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Protein Profiling of Resistant and Susceptible Mutant Lines of Rice Variety Swarna in Response to *Rhizoctonia solani AG1 IA* Infection

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A B S T R A C T

Rice sheath blight, caused by *Rhizoctonia solani*, is one of the most devastating diseases for stable rice production in most rice-growing regions of the world. Currently, studies of the molecular mechanism of rice sheath blight resistance are scarce. Here, we used mutated rice population of the variety swarna induced by the sodium azide (NaN_3). The variability in disease reaction was observed among mutated rice lines. Out of the total 1000 mutant plants, 47 plants were screened that show low disease index, ranged between 0 to 5 were selected for next generation. In M_1 generation the selected genotypes show differences for disease index, only 12 lines shows resistance in M_1 generation in field condition and in humidity chamber condition only 8 lines shows resistance. The protein analysis by SDS-PAGE showed that the resistance lines have one extra band in between 33 kDa and 43 kDa that is not showing in moderate and susceptible lines. Thus, these resistance lines could be considered a potential source for disease resistance against the sheath blight of rice and could be used further in the crossing programme for development of sheath blight resistant rice variety.

Introduction

Rice is the most important cereal crop and it is a staple food for millions of people in the world (Chakravarti *et al*., 2012; Davla *et al.*, 2013). Consumption of rice accounts for over 90% of the world's population in Asia, with China, India and Indonesia producing 30.85%, 20.12% and 8.21%, respectively of total global rice production (Kadu, *et al.*, 2015). More than 70 diseases caused by fungi, bacteria, viruses or nematodes have been recorded on rice, among which rice blast (*Magnaporthe grisea*), bacterial leaf blight (*Xanthomonas oryzae pv. oryzae*) and sheath blight are the most serious constraints on high

productivity (Ou, 1985). *Rhizoctonia solani* Kühn is a widespread soil-borne pathogen that causes economically important diseases in many crops (Adams, 1988). Rice sheath blight caused by *R. solani* is one of the most serious diseases of rice worldwide, causing considerable yield losses (Sudhakar *et al*., 1998). The widespread adoption of new, susceptible, high yielding cultivars with large numbers of tillers, and the changes in cultural practices associated with these cultivars, favor the development of sheath blight and contribute greatly to the rapid increase in the incidence and severity of this disease in rice-producing areas throughout the world (Groth *et al*., 1991). Furthermore, environmental conditions such as low light, cloudy days, high temperature and high relative humidity also favor the disease (Ou, 1985).

Mutation breeding involves the development of new varieties by generating and utilizing genetic variability through chemical and physical mutagenesis (Kharkwal *et al.,* 2009 and Forster *et al.,* 2012). Sodium azide (NaN_3) is a chemical mutagen and has been one of the most powerful mutagens in crop plants (Wen and Liang, 1995). It is known to be highly mutagenic in several organisms, including plants and animals (Rines, 1985; Raicu and Mixich, 1992; Grant and Salamone, 1994) and its mutagenic potential has been reported in several screening assays. Sodium azide is marginally mutagenic in different organisms (Arenaz *et al.,* 1989). The mutagenicity is mediated through the production of an organic metabolite of azide compound (Owais and Kleinhofs, 1988). This metabolite enters into the nucleus, interacts to DNA, and creates point mutation in the genome (Kleinhofs *et al.,* 1978; Gichner and Veleminsky, 1977). Being a strong mutagen in plant, it affects the different parts of the plants and their growth developmental phenomena by disturbing the metabolic activities (Salim *et al*., 2009).

Materials and Methods

Plant materials

The seeds of rice variety Swarna were collected from Department of Genetics and Plant Breeding, College of Agriculture, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya. India. Chemical mutagen sodium azide was used as a mutagen in this experiment. The 0.01%, 0.02%, 0.03%, 0.04%, 0.05% NaN_3 solution were prepared and record the lethal dose 50 (LD_{50}) and pH adjusted with ortho-phosphoric acid. The mutated seeds treated with 0.03% NaN_3 were grown in nursery and later on healthy seedlings were transplanted in main fields at 21days of growth stage. The genotypes were screened under field conditions and humidity chamber condition in *Kharif* season for two consecutive years i.e. 20016-17 and 2017-18 for selection of resistant genotypes against *R. solani.* In M1 generation, individual plant represents one single genotype. Some potent mutant genotypes were selected based on the disease evaluation from 1000 plant population, consisting both mutant and control lines and their seeds were harvested separately. In M_2 generation, disease resistance of selected mutants was confirmed by growing progeny to row method. The genotypes depicting very disease index value have been advanced to next generation (M_2). Control lines of swarna (non-mutated) were grown along with the mutated lines for resistance screening.

