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Biological Activity of Plant Tuber (RVL1) and Fungal Lectin (SRL1) against *Sclerotium rolfsii*

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Efficacy of *E.coli* expressed *R. vivipara* lectin (RVL1) and *S. rolfsii* lectin (SRL1) was studied against *S. rolfsii*. Protein Expression checked through SDS-PAGE, showed respectively an extra protein band corresponding to molecular weight of 28.2 kDa for RVL1 and 19.5 kDa for SRL1. *E. coli* expressed RVL1 and SRL1 showed agglutination with trypsinised rabbit erythrocytes indicating the presence of lectin. Compared to bacterial control Bl21 and without protein white, smooth, sparse radiating mycelial growth of *S.rolfsii* was obseved in different concentrations of lectins and the rate of formation of sclerotial bodies increased with increasing concentration of both the lectins. SRL1-treated PDA plates produced bigger, heavier and more number of sclerotial bodies with increasing concentrations of SRL1. Compared to SRL1 treated plates, RVL1 treated plates produced small, lighter and less number of sclerotial bodies. Further, these sclerotial bodies failed to germinate on the medium. This indicates that lectins play a major role in early formation sclerotilal bodies of lectins but inhibited their germination.

Key words: Plant *Remusatia vivipara* lectin, fungal *Sclerotium rolfsii* lectin,

Lectins are proteins of non-immune origin that specifically and reversibly bind to carbohydrates. The physiological functions of lectins are very diverse[1]. A common theme that emerges from the described functions of many plant and animal lectins pertain to their role as effectors or regulators in the interaction with other organisms, be it symbiosis or defence. Many lectins have been identified and isolated from fruiting body-forming fungi of Basidiomycota and Ascomycota phyla [2,1]. Some of these lectins have been characterized biochemically and structurally. Lectins from plants have been extensively studied. Many plant lectins have been demonstrated to interact with carbohydrates of other organisms either in symbiosis or in defence processes [5-7]. One of the most important functions of plant lectins is their role as effectors in the defence against parasites and herbivores [8-9]. It has been shown that plant lectins possess fungicidal, insecticidal and nematicidal properties and are also toxic to higher animals [5, 10]. The expression of these lectins is regulated both temporally and spatially. It can be tissue-specific or systemic, constitutive or induced upon stress, herbivory or pathogen infection[11]. Fungivory plays a significant role in shaping the structure and function of natural fungal

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communities and represent a strong selective force for the evolution of chemical defence systems in fungi[15]. Accordingly, a wide variety of chemical compounds, either constitutively produced or wound-activated have been identified in fungi[14], and many of these secondary metabolites are believed to have evolved to protect saprophytic fungi from being used as a food source by amoebozoa, nematodes and other invertebrates[16]. On the other hand, it has been shown that proteins are responsible for most of the insecticidal activity of mushrooms[17]. In this work, we show the effect of *E. coli* expressed *Remusatia vivipara* lectin (a plant lectin) and *Sclerotium rolfsii* lectin (a fungal lectin) on the development and germination of a fungus *Sclerotium rolfsii*.

MATERIAL AND METHODS

Expression of RVL1 and SRL1 in E. coli and its isolation

In present study, we selected plant tuber lectin (RVL1) from *Remusatia vivipara* and fungal fruiting body specific lectin (SRL1) from *Sclerotium rolfsii* (both previously characterized). Both the lectin genes were cloned into pET vector (Invitrogen) under the control of the isopropyl-b-D-thiogalactopyranoside (IPTG)-inducible T7-promoter. Cloning of *rvl1* and *srl1* was described previously[19-20]. *E. coli* strain BL21 (DE3) transformants containing *rvl1* and *srl1* were cultivated in LB medium containing 50 µgD mL kanamycin at 37^0C to $OD_{600} = 1$, induced with 1mM IPTG and incubated overnight at 23^0C. Solubility of the recombinant lectins was tested by disrupting the *E. coli* cells in PBS using a sonicator instrument and checking the lectin content in the supernatant after centrifugation (16,000 g for 20 min) by haemagglutination assay and SDS-PAGE.

