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Screening for sheath blight resistance lines of rice (*Oryza sativa* L.) variety swarna by using of chemical mutagen

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Abstract

The present investigation was undertaken to identify the resistant lines amongst mutated rice population of the variety swarna induced by the sodium Azide. 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 M generation. In M2 generation the selected genotypes show differences for disease index, only 12 lines shows resistance in M2 generation in field condition and in humidity chamber condition only 8 lines shows resistance. The number of resistance lines were decreases in humidity condition. Non significant variation in morphological expression and in yield attributes among the selected mutants was also reported. Thus, these 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.

Keywords: Screening, sheath blight, chemical, *Oryza sativa* L.

Introduction

Rhizoctonia solani Kuhn [Teleomorph: *Thanatephorus cucumeris* (Frank) Donk] is most widely distributed and destructive pathogen of rice causing sheath blight. It is prevalent in almost all rice growing areas of the world (Dasgupta, 1992) [2] as well as India (Reddy and Reddy, 1986) [14] and has become a major constraint to rice production during the last two decades (Kobayashi, 1997). Due to its wider host range (Roy, 1973; Tsai, 1974; Kozoka, 1975) [15, 16], it is very difficult to manage through other disease management methods, except resistance breeding. But, the complex genetic nature of resistance to sheath blight and genetic variability of the pathogen increases the difficulty in developing resistant host genotypes, as well as in effectively deploying available tolerant cultivars. Unfortunately, at present, there is no known rice variety which is either immune or possesses high degree of resistance to sheath blight disease in the eastern part of India, only moderate or low level of resistance is present (Dwivedi, 2004) [3]. Mutation would be better option for creating variability and to develop resistant genotypes against sheath blight disease of rice. Mutation breeding is one of the most effective ways of inducing genetic variability and creating new mutant lines with desirable traits. Induced mutations have played a vital role for the improvement of rice by developing a large number of semi-dwarf and high yielding varieties around the world.

The Swarna is a popular rice variety in India but it is highly susceptible to sheath blight disease (*R. solani*). Completely resistant sources to rice sheath blight are yet to be identified from cultivated rice genotypes (Groth and Lee, 2003) [5]. Induced mutation breeding may play an important role in identifying individual mutants for rice germplasm improvement (Hu and Rutger, 1992) [8]. There are the reports of improvement of different rice genotypes for qualitative (leaf colour, reducing plant height, early maturation period, starch content etc.) and quantitative traits (disease resistance) (Alias *et al.*, 1988; Hadzim *et al.*, 1988) [1, 6]. Thus, during present investigation we have tried to generate sheath blight resistant genotypes amongst mutated population of rice var. Pusa Basmati-1 that may be effectively used in developing sheath blight resistant rice genotypes in future.

Materials and Methods

Plant materials: The seeds of rice variety Swarna were collected from Agricultural Research Farm, Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad, India. Chemical mutagen sodium azide was used as a mutagen in this experiment. The 0.03% NaN₃ solution were prepared and pH adjusted with ortho-phosphoric acid. The mutated seeds were grown in nursery and later on healthy seedlings were transplanted in main fields at 21 days 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 10,00 plant population, consisting both mutant and control lines and their seeds were harvested separately. In M2 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 (i.e. M3 and M4). Control lines of Swarna (non-mutated) were grown along with the mutated lines for resistance screening.

Fungal isolate and inoculum 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°C under continuous light. Mycelial bits or immature sclerotia taken from 7-day-old culture were cut and used as inoculum.

Morphological observation

Observations on plant height, number of tillers were recorded on individual plants of M1 and five plants of M2 generation for screening of potential yielding genotypes.

Experimental design and statistics

The experimental design was completely randomized design with four replicates. Mean and standard error were calculated from the data obtained. Data were analyzed following two way analysis of variance using GENSTAT (8TH edition) statistical software package. Where significant F value were obtained, differences between means were separated using Student Newman Keuls test (Alika 2006).

Results

Table 1: Shows the field screening data of selected mutants rice genotypes of Swarna showing low Disease index in M1 generation

