11.	Kali*Joha*	No fragrance	++++
12.	Kon*Joha* I	fragrance	+++
13.	Kon*Joha* II	fragrance	+++
14.	Kon*Joha* III	fragrance	+++
15.	Kopow*Joha*	fragrance	+++
16.	Krishna*Joha*	fragrance	+++
17.	Kotari*Joha*	No fragrance	++++
18.	Nepali *Joha*	No fragrance	+
19.	Rampal *Joha*	fragrance	++
20.	Tulsibhog	fragrance	+++
21.	KDML	fragrance	
22.	Tulsi *Joha*	fragrance	++
23.	Bogi*Joha*	fragrance	+++
24.	Boga Manikimadhuri *Joha*	fragrance	++
25.	Boga Tulsi*Joha*	fragrance	++
26.	Borsal *Joha*	No fragrance	+++
27.	Goalporia *Joha* II	fragrance	++
28.	*Joha*bora	fragrance	++
29.	Kunkuni *Joha*	fragrance	+++
30.	Khorika*Joha*	fragrance	+
31.	Kon Bogi*Joha*	fragrance	++
32.	Kola*Joha* I	fragrance	++++
33.	Kola*Joha* II	No fragrance	++++
34.	Kaljira	fragrance	+++
35.	Kamini*Joha*	fragrance	+++
36.	Manikimadhuri *Joha*	fragrance	++
37.	Monipuri *Joha*	No fragrance	+
38.	Ronga *Joha* II	fragrance	++
39.	Govindabhog	No fragrance	+++

Joha showed the presence of only one blast resistance gene, *Pita*. So it can be assumed that the resistance showed by the other *joha* germplasms is may be due to the presence of other blast resistance genes, but needs further studies to identify and confirm the presence of other *R* genes.

Screening for brown spot disease showed that seedlings of the susceptible check Prafulla were severely infected indicating high incidence of the infection in the experimental plots. Out of 39 genotypes under the study, eight exhibited resistant reaction, nine moderately resistant, 20 moderately susceptible, and one each susceptible and highly susceptible reaction (Table 4).

On the basis of phenotypic screening the germplasm were categorized into four different groups as listed below:-

The findings from the present study suggest that these resistant cultivars may be used as a source of resistance in different breeding programs. SNP analysis for Badh2 confirms the presence of the gene in 30 numbers of *Joha* germplasm. However the fragrance in some *Joha* germplasms- Bengali *Joha*, Bokul *Joha*, Kali *Joha*, Kotari *Joha*, Nepali *Joha*, Borsal *Joha*, Kola *Joha* II, Monipuri *Joha*, Govindabhog is not associated with the *Badh2*. The factor responsible for the fragrance in these *Joha* germplasm needs to be identified. Further study is required to know the genes responsible for blast resistance in other rice *Joha* germplasm.

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