Agglutination assay

The activity of both *Remusatia vivipara* lectin and *Sclerotium rolfsii* lectin expressed in *E. coli* was determined by haemagglutination assay[21] using trypsinised rabbit erythrocytes. Haemagglutination activity was assayed in an ELISA microtiter plate by the serial two fold dilution technique of [22] with some modifications. Phosphate buffer saline (PBS; 50 µl of 150 mM NaCl) was added to 12 wells in the first row, and lectin sample (50 µl) was added only to the first well of the assay plate. The contents of the first well (100 µl) were mixed well and 50 µl of it was transferred to the second well and the process was repeated serially for the remaining wells. Thus, the lectin extract was serially two fold diluted to which 50µl of trypsinized erythrocytes suspension was added and gently mixed on a rotary shaker. After incubation for 1 hr at 37°C, the plates were visually examined for haemagglutination. The highest dilution of the extract causing visible haemagglutination was regarded as the 'titre'. The protein content in the highest dilution causing visible agglutination was referred to as 'one unit' of haemagglutination activity. It is otherwise expressed as MCA (minimum concentration of protein required for agglutination). The specific haemagglutination activity was expressed as units of activity per mg of protein.

SDS-PAGE for *E. coli* expressed RVL1and SRL1

Crude protein (50 µl containing 0.4 mg) was mixed with 2 X SDS gel loading buffer (50 µl). The mixture was heated at 98°C for 5 min and thoroughly mixed. Protein (20 µl) was loaded on 12 per cent polyacrylamide gel along with suitable control protein. The gel was run at 60 V for 1 hr and subsequently at 120 V for 3 hr. After staining with commassie brilliant blue, the excess dye was removed by repeated washing every 2-3 hr with de-staining solution till blue colour band appeared. The gel was sealed in polyethylene bag and stored at 4°C.

In vitro Antifungal Assay

Biotoxicity assays of plant (RVL1) and fungal (SRL1) lectins expressed in *E. coli* were performed against *Sclerotium rolfsii* to know the efficacy of lectins in inhibiting the pathogen on Potato dextrose agar using spread plate under *in vitro* condition. The crude proteins of RVL1 and SRL1 with two controls, one bacterial control (BL21) and another one, untreated were tested at different protein quantity 1.2 mg/ml, 2.4 mg/ml, 3.6 mg/ml and 4.8 mg/ml. *S. rolfsii* was isolated from collar rot-infected tomato plants using standard tissue isolation method. Pure culture was maintained on PDA slants for further use. To study the efficacy of lectins, 2 days old culture was used for this study. Further sclerotial bodies were collected from the tested treatments after 12 days, and were observed for the colour, size, the number of sclerotial bodies and weight of 50 sclerotial bodies. Sclerotial bodies developed on PDA containing RVL1 and SRL1 lectins were used for testing germination of sclerotial bodies by plating them on 2% water agar.

RESULTS AND DISCUSSION

Haemagglutination and SDS-PAGE

The gels stained with Commassie brilliant blue showed an extra protein band corresponding to molecular weight of 28.2 kDa of RVL1 and 19.5 kDa for SRL1. Minimum concentration of agglutinin required for agglutination was 1.37 µg and 2.73 µg for SRL1 and RVL1, respectively. The specific activity for SRL1 and RVL1 was found to be 7.29×10^2 and 3.66×10^2, respectively with rabbit erythrocytes. These results were verified with Neekhra et al., and Bhat et al., (ref[19-20]).

Antifungal activity of RVL1 and SRL1

Two days after inoculation of the pathogen on PDA, differences in mycelial growth in different treatments were seen. *Remusatia vivipara* lectin treated plate showed smooth, pure white, sparse radiating mycelium. Similarly, *Sclerotium rolfsii* lectin treated plate showed white, sparse mycelium. But in both the controls, luxurious radiating and aerial growth of the fungus was noticed. Mycelium diameter 90 mm in all lectin treated PDA plates as well as on the PDA plates without lectins (Figure 1), reached on third day. In SRL1 treated plates, it was found that pre-sclerotial bodies formation started on second day of inoculation. On fourth day, white pre-sclerotial bodies formation was seen in all lectin treated plates except that of control. In both control PDA plates, sclerotial bodies started forming on 7th day of inoculation.

