International Journal of Agriculture, Environment and Biotechnology
Citation: IJAEB: 9(6): 937-946, December 2016
DOI: 10.5958/2230-732X.2015.00092.3

BIOTECHNOLOGY

Detection of salt gene expression in resistant rice lines to brown spot disease

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Paper No. 523 Received: 14 June 2016 Accepted: 20 December 2016

Abstract

Brown spot is one of the most common and damaging rice diseases and it has been reported to occur in all the rice growing countries. This disease infects the coleoptile, leaves, leaf sheath, panicle branches, glumes, and spikelets. It occurs at all crop stages, but infection is more severe during maximum tillering up to the ripening stages of the crop. In South and Southeast Asia, this disease causes 5% yield loss across all lowland rice production. In this study, 611 rice germplasm lines were evaluated for identification of resistant line against leaf brown spot disease at field level. Among them, 52 lines were resistant, 157 lines were moderately resistant and 408 rice lines were susceptible. In gene expression analysis, strong expression of SalT gene linked with Abscisic acid (ABA) signalling pathway was found in resistant and moderately resistant rice lines. Thus selected resistant rice lines will be useful in breeding programme to improve rice cultivars against brown spot disease.

Highlights

- 611 rice germplasm lines were evaluated against leaf brown spot disease at field level. Among them, 52 lines were resistant, 157 lines were moderately resistant and 408 rice lines were susceptible.
- In gene expression analysis, strong expression of SalT gene linked with Abscisic acid (ABA) signalling pathway was found in resistant and moderately resistant rice lines.
- Thus selected resistant rice lines will be useful in breeding programme to improve rice cultivars against brown spot disease.

Keywords: Brown spot, gene expression, rice germplasms, salt, abscisic acid, pathogen-related genes

Brown spot is one of the most common and damaging rice diseases. It is a fungal disease caused by *Cochliobolus miyabeanus* (*Bipolaris oryzae*, *Drechslera oryzae*, *Helminthosporium oryzae*). This fungus is classified in the subdivision Deuteromycotina (imperfect fungi), class Deuteromycetes, order Moniliales, and family Dematiaceae. It can survive in the seed for more than 4 years. This disease has been reported to occur in all the rice growing countries including Japan, China, Burma, Sri Lanka, Bangladesh, Iran, Africa, South America, Russia, North America, Philippines, Saudi Arabia, Australia, Malaya and Thailand (Khalili *et al*. 2012).

The symptoms of brown spot mainly appear on the leaves at the early stage. Leaf lesions reduces nutrient absorption and photosynthetic area, which result in the decrease of tillering nodes. And also, it infects other parts of the plants like the coleoptile, leaf sheath, panicle branches, glumes, and spikelets. This disease causes severe damage under the conditions of cool summer and nitrogen deficiency. High humidity (>92.5%), leaf wetness and temperature (24-30°C) are favorable conditions for disease development (Picco and Rodolfi 2002). Wind and rainfall can spread the spores to other organs of the same individual and other plants.

Losses can be severe if weather and field conditions are favorable for disease spreading. Major sources of brown spot in the field are the infected seed, which give rise to infected seedlings, infected rice debris and weeds.

Brown spot occurs at all crop stages, but infection is more severe during maximum tillering up to the ripening stages of the crop. It causes 5% yield loss across all lowland rice production in South and Southeast Asia and severely infected field can have as high as 45% yield loss. Heavily infected seeds cause seedling blight and lead to 10–58% seedling mortality. It also affects the quality and the number of grains per panicle, and reduces the kernel weight. Therefore, Brown spot disease should be considered as a major factor in rice cultivating areas since it has contributed to the Great Bengal Famine in 1943 (Padmanabhan 1973). To manage the loss of brown spot disease, rice farmers are advised to use different types of chemicals.