Fungal isolate and inoculums preparation

The most aggressive isolate A-1 of *Rhizoctonia solani*, isolated from the Rice Pathology Laboratory, Indian Institute of Rice Research, Hyderabad (Telangana) India, was taken for resistance screening. After placing sclerotia of *R. solani* onto Potato Dextrose

Agar (PDA) under aseptic conditions, cultures were grown at 25± 2^0C under continuous light. Mycelial bits or immature sclerotia taken from 7 day old culture were cut and used as inoculums.

Screening of sheath blight (ShB) under field condition

Inoculation procedure for infection in cultivated varieties of rice and wild rice by *R. solani* isolates was performed according to Park *et al.*, (2008). Immature sclerotia developed on 4-6 days old mycelia of *R. solani* strain D-14 was grown on Potato Dextrose Agar (PDA) medium. An immature sclerotium of *R. solani* was placed underneath the leaf sheath with 10 ml of sterilized water. Inoculated and non-inoculated plants were placed in humidified chamber condition at 28± 1º C for 24 hrs. Scoring of disease was carried out on 0-9 rating scale (Standard Evalution System of IRRI) as described below:

0= absolutely free from infection
1= lesions limited to lower 20% of plant height
3= 20-30% disease
5= 31-45% disease
7= 46-65% disease
9= more than 65% disease.

Protein profiling from leaves of mutant rice plant

All chemicals for analytical work were of AR grade. Sodium phosphate buffer (0.25 M, pH 7.0), containing 0.15N NaCl, mutant rice leaves, pestle and mortar, ice, centrifuge tubes etc. The rice leaf protein was isolated as method described by (Laemmli *et al.*, 1970). The fresh plant leaf were cut into small pieces using razor and crushed in sodium phosphate buffer (0.25M, pH 7.0) containing 0.15 N. It was homogenized mechanically and centrifuged at 10,000 g at 4^0C for 20 minutes. This process was done twice. After centrifugation the supernatant was collected. This supernatant was crude rice leaf protein.

Gel electrophoresis

The separating gel were put between glass plates up to proper mark and wait for 30- 40 minutes for proper polymerization of gel. The stacking gel was cast, after polymerization of separating gel, insert the Teflon comb (13 well) in the gap between the glass plates and wait for proper polymerization of the stacking gel. After proper polymerization the Teflon comb was carefully removed from the gel and plates were assembled into electrophoresis unit and electrode buffer was filled both in lower and upper tank of electrophoresis unit. After this the electrophoresis unit was attached with power pack and placed the gel for 8-10 hours with a supply of 25 mA and 160 volt current. When the tracking dye reached the end of the running gel after complete separation of protein molecules, power supply turned off. The gel was gently removed from the space between

Gel analysis of protein

The relative mobility of the different protein bands were recorded by comparing the bands with standard protein marker loaded with gel.

Results and Discussion

Disease screening of sheath blight (ShB) of M_1 generation under field condition

Out of 1000 mutant rice plants of Swarna, 47 rice mutants plant showed resistance (R), in M_1 population. The disease reaction and phenotypic expression were observed among the mutant population. Similarly mutation induced lines of variety Mahsuri were released for blast resistance with improved

cooking and eating qualities. (Hadzim, *et al*., 1994; Hadzim, *et al*., 1988; Faruq *et al*., 2003). The screening of mutant line Zhe-101 selected from the mutant progenies of Indica rice cultivar showed the significant improvement in disease resistance to blast and bacterial blight (Wen-chao *et al*., 2004).

Figure.1 Showing disease infection in field condition of mutant rice plant

Figure.2 Protein profiling of control, moderately susceptible, susceptible and resistance mutant lines. M-Marker, C- Control, MS- Moderately Susceptible, S- Susceptible, HS- highly Susceptible, R-Resistance (1R, 2R,3R,4R are showing different resistant mutant lines)