Genotypes	Disease index	Visual rating	Plant height(cm)	Tillers/hill	Genotypes	Disease index	Visual rating	Plant height(cm)	Tillers/hill
L1	4.32	4.08	98	12	L25	3.69	2.19	104	7
L2	2.60	3.34	108	14	L26	3.68	3.44	97	8
L3	3.54	3.48	100	18	L27	3.68	2.19	103	8
L4	4.69	4.92	99	06	L28	5.40	4.42	98	13
L5	1.50	1.32	105	11	L29	1.22	1.67	105	14
L6	3.13	3.49	100	16	L30	3.27	3.34	89	6
L7	4.83	4.58	105	5	L31	4.53	3.59	97	7
L8	2.69	1.35	107	7	L32	1.78	1.60	90	12
L9	3.16	2.24	101	6	L33	4.72	3.48	98	12
L10	1.23	1.62	112	3	L34	2.46	1.98	100	11
L11	4.62	3.38	104	11	L35	4.23	3.54	98	10
L12	3.41	3.96	120	7	L36	1.80	1.84	91	6
L13	1.68	1.39	100	12	L37	2.82	2.56	89	10
L14	4.68	4.52	108	12	L38	3.80	4.65	97	6
L15	4.82	3.95	95	7	L39	5.56	4.76	87	8
L16	3.90	3.58	113	12	L40	5.60	4.52	102	6
L17	4.68	3.64	110	6	L41	1.20	1.22	108	6
L18	2.65	1.54	104	4	L42	4.23	3.76	102	11
L19	1.89	1.87	108	5	L43	2.62	1.49	97	7
L20	1.64	1.46	108	8	L44	4.80	4.03	105	4
L21	2.94	2.06	101	7	L45	2.49	1.16	82	10
L22	3.28	2.58	108	7	L46	3.34	3.06	102	8
L23	3.89	3.07	40	13	L47	5.87	4.85	96	10
L24	3.80	3.29	47	7	Control	7.75	7.67	100	10
					CD 5%	0.229	0.204	6.119	0.554
					S.Em ±	0.078	0.069	2.081	0.188
					CV	4.394	4.449	4.216	4.245

A total of 1000 mutants rice plant of swarna, 47 rice mutants were screened with better disease resistance from an M1 population and were advanced to next M₂ generation. Observed disease reaction and phenotypic expression among the mutant population consisting of 1000 plants as compared to the control consisting 25 plants. 47 mutant plants were screened based on the field data for disease resistance and the average plant height and number of tillers per hills were also recorded (Table). The disease index of mutated rice population ranged between 0 to 9 whereas in control

(untreated) it was calculated between 7 to 9 in M1 generations.

The whole plant population were categorized into different classes based on differential disease response and those showing disease index >5 were selected for advancement to next generation (M2). The genotypes showing disease index value zero (0) were discarded because it was assumed that it may be due to disease escape. The panicle of the selected plants were harvested individually, threshed and dried to bring optimum moisture for storage.

Table 2: Shows the screening of M1 selected rice genotypes in M2 generation in field condition:

Genotypes	Disease index	Visual rating	Plant height(cm)	Tillers/hill	Genotypes	Disease index	Visual rating	Plant height(cm)	Tillers/hill
L1	6.23	5.48	94	11	L25	4.49	3.89	105	9
L2	5.60	5.34	100	10	L26	7.48	6.49	96	9
L3	3.64	3.85	105	14	L27	5.98	4.19	108	11
L4	4.79	4.52	95	10	L28	5.40	4.42	94	14
L5	1.70	1.82	103	9	L29	1.82	1.97	103	13
L6	3.23	3.94	110	16	L30	4.77	3.44	89	9
L7	6.38	6.78	106	8	L31	4.13	4.59	97	6
L8	2.69	1.35	105	12	L32	2.48	1.64	98	8
L9	3.86	2.44	98	6	L33	4.76	4.48	98	10
L10	1.43	1.92	102	10	L34	2.45	1.86	112	8
L11	4.82	4.78	100	7	L35	6.63	5.94	96	8
L12	6.45	5.98	107	7	L36	1.81	1.98	95	8
L13	2.58	2.59	108	12	L37	7.84	6.53	94	12
L14	4.87	4.55	103	10	L38	5.80	5.65	100	6
L15	7.42	6.95	98	8	L39	6.56	5.76	82	9
L16	4.93	4.58	111	12	L40	5.60	4.52	103	11
L17	5.48	4.44	97	6	L41	3.20	3.22	98	7
L18	1.65	1.48	103	9	L42	5.23	4.76	106	12
L19	5.99	5.47	102	7	L43	2.62	1.49	98	10
L20	1.84	1.86	107	8	L44	7.80	6.03	111	8
L21	2.24	2.16	101	7	L45	4.49	4.16	87	7
L22	7.48	6.18	104	8	L46	4.74	3.06	98	11
L23	5.49	5.97	87	12	L47	5.87	4.85	102	9
L24	3.38	3.39	47	7	Control	7.95	7.67	102	12
					CD 5%	0.296	0.217	5.632	0.547
					SEm ±	0.089	0.064	2.013	0.163
					CV	4.407	4.328	4.088	4.125

Table 3: Scoring system used to evaluate mutant lines for sheath blight resistance in field condition:

Scale	Description	Disease leaf area %	Genotypes
1	R	lesions limited to lower 20% of plant height	L5,L8,L10,L13,,L18,L20,L21,L29,L32L34,L36,L43 (Total-12 genotypes)
3	MR	20-30% disease	L3,L4,L6,L9,L11,L14,L16,L24,L25,L30,L31,L33,L41,L45,L46 (Total-15 genotypes)
5	MS	31-45% disease	L1,L2,L7,L12,L17,L19,L23,L27,L28, L35,L38.L39,L40,L42,L47 (Total-15 genotypes)
7	S	46-65% disease	L15,L22,L26,L37,L44 (Total-5 genotypes)

R- Resistance, MR-Moderate resistance, MS-Moderate susceptible, S- Susceptible

Screening in M2 generation from M1 selected plants in field condition, four types of genotypes were found on the basis of disease reaction of various mutants lines. Out of 47 mutants genotypes, 12 genotypes resistance, 15 genotypes moderate resistance, 15 genotypes moderate susceptible and 5

genotypes were susceptible in M2 generation. This data was recorded by taking mean value of 5 plant from every row of specific genotype. These identified mutant genotypes could be considered being a potential source for disease resistance against the sheath blight of rice.