In this case, application of fungicides for the control of brown spot is the most effective management option, but under high disease pressure effective control is not achieved. Additionally, use of chemicals is known to cause undesirable effects such as residual toxicity, development of pathogen resistance to fungicides, environmental pollution, health hazards to humans and animals and increased expenditure for plant protection. Besides, plant pathologists focus their attention to develop environmentally safe, long-lasting and effective biocontrol methods for the management of this diseases. However, inconsistant effect of biocontrol agents at field level makes the farmers unhappy. In this context, use of host resistant rice varieties is the most effective and economical way to control disease (Delteil *et al*., 2010).

Host resistant plants encounter a vast array of pathogenic microorganisms (fungi, oomycetes, bacteria, viruses and nematodes) at various level by inducing constitutive plant hormones, such as salicylic acid (SA), jasmonates (JA) and ethylene (ET), abscisic acid (ABA), auxin, gibberellic acid (GA), cytokinin (CK) and brassinosteroids (BR). SA signalling pathway mediates chemical-induced resistance to multiple pathogens, including Magnaporthe oryzae and *Xanthomonas oryzae* pv. *oryzae* and its crosstalk with other plant harmones plays a crucial role in the defence responses of rice (De Vleesschauwer *et al*. 2013; Takatsuji and Jiang 2014; Kazan and Manners 2011). JA mediates responses to several biotic and abiotic stresses (Zheng *et al*., 2015; Peleg and Blumwald 2011). JA level in rice increase significantly in stressed condition however under heat stress JA level decreases and JA biosynthetic genes downregulated, implying that different JA regulating mechanisms may function under different abiotic stresses. Molecular assays revealed that the expression levels of several pathogenesis related (*PR*) genes, are upregulated in rice upon JA treatment confirming that JA functions as an important signaling molecule in pathogen resistance(Du *et al*. 2013; Yang *et al*. 2013). JA signaling and SA signaling synergistically augments against pathogens defense in rice (Tong *et al*. 2012). ABA plays a negative role by antagonizing JA biosynthesis and the signaling pathway, thus making the rice plants more susceptible to the nematode (Nahar *et al*. 2011; Nahar *et al*. 2012).

Association of some plant microbes such as Plant Growth Promoting Bacteria (PGPB) with rice plant root system enhances salt tolerant ability as the plant part affected first by salinity, so it serve as a useful tool for alleviating salinity stress. Antioxidative defense mechanism is effective in providing tolerance to salt stress in rice plant (Jha *et al.,* 2014; Thamodharan *et al*., 2014). The objective of our was to identify tolerant rice germplasms lines against brown spot disease from 611 rice lines at natural condition and to find the role of genes linked with SA, JA and ABA pathway in tolerant lines.

Materials and Methods

Plant materials

In this study, a number of 611 rice germplasms were used to select tolerant line against brown spot disease (Table 1).

Disease scoring

Following the seed sowing of 611 rice germplasms directly in soil, the field was irrigated. In two lines, rice seedlings of each germplasm were maintained at 10 × 15cm distance and each line consisted of 20 plants. The disease scoring was done at maximum tillering stage based on IRRI's standard evaluation scale (SES scale). Three leaves per plant were taken for disease scoring. Identification of tolerant lines

to brown spot was done in rain-fed upland areas in CRRI, Odisha during summer season-2014.

RNA extraction and reverse-transcriptase (RT)-PCR

Gene expression analysis was done in rice lines which characterized as tolerant, moderaely tolerant and susceptible to brown spot. For RNA extraction, 100mg leaf tissue were ground in liquid nitrogen using mortar and pestle and total RNA was extracted using TRIzol according to manufacturer's instructions. The RNA pellet was dissolved in 50µl RNase free water and stored at –20ºC. The quality and quantity of total RNA were analysed by gel visualization in a 1.5% Tris–boric–EDTA–agarose gel stained with ethidium bromide and by spectrophotometric analysis. cDNA sysnthesis was done using SuperScriptTM III Reverse Transcriptase according to manufacturer's protocol (Invitrogen, California, USA) in a reaction mixture containing 50–75 ng RNA with the final volume completed to 20µL using RNase free water. PCR amplification for cDNA was done at 56ºC using primer sequences of OsPR1b and PR10/PB21 for SA pathway, JIOsPR10 for JA pathway, OsMPK5 and SalT for ABA pathway (Vleesschauwer *et al*. 2010)**.** The primer sequence of ACTIN1 was used as a loading control.