On 6th day of inoculation, colour of sclerotial bodies turned to dark brown in PDA plate treated with 3.6 mg/ml and 4.8 mg/ml of *E. coli* protein containing SRL1. Formation of dark brown sclerotial bodies was observed on 7th day of inoculation in PDA plate treated with 1.2 mg/ml and 2.4 mg/ml of *E. coli* protein containing SRL1 and PDA plate treated with 1.2 mg/ml, 2.4 mg/ml, 3.6 mg/ml and 4.2 mg/ml of *E. coli* protein containing RVL1. Compared to SRL1 and RVL1 treated PDA plates, control plates took 12 days for formation of dark brown sclerotial bodies (Table 1). Rate of formation of sclerotial bodies increased

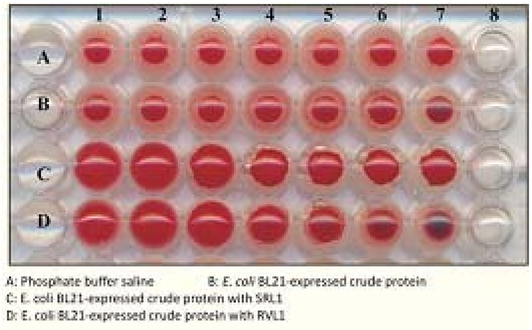

Fig.1. Heamagglutination assay of *E. coli*-expressed RVL1 and SRL1

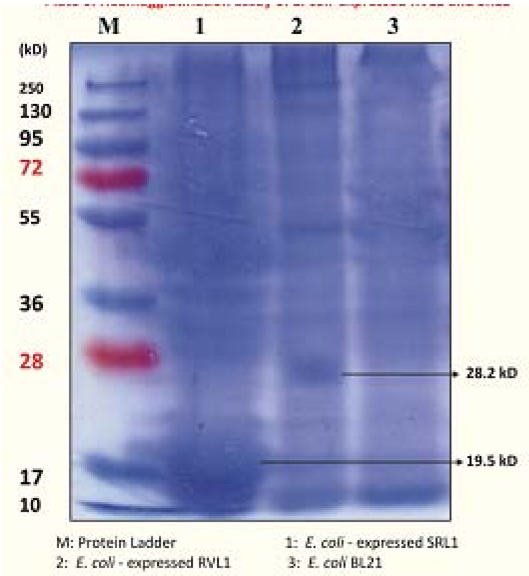

Fig. 2. SDS-PAGE analysis *of E. coli*-expressed RVL1 and SRL1

Fig. 3. *In vitro* evaluation of RVL1 and SRL1against *S. rolfsii*

Table 1. Efficacy of RVL1 and SRL1 lectin on growth parameters of *Sclerotium rolfsii*

Treatment	Quantity of protein (mg/ml)	Mycelial growth	Diameter (mm)	Sclerotial initiation	Sclerotial bodies colour	Total Number of Sclerotial bodies	Size of Sclerotial bodies	Weight of 50 Sclerotial bodies (mg)	Germination of Sclerotial bodies (Hyphal length in mm)
RVL1	1.2	Pure White, sparse	90	4th day	Initially pure white later turns to pale brown and turns to dark brown on 8th day of inoculation	52	Medium	0.040	0.0
	2.4	Pure White, sparse	90	4th day	-do-	58	Medium	0.042	0.0
	3.6	Pure white, sparse	90	3rd day	Initially pure white later turns to pale brown and turns to dark brown on 7th day of inoculation	64	Medium	0.050	0.0
	4.8	Pure white, sparse	90	3rd day	-do-	72	Medium	0.060	0.0
SRL1	1.2	White, sparse	90	3rd day	Initially pure white later turns to pale brown and turns to dark brown on 7th day of inoculation	103	Medium – big	0.090	12.0
	2.4	White, sparse	90	2nd day	-do-	116	Medium - big	0.080	10.0
	3.6	Pure white, sparse	90	2nd day	Initially pure white later turns to pale brown and turns to dark brown on 6th day of inoculation	148	Medium - big	0.080	7.0
	4.8	Pure white, sparse	90	2nd day	-do-	174	Big	0.150	5.0
Control (BL21)	1.2	Pure white, radiating mycelium	90	7th day	Initially white later turns to pale brown and turns to dark brown on 12th day of inoculation	50	Small	0.020	90
	2.4	Pure white, radiating mycelium	90	7th day	-do-	50	Small	0.020	90
	3.6	Pure white, radiating mycelium	90	7th day	-do-	55	small	0.030	90
	4.8	Pure white, radiating mycelium	90	7th day	-do-	52	Small	0.030	90
Control	-	Pure white, radiating mycelium	90	7th day	-do-	62	Small	0.036	90

with increasing concentration of both lectins. SRL1 treated PDA plates produced bigger, heavier and more number of sclerotial bodies with increasing concentrations of SRL1. Compared to SRL1 treated plates, RVL1 treated plates produced small, lighter and less number of sclerotial bodies. Both control PDA plates containing bacterial protein without lectin, and PDA plate without protein showed small sized and less number of sclerotial bodies.