Table 4: Shows the Screening of M1 selected rice genotypes in M2 generation in humidified chamber

Genotypes	Disease index	Visual rating	Plant height(cm)	Tillers/hill	Genotypes	Disease index	Visual rating	Plant height(cm)	Tillers/hill
L1	6.73	6.84	94	11	L25	5.69	4.87	105	9
L2	7.60	7.48	100	10	L26	7.47	6.79	96	9
L3	4.41	4.85	105	14	L27	5.96	4.78	108	11
L4	6.69	6.54	95	10	L28	7.48	6.49	94	14
L5	1.77	1.89	103	9	L29	1.88	1.93	103	13
L6	3.20	3.91	110	16	L30	6.74	6.45	89	9
L7	6.78	6.72	106	8	L31	4.17	4.67	97	6
L8	5.89	4.75	105	12	L32	4.47	3.69	98	8
L9	4.80	2.44	98	6	L33	6.76	5.44	98	10
L10	2.43	1.90	102	10	L34	2.48	1.86	112	8
L11	4.84	4.28	100	7	L35	6.63	5.94	96	8
L12	6.47	6.90	107	7	L36	2.85	1.98	95	8
L13	5.68	4.51	108	12	L37	7.85	6.58	94	12
L14	4.57	5.52	103	10	L38	6.83	6.60	100	6
L15	7.48	6.25	98	8	L39	7.56	6.73	82	9
L16	5.98	5.58	111	12	L40	5.63	4.57	103	11
L17	6.47	6.45	97	6	L41	3.78	2.23	98	7
L18	1.55	1.28	103	9	L42	7.56	6.78	106	12
L19	6.95	5.41	102	7	L43	2.78	1.67	98	10

L20	3.85	3.89	107	8	L44	7.80	6.03	111	8
L21	2.84	2.26	101	7	L45	5.42	5.16	87	7
L22	7.58	6.68	104	8	L46	4.74	3.06	98	11
L23	6.49	5.98	87	12	L47	6.88	6.85	102	9
L24	3.37	3.79	47	7	Control	7.65	7.88	100	98

Table 5: Scoring system used to evaluate mutant lines for sheath blight resistance in field condition:

Scale	Description	Disease leaf area %	Genotypes	Total Genotypes
1	R	lesions limited to lower 20% of plant height	L5,L10,L18,L21,L29,L34,L36,L43	8
3	MR	20-30% disease	L3,L6,L9,L11,L14,L20,L24,L31,L32,L41,L46	11
5	MS	31-45% disease	L1,L4,L7,L8,L12,L13,L16,L17,L19,L23,L25,L27,L30,L33,L35,L38,L40,L45,L47	19
7	S	46-65% disease	L2,L15,L22,L26,L28,L37,L39,L42,L44	9

R-resistance, MR-Moderate resistance, MS-Moderate susceptible, S-Susceptible

The 47 selected mutants with low disease index (> 5) in M1 generation were selected for screening in M2 generation in humidity condition. In this screening data the only 8 genotypes were resistance and the number of susceptible genotypes were increases from 5 to 9 as compare to M2 generation in field condition. The moderate resistance were 11 and moderate susceptible were 19 in humidity chamber. In this condition the resistance genotypes decreases and number of susceptible were increases as compare to field condition in M2 generation. Thus these identified mutant genotypes could be considered being a potential source for disease resistance against the sheath blight of rice.

Discussion

These identified mutant genotypes could be considered being a potential source for disease resistance against the sheath blight of rice. Similarly mutation induced lines of variety Mahsuri were released for blast resistance with improved cooking and eating qualities (Hadzim *et al.*, 1988; Hadjim *et al.*, 1994; Faruq *et al.*, 2003) [6, 7, 4]. 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) [17]. The elite US rice cultivar “Katy” having “*Pi-ta*” gene for blast (*Magnaporthe grisea*) resistance was mutated by using chemical (EMS) and physical mutagens (Fast neutrons and Gamma rays) to explore the molecular basis of disease resistance (Jia *et al.*, 2004) [9]. Several researchers have used mutant lines in different crops for disease resistance. Phadvibulya *et al.*, 2009 [13] obtained YVMD (Yellow vein mosaic disease) resistant okra lines advanced up to 7th generation. However, only a small portion of the plants of the mutant lines appeared to be resistant throughout the whole growth duration; others eventually exhibited the yellow vein symptoms. Genetic analysis using 72 molecular markers revealed that 45 resistant accessions were indica type. Three accessions were identified two as aromatic, and one each as temperate japonica and tropical japonica. Breeders could use these findings to choose sheath blight resistant accessions for cultivar improvement (Limeng Jia *et al.*, 2011) [11]. Mosaddeque *et al.*, (2008) [12] 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.

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