Results and Discussion

Disease scoring for Identification of resistant line against leaf brown spot disease

Historically, leaf brown spot disease of rice is of great importance and has terified through Great Bengal Famine during 1942 (Padmanabhan 1973). In India, brown spot occurs every year on most on the cultivated rice varieties particularly the disease is more severe in dry/direct seeded rice in the states of Bihar, Chhatisgarh, Madhya Pradesh, Orissa, Assam, Jharkhand and West Bengal. Presently, there are very limited strategies for the control of brown spot and cultivars with an adequate level of resistance are not available. It can cause enormous loss in grain yield (up to 90%) particularly when leaf spotting phase assumes epiphytotic proportions.

In this study, we scored the disease incidence of leaf brown spot which occurred naturally on 611 germplasm lines in the range of resistance to susceptible (SES score 3-9) during the summer season-2014. Among them, 52 germplasm lines were resistant (score 3), 157 germplasm lines were moderately resistant (score 5), 408 germplasm lines were susceptible (score 7) (Fig.1A, B; Table 1). In this selection, we noted that out of 611 rice lines, most of the lines showed susceptible reaction against brown spot as compared to lines which showed resistant reaction. Generally, brown leaf spot disease is associated with rice growing under conditions of some form of nutrients or other stresses i.e., when Phosphorus (P) level is low plants are less susceptible and the disease incidence becomes more severe when Nitrogen (N) is deficient after the middle of the growth period. Seedling blight and leaf spot symptoms have been shown to increase under condition of deficiency and excess of NH+4-N. In India, foliar application of some specific micronutrients such as $FeSO_4$ and $CuSO_4$ were also shown to influence the incidence of brown leaf spot and low levels of disease (Phelps and Shand 1995). In this study, there was no significant micronutrients influence on rice germplasm lines because intensity of brown spot disease were observed at various level on rice germplasms lines (SES score 3-7). It indicates that the disease intensity is associated not only with nutrient deficiency but also with variation of rice genotypes. Here, the rice lines which categorized as tolerant and moderately tolerant to brown spot disease have also accounted for drought tolerance (data not given).

Reverse-transcriptase (RT)-PCR

In gene expression analysis, expression of OsPR1b, PR10/PB21, JIOsPR10, OsMPK5 and SalT) genes were observed at different level in rice lines which were scored to 3-7 scale against brown spot disease (Fig. 2A, B). Strong expression of OsPR1b gene was detected only in moderately tolerant and susceptible lines but not in tolerant lines. In case of PR10/PB21, the gene expression was stronger in susceptible line than in tolerant and moderately tolerant lines. Increased level of SA in plants either due to pathogen infection or exogenous application involves in expression of PR genes and enhances resistance to a broad range of pathogen (Grant and Lamb 2006). Here, expression of PR genes was not significant in the disease control because its strong gene expression was found only in moderately tolerant and susceptible lines but not in tolerant lines. Also,

Table 1: Rice germplasm lines and their level of disease reason to leaf brown spot under field condition