Germination of sclerotial bodies is another event in the development of fungus. Sclerotia were plated on water agar to know the role of both the lectins on their germination. Results revealed that there was no germination of sclerotial bodies in all

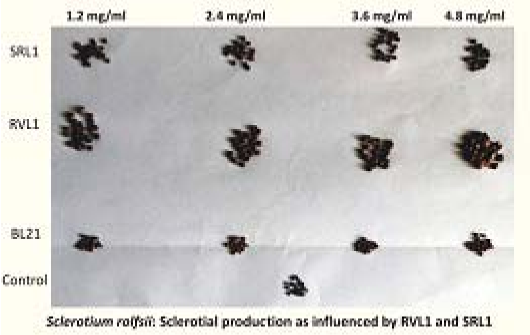

Fig. 4. *Sclerotium rolfsii*: Sclerotial Production as influenced by RVL1 and SRL1

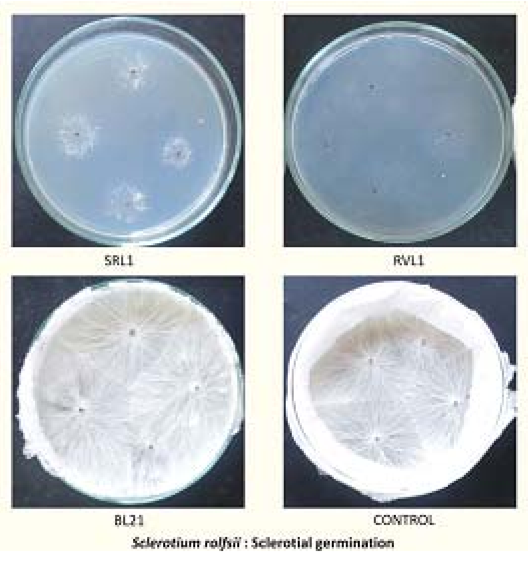

Fig. 5. *Sclerotium rolfsii:* Sclerotial germination inhibition as influenced by RVL1 and SRL1

concentrations of RVL1 treatment compared to SRL1 and control (Table 1, Figure 3). In both the controls, (bacterial protein without lectin and untreated sclerotial bodies), sclerotia started germinating after two days of inoculation. Pure white, smooth, radiating mycelial growth was seen after four days. At the same time, both SRL1 and RVL1 treated PDA plates failed to show germination of sclerotial bodies. But after five days of inoculation, slight germination of sclerotial bodies and dull white, sparse thread-like mycelial growth (5 to 12 mm length) was observed in all concentrations of SRL1. There was no sclerotial germination in all concentrations of RVL1. These results are correlated with[23] where they have shown that capping of the lectin sites by anti-SRL strongly inhibited the germination of sclerotial bodies. Similar inhibition was found by treating these bodies with mucin or fetuin, with which SRL strongly binds[24].

CONCLUSIONS

Since plant lectins cannot bind to glycoconjugates on the fungal membranes or penetrate the cytoplasm of the cells because of the presence of a thick and rigid cell wall, a direct interference with the growth and development of these organisms (i.e. through an alteration of the structure and/or permeability of the membrane or a disturbance of the normal intracellular processes) seems unlikely. However, indirect effects based on the binding of lectins to carbohydrates exposed on the surface of the fungal cell wall are possible. By virtue of their specificity, chitin-binding lectins seemed likely to have a role in the plant's defense against fungi. Fungal lectins are directly involved in morphogenesis and development of fungi and mediate host-parasite interactions. Our *in vitro* studies demonstrated that both RVL1 and SRL1 playing a role in early formation of sclerotial bodies rather than simply serving as reserve storage protein. Germination of sclerotial bodies is another event in development of the fungus, which was strongly inhibited by SRL1 and RVL1 lectins. In contrast to an endogenous function, the cytoplasmic localization of fruiting body lectins is ideally suited for the proposed role of these proteins in the defence of fungi against pathogen. These studies suggest that lectin-mediated defence is an ancient form of defence that is conserved across all kingdoms including animals, plants and fungi. Studies of this type of defence may open new avenues for the management of pests and parasites. Additionally, this study emphasizes the potential of studying specific lectin–carbohydrate interactions as a molecular tool to understand interspecific trophic and evolutionary relationships in nature. Both fungal and plant lectins are playing a role in inhibiting the pathogen. To our knowledge, this is the first report on plant lectin RVL1 inhibiting sclerotial bodies germination.