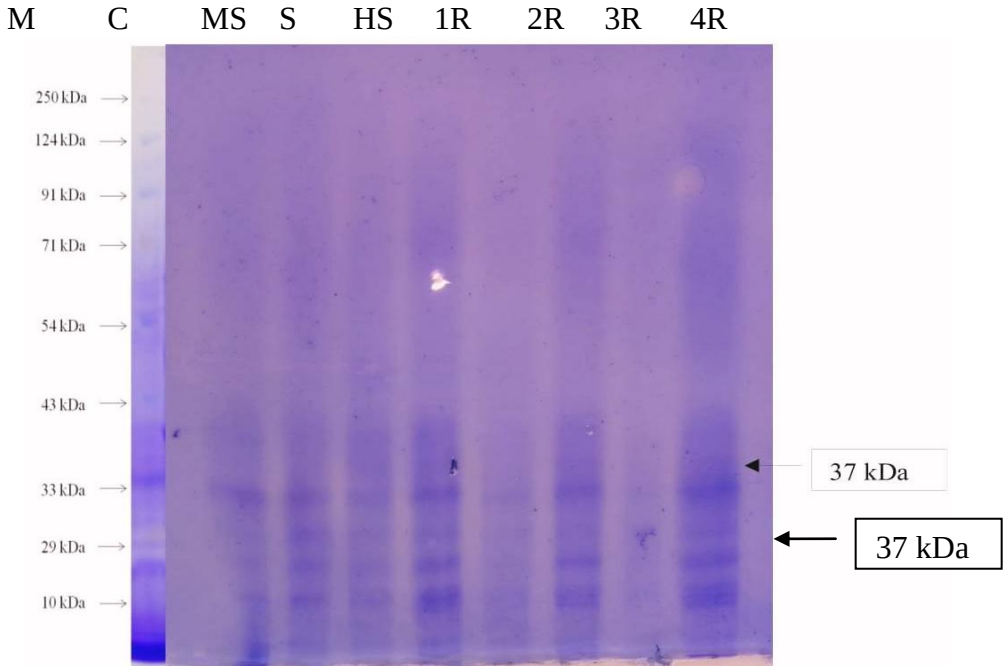

Table.1 Showing different banding pattern of mutant rice plant in kDa

S.No.	M	Control	MS	S	HS	1R	2R	3R	4R
1	250	-	-	-	-	-	-	-	-
2	240	-	-	-	-	-	-	-	-
3	200	-	-	-	-	-	-	-	-
4	190	-	-	-	-	-	-	-	-
5	150	-	-	-	-	-	-	-	-
6	124	-	-	-	-	-	-	-	-
7	110	-	-	-	-	-	-	-	-
8	92	-	-	-	-	-	-	-	-
9	91	-	-	-	-	-	-	-	-
10	90	-	-	-	-	-	-	-	-
11	89	-	-	-	-	-	-	-	-
12	85	-	-	-	-	-	-	-	-
13	80	-	-	-	-	-	-	-	-
14	79	-	-	-	-	-	-	-	-
15	75	-	-	-	-	-	-	-	-
16	72	-	-	-	-	-	-	-	-
17	71	-	-	-	-	-	-	-	-
18	60	-	-	-	-	-	-	-	-
19	55	-	-	-	-	-	-	-	-
20	54	-	-	-	-	-	-	-	-
21	53	-	-	-	-	-	-	-	-
22	44	-	-	-	-	-	-	-	-
23	43	-	-	-	-	-	-	-	-
24	40	-	-	-	-	-	-	-	-
25	37					37		-	37
26	34	-	-	-	-	-	-	-	-
27	33	33	33	33	33	33	33	-	33
28	32	-	-	-	-	-	-	-	-
29	30	-	-	-	-	-	-	-	-
30	29	29	29	29	--	--	39	-	29
31	28	-	-	-	-	-	-	-	-
32	25	25	-	25	25	25	-	-	25
33	22	-	-	-	-	-	-	-	-
34	20	-	-	-	-	-	-	-	-
35	19	-	-	-	-	-	-	-	-
36	18	-	-	-	-	-	-	-	-
37	15	-	-	-	-	-	-	-	-
38	14	-	-	-	-	-	-	-	-
39	12	-	-	-	-	-	-	-	-
40	11	-	-	-	-	-	-	-	-
41	10	10	10	10	10	10	10	10	10

Disease screening of sheath blight in M_2 generation of M_1 selected plants under field condition

Screening in M_2 generation of M_1 selected plants in field condition four types of genotypes were found on the basis of disease reaction. In M_2 generation out of 47 mutants genotyped we found 12 resistance (R), 15 moderate resistance (MR), 15 moderate susceptible (MS) and 5 susceptible (S) lines. This data was recorded by taking mean value of 5 plants from every row of specific genotype. These identified mutant lines could be considered being a potential source for disease resistance against the sheath blight of rice according to Mosaddeque *et. al.*, (2008) conducted that studies on forty-four test entries of parental lines of rice with one susceptible and one resistant check were screened against sheath blight. Ten lines were resistant, 31 were moderately resistant and 3 showed moderately susceptible reaction at maximum tillering stage (Fig. 1 and 2; Table 1).

Protein profiling of mutant rice plant on basis of diseases reaction

The protein profiling showed that the not mutant and mutant line. Mutant line classified on the basis of disease reaction against sheath blight resistance and susceptible lines. Maximum lines shows similar band, only 1R and 4R mutant line show extra band in between 33 kDa and 43 kDa.

The band intensity is high in resistance as compare to moderate and susceptible mutant lines of rice variety Swarna result supported by Li *et al* (2011).The result on the banding pattern of the protein profiles suggested that the specific genotype could be differentiated either based on the position or intensity of bands but not on number, as some of the varieties expressed similar number of bands, similar report supported that Netra and Prasad, (2007).

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