Rice line	SES	Rice line	SES	Rice line	SES	Rice line	SES	Rice line	SES	Rice line	SES
AC 36308	7	ARC 6612	7	ARC 10618	7	AC 43785	5	ARC 7093	7	ARC 10690	7
AC 36763	5	ARC 6630	7	ARC 10619	7	AC 43801	5	ARC 7094	7	ARC 10695	7
AC 38392	5	ARC 6631	7	ARC 10625	7	AC 43809	7	ARC-7104	7	ARC 10696	5
AC 38407	7	ARC 6633	7	ARC 10632	7	AC 43852	7	ARC-7105	7	ARC 10698	5
AC 38422	5	ARC 6634	7	ARC 10635	7	AC 43858	7	ARC-7106	7	ARC 10699	7
AC 38448	5	ARC 6647	7	ARC 10636	7	AC 43872	5	ARC-7107	7	ARC 10700	7
AC 38465	5	ARC 6648	7	ARC 10640	7	AC 43876	7	ARC-7109	7	ARC 10702	5
AC 38474	5	ARC 7008	7	ARC 10645	7	AC 43884	5	ARC-7110	7	ARC 10703	5
AC 38517	5	ARC 7009	7	ARC 10646	7	AC 43891	5	ARC-7118	7	ARC 10714	5
AC 38556	7	ARC 7024	7	ARC 10647	7	AC 43901	7	ARC-7119	7	ARC 10744	5
AC 38570	5	ARC 7028	7	ARC 10650	7	AC 43903	5	ARC-7120	7	ARC 10753	5
AC 38571	5	ARC 7029	7	ARC 10651	7	AC 43909	7	ARC-7124	7	ARC 10776	5
AC 38659	5	ARC 7032	7	ARC 10653	7	AC 43917	7	ARC-7126	7	ARC 10790	3
AC 38684	7	ARC 7038	7	ARC 10654	7	AC 43956	5	ARC-7130	7	ARC 10797	3
AC 38732	7	ARC 7039	7	ARC 10655	7	AC 44025	5	ARC-7133	7	ARC 10827	5
AC 38758	5	ARC 7044	7	ARC 10656	5	AC 43091	5	ARC-7134	7	ARC 10834	5
AC 38892	3	ARC 7050	7	ARC 10657	7	AC40452	3	ARC-7147	7	ARC 10838	5
AC 39416	7	ARC 7054	7	ARC 10661	7	AC41685	5	ARC-7150	7	ARC 10840	5
AC 42374	7	ARC 7071	7	ARC 10663	7	Azucena	3	ARC-7204	7	ARC 10841	5
AC 42375	5	ARC 7074	7	ARC 10664	7	AC 41299	3	ARC-7210	7	ARC 10844	3
AC 42376	7	ARC 7075	7	ARC 10666	7	AC 43633	5	ARC-7211	7	ARC 10843	3
AC 42379	7	ARC 7076	7	ARC 10667	7	IR72	5	ARC-7218	7	ARC 10845	3
AC 42380	7	ARC 7080	7	ARC 10669	7	Sabita	7	ARC-7219	7	ARC 10846	3
AC 42381	5	ARC 7083	7	ARC 10670	7	ARC 5751	7	ARC-7220	7	ARC 10847	3
AC 43694	5	ARC 7084	7	ARC 10672	7	ARC 5757	7	ARC-7225	7	ARC 10851	3
AC 43748	5	ARC 7085	7	ARC 10682	7	ARC 5758	7	ARC-7234	7	ARC 10857	3
AC 43765	5	ARC 7086	7	ARC 10689	7	ARC 5759	7	ARC-7235	7	ARC 10873	3
ARC 5764	7	ARC-7243	7	ARC 10878	3	ARC 5840	5	ARC-7341	7	CO 51	7
ARC 5767	5	ARC-7244	7	AC 35004	3	ARC 5841	5	ARC-7342	7	TJ 1	7
ARC 5768	5	ARC-7248	7	AC 35633	3	ARC 5842	5	ARC-7343	7	TJ 2	7
ARC 5769	5	ARC-7250	7	AC 37938	3	ARC 7135	5	ARC-7408	5	AC 11322	7
ARC 5772	5	ARC-7255	7	AC 4148	7	ARC 5846	5	ARC-7410	5	AC 34245	7
ARC 5774	5	ARC-7259	7	AC 41620	7	ARC 5848	5	ARC-7412	5	SAP 1	7
ARC 5776	5	ARC-7263	7	AC 43967	7	ARC 5850	5	ARC-7414	5	SAP 2	7
ARC 5778	5	ARC-7268	7	AC 44012	7	ARC 5906	5	ARC-7415	5	SAP 3	7
ARC 5779	5	ARC-7269	7	AC 44013	5	ARC 5911	5	ARC-7416	5	SAP 4	7

ARC 5780	5	ARC-7270	7	AC 44014	7	ARC 5912	5	ARC-7432	5	SAP 5	7
ARC 781	7	ARC-7271	7	AC 44018	5	ARC 5913	5	ARC-10882	5	SAP 6	7
ARC 5783	7	ARC-7275	5	AC 44052	7	ARC 5914	3	ARC-10884	5	SAP 7	7
ARC 784	7	ARC-7279	5	AC 44071	7	AC 36308	5	ARC-10902	5	SAP 9	7
ARC 5786	7	ARC-7282	5	AC 44087	7	AC 36763	5	ARC-10913	5	SAP 10	7
ARC 5787	7	ARC-7283	5	AC 44099	7	ARC 5922	5	ARC-10922	5	SAP 11	7
ARC 5791	7	ARC-7284	7	AC 44100	7	ARC 5923	5	ARC-10925	5	SAP 12	7
ARC 5793	5	ARC-7308	5	ANJALI	5	ARC 5927	5	ARC-10926	5	SAP 13	7
ARC 5795	5	ARC-7312	5	F5-444-2-2-5	7	ARC 5928	5	ARC-10927	5	SAP 14	7
ARC 5797	5	ARC-10691	5	F5-444-3-1-1	7	ARC 5937	3	ARC-10934	5	SAP 15	7
ARC 799	5	ARC-7317	5	F5-444-3-1-1-2	7	ARC 5940	5	ARC-10937	5	SAP 17	7
ARC 5801	5	ARC-7318	5	F5-444-3-1-1-3	5	ARC 5944	5	ARC-10940	3	SAP 18	5
ARC 5813	3	ARC-7320	5	Fortuna	7	ARC 5946	5	ARC-10944	7	SAP 19	7
ARC 5823	7	ARC-7323	5	Piyari	7	ARC 5951	5	ARC-10946	7	SAP 20	7
ARC 5828	7	ARC-7328	5	CR Dhan 201	7	ARC 5956	5	ARC-10954	7	SAP 21	5
ARC 5832	5	ARC-7329	5	CR Dhan 204	7	ARC 5965	5	ARC-10957	7	SAP 22	7
ARC 5833	5	ARC-7335	5	CR Dhan 202	7	ARC 5971	5	ARC-10958	7	SAP 23	7
ARC 5839	5	ARC-7339	7	CR Dhan 601	5	ARC 5972	5	ARC-10960	7	SAP 24	7
ARC 5973	5	ARC 10023	7	SAP 26	7	ARC 6039	3	ARC 10245	7	AG 13	7
ARC 5975	5	ARC 10059	7	SAP 27	7	ARC 6040	3	ARC 10248	7	AG 14	7
ARC 5976	5	ARC 10061	7	SAP 29	7	ARC 6043	3	ARC 10254	7	AG 15	7
ARC 5977	5	ARC 10062	7	SAP 30	7	ARC 6053	3	ARC 10258	7	AG 16	7
ARC 5982	5	ARC 10088	7	SAP 31	7	ARC 6058	3	ARC 10259	7	AG 17	7
ARC 5985	5	ARC 10090	7	SAP 32	7	ARC 6060	3	ARC 10260	7	AG 18	7
ARC 5993	3	ARC 10118	7	SAP 33	7	ARC 6076	3	ARC 10262	7	AG 19	7
ARC 5994	5	ARC 10120	7	SAP 34	7	ARC 6082	3	ARC 10264	7	AG 20	7

ARC 5995	5	ARC 10148	7	SAP 35	7	ARC 6088	3	ARC 10266	7	AG 21	7
ARC 5999	5	ARC 10152	7	SAP 37	7	ARC 6091	3	ARC 10269	7	AG 22	7
ARC 6001	5	ARC 10157	7	SAP 38	7	ARC 6093	3	ARC 10270	7	AG 23	7
ARC 6004	5	ARC 10168	7	SAP 39	7	ARC 6096	3	ARC 10271	7	AG 24	7
ARC 6005	5	ARC 10187	7	SAP 40	7	ARC 6097	3	ARC 10281	7	AG 25	7
ARC 6006	5	ARC 10194	7	SAP 41	7	ARC 6099	3	ARC 10276	7	AG 26	7
ARC 6007	5	ARC 10197	7	SAP 42	7	ARC 6101	3	ARC 10287	7	AG 27	7
ARC 6009	3	ARC 10162	7	SAP 43	7	ARC 6102	3	ARC 10304	7	AG 28	7
ARC 6017	3	ARC 10156	7	AG 1	7	ARC 6110	3	ARC 10317	7	AG 29	7
ARC 6018	3	ARC 10171	7	AG 2	7	ARC 6115	3	ARC 10321	7	AG 30	7
ARC 6023	3	ARC 10191	5	AG 3	7	ARC 6117	3	ARC 10333	7	AG 31	7
ARC 6025	3	ARC 10178	7	AG 4	5	ARC 6123	3	ARC 10342	7	AG 32	7
ARC 6026	5	ARC 10220	7	AG 5	5	ARC 6127	7	ARC 10344	7	AG 33	7
ARC 6027	5	ARC 10222	7	AG 6	5	ARC 6130	7	ARC 10363	7	AG 34	7
ARC 6029	5	ARC 10223	7	AG 8	7	ARC 6135	7	ARC 10392	7	AG 35	7
ARC 6033	5	ARC 10229	7	AG 9	7	ARC 6139	5	ARC 10393	7	AG 36	7
ARC 6035	3	ARC 10235	7	AG 10	7	ARC 6143	7	ARC 10399	7	AG 37	7
ARC 6037	3	ARC 10243	7	AG 11	7	ARC 6144	7	ARC 10405	7	AG 38	7
ARC 6038	3	ARC 10244	7	AG 12	7	ARC 6147	7	ARC 10416	7	AG 39	7
ARC 6153	7	ARC 10419	7	AG 40	7	ARC 6567	5	ARC 10571	7	AG 67	5
ARC 6154	7	ARC 10424	7	AG 41	7	ARC 6571	7	ARC 10595	7	AG 68	7
ARC 6156	7	ARC 10426	7	AG 42	7	ARC 6581	7	ARC 10599	7	AG 69	5
ARC 6161	7	ARC 10438	7	AG 43	7	ARC 6582	7	ARC 10600	7	AG 70	5
ARC 6170	7	ARC 10444	7	AG 44	7	ARC 6588	7	ARC 10601	7	AG 71	5
ARC 6171	7	ARC 10468	7	AG 45	5	ARC 6591	7	ARC 10603	7	AG 72	7

ARC 6172	7	ARC 10491	7	AG 46	7	ARC 6592	7	ARC 10604	7	AG 73	7
ARC 6173	7	ARC 10421	7	AG 47	7	ARC 6595	7	ARC 10606	7	AG 74	7
ARC 6174	7	ARC 10446	7	AG 48	7	ARC 6596	7	ARC 10608	7	AG 76	7
ARC 6175	7	ARC 10450	7	AG 49	7	ARC 6598	7	ARC 10609	7	AG 77	5
ARC 6180	7	ARC 10451	7	AG 50	7	ARC 6249	7	ARC 10519	7	AG 64	7
ARC 6183	7	ARC 10455	7	AG 51	7	ARC 6555	7	ARC 10525	7	AG 65	7
ARC 6202	7	ARC 10457	7	AG 52	7	ARC 6557	7	ARC 10527	7	AG 66	7
ARC 6206	7	ARC 10459	7	AG 53	7	ARC 6558	7	ARC 10544	7	AG 78	5
ARC 6218	7	ARC 10471	7	AG 54	7	ARC 6562	5	ARC 10563	7	AG 79	5
ARC 6220	7	ARC 10481	7	AG 55	7	ARC 6603	7	ARC 10610	7	AG 80	3
ARC 6221	5	ARC 10487	5	AG 56	7	ARC 6605	7	ARC 10611	7	ARC 10625	7
ARC 6225	7	ARC 10493	7	AG 57	7	ARC 6606	7	ARC 10612	7		
ARC 6230	7	ARC 10504	7	AG 58	7	ARC 6608	7	ARC 10614	5		
ARC 6234	7	ARC 10505	7	AG 59	7	ARC 6609	7	ARC 10616	7		
ARC 6235	7	ARC 10508	7	AG 60	7	ARC 6611	7	ARC 10617	7		
ARC 6237	7	ARC 10518	7	AG 61	7	AG 62	7	AG 63	7		

SES score: 1-highly resistant; 3-resistant; 5-moderately resistant; 7-susceptible; 9-highly susceptible

in a previous study, expression of OsPR1b and PB21 in rice line CO39 has been reported in the NPR1 (non-pathogen related)-dependent SA pathway in response to BTH (benzothiadiazole) (Shimono *et al*. 2007). Moreover, expression of JA responsive gene JIOsPR10 was found strongly in tolerant, moderately tolerant and susceptible rice lines. Contrasting to SA, JA is associated with defence against necrotrophic pathogens and herbivorous insects. It increases locally in response to pathogen infection or tissue damage or exogenous application of JA and induces the expression of defence related genes. In this study, expression of JIOsPR10 gene in all rice lines regardless of disease intensity indicates that JA is not a major signal for the activation of defense against brown spot (Vleesschauwer *et al*., 2010).

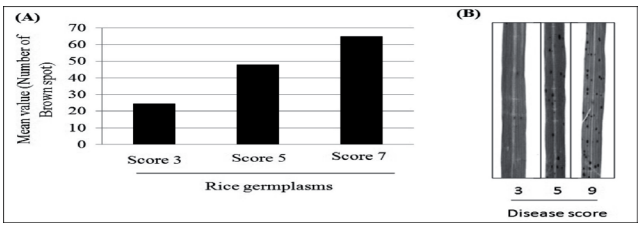

Fig. 1: (A) Graph representing the level of disease incidence of brown spot on rice lines. **(B)** depicting the number of disease spots on rice leaves at different level

However, expression of genes linked with SA and JA signalling pathway in these lines indicates the chance of cross talk between the SA and JA

signalling pathways. Furthermore, it has been reported that this cross talk is mediated by fatty acid-derived signals and/or glutaredoxin genes (Ndamukong *et al*., 2007). Additionally, not only interaction of SA and JA in disease control, there is overwhelming evidence that ABA modulates ethylene (ET) signalling (Tanaka *et al*., 2005) via MAPK (mitogen activated protein kinase) gene OsMPK5 (Vleesschauwer *et al*. 2010) and antagonizes pathogen. MAPKs are activated by fungal elicitors (Suzuki and Shinshi 1995), SA (Zhang and Klessig 1997), JA (Seo *et al*., 1999), and ABA (Heimovaara *et al*., 2000). Here, expression of OsMAPK5 gene was not strong in all lines (tolerant, moderately tolerant and susceptible lines, but suppression of OsMAPK5 gene expression has resulted in the constitutive expression of PR genes and enhanced resistance to fungal and bacterial pathogens (Xiong and Yang 2003).

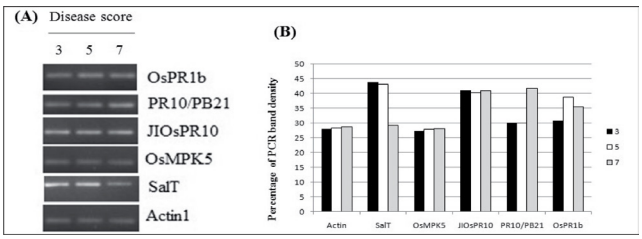

Fig. 2: (A) representing the expression of genes linked with salicyclic acid (SA) and Jasmonic acid (JA) and abscisic acid (ABA) signaling pathways in rice lines with brown spot disease score 3, 5 and 7. **(B)** depicting gene expression level in rice lines with disease score 3, 5 and 7 in percentage. SES Score: 3- Tolerant; 5- Moderately tolerant; 7-Susceptible

Hence, this study coincides with the previous report (Hofmann 2008) that SA and ABA signalling mutually antagonistic to each other. Even though, suppression of OsMAPK5 gene expression and induces of PR gene expression in rice lines are not associated with the disease control in rice lines as expression of PR genes was detected in rice lines which categorized as susceptible also. Meanwhile, one of ABA responsive genes SalT expressed strongly in resistant and moderately resistant rice lines as compared to OsMAPK5 gene expression. SalT is a jacalin-like lectin protein and it is one of the most prominent proteins induced by high salt conditions in roots (Clas 1990).

Also, the accumulation of SalT mRNA is reported in rice lines having *Sub1* locus for submergence tolerance under water stress condition (Fukao *et al*. 2011), because ABA is a key signalling molecule that coordinates water balance, expression of stress-inducible genes and metabolic adjustant under water deficit condition. Under water deficit condition, the process of stomatal closing by ABA is one of the water saving mechanisms in drought tolerant rice lines and this process also supports the plants through prevention of entry of plant pathogens into plants (Melotto *et al*. 2006). In the present study also, mild disease incidence of brown spot (score 3) was observed in rice lines which associated with drought tolerance (data not shown).

In this case, SalT expression in resistant and moderately resistant lines indicates that its expression is associated with water stress but not with the disease incidence because the plant hormone ABA is involved in adaptation to environmental stresses. And also, it is reported that ABA negatively regulates disease resistance (Mauch-Mani and Mauch 2005). Furthermore, tolerant lines may involve in stomatal closing process under water deficit condition and this process may lead to avoid the entry of pathogen into rice plants. In this way, drought tolerant lines may protect plants from the infestation of pathogens in rain-fed upland areas. Thus, Salt gene expression indirectly associated with disease control in drought tolerant rice lines.

Conclusion

In conclusion, among 611 germplasm lines, 52 lines were identified as resistant to Brown spot under natural disease incidence condition at field level. Expression of PR genes related to SA signalling pathway was found for not associated with disease suppression in lines categorized as susceptible. At the same time, limitation in expression of PR genes was found in tolerant rice lines. But, we found no difference in disease scoring level of rice lines by suppression of gene related to JA signalling pathway. Similarly, insignificant expression of OsMAPK5 gene related to ABA pathway yielded no difference in disease scoring level of rice lines. However, expression of SalT gene linked with ABA pathway is associated with the difference of disease scoring level in tolerant lines and also in moderately tolerant lines. Perhaps, SalT gene expression might associate with stomatal closing process via ABA pathway and suppressed the pathogen entry into plants.

In this study, JIOsPR10 gene expression (JA) is not antagonistic to SalT gene expression as well as OsPR1b (SA) but SalT gene expression is antagonistic to PR10/PB21 (SA). This study suggests that rice lines associated with drought tolerance can reduce the intensity of brown spot disease via ABA pathway and it will be appropriate to select drought tolerant lines to increase the rice production in rain-fed lowland and upland areas. Thus selected resistant rice lines will be useful source in breeding programme to improve rice cultivars against brown spot disease.

Acknowledgements

We sincerely thank the Department of Biotechnology (DBT), INDIA for financial support and the Director, CRRI for providing facilities to carry out this study.

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