REFERENCES

1. Dam, T. K and Brewer, C. F., Fundamentals of lectin-carbohydrate interactions. *In: Comprehensive Glycoscience* (ed. Kamerling H), Elsevier, Oxford, 2007; 397–452.
2. Guillot, J. and Konska, G., Lectins in higher fungi. Biochem. *Syst. Ecol*., 1997; **25**: 203-230.
3. Singh, R. S., Bhari, R. and Kaur, H. P., Mushroom lectins: Current status and future perspectives. *Crit. Rev. Biotechnol*., 2010; **30** (2): 99-126.
4. Walti, M. A., Walser. P. J. and Thore, S., Structural basis for chitotetraose coordination by CGL3, a novel galectin-related protein from *Coprinopsis cinerea. J. of Mole. Biol.,* 2008; **379**: 146–159.
5. Peumans, W. J. and Van Damme, E. J. M., Lectins as plant defense proteins. *Pl. Physiol.*, 1995; **109**: 347–352.
6. Van Damme, E. J. M., Barre, A., Rouge, P. and Peumans, W. J. Cytoplasmic D nuclear plant lectins: a new story. *Trends in Pl. Sci.*, 2004; **9**: 484–489.
7. De Hoff, P. L., Brill, L. M. and Hirsch, A. M., Plant lectins: the ties that bind in root symbiosis and plant defense. *Mol. Gen. and Genotype.*, 2009; **282**: 1–15.
8. Murdock, L. L. and Shade, R.E., Lectins and protease inhibitors as plant defenses against insects. *J. of Agri. and Food Chem.*, 2002; **50**: 6605-6611.
9. Vasconcelos, I. M. and Oliveira, J. T. A., Antinutritional properties of plant lectins. *Toxicol.*, 2004; **44**(4): 385-403.
10. Macedo, M. L. R., Freire, M. D. M., Novello, J. C. and Marangoni, S., Talisia esculenta lectin and larval development of Callosobruchus maculatus and Zabrotes subfasciatus (Coleoptera: Bruchidae). *Biochimica et biophysica acta*, 2002; **1571**: 83-88.
11. Vandenborre, G., Miersch, O. and Hause, B., Spodoptera littoralis-induced lectin expression in tobacco. *Pl. and Cell Physiol.*, 2009; **50**: 1142–1155.
12. Ripoll, C., Favery, B., Lecomte, P., Van Damme, E., Peumans, W., Abad, P. And Jouanin, L., Evaluation of the ability of lectin from snowdrop (*Galanthus nivalis*) to protect plants against root-knot nematodes. *Pl. Sci*., 2003; **164**: 517-523.
13. Trebicki, P., Harding, R.M. and Powell, K. S., Anti-metabolic effects of Galanthus nivalis agglutinin and wheat germ agglutinin on nymphal stages of the common brown leafhopper using a novel artificial diet system. *Entomologia Experimentalis Et Applicata*, 2009; **131**: 99–105.
14. Spiteller, P., Chemical defence strategies of higher fungi. *Chem.*, 2008; **14**: 9100-9110.
15. Mc Gonigle, T. P., Effects of animals grazing fungi. *In: Environmental and Microbial Relationships* (ed. Kubicek, C. P., Druzhinina, I. S.), Springer-Verlag, Berlin, Heidelberg. 2007; 201–212.
16. Fox, E. M., Howlett, B. J., Secondary metabolism: regulation and role in fungal biology. *Curr. Opin. in Microbiol*, 2008; **11**: 481–487.
17. Wang, M., Triguéros, V., Paquereau, L., Chavant, L. and Fournier, D., Proteins as active compounds involved in insecticidal activity of mushroom fruitbodies. *J. Econ. Entomol.*, 2002; **95**(3): 603-607.
18. Hamshou, M., Smagghe, G. and Shahidi-Noghabi, S., Insecticidal properties of Sclerotinia sclerotiorum agglutinin and its interaction with insect tissues and cells. *Insec Biochem. and Mol. Biol.*, 2010; **40**: 883-890.
19. Neekhra, V., Bhat, G. G., Bhagat, Y. S., Lingaraju, S. and Bhat, R. S., , Nematicidal activity of *Remusatia vivipara* lectin expressed in *Escherichia coli. Curr. Sci*, 2011; **101**(2): 150-151.
20. Bhat, R. S., Chandrashekar, T. M., Basingi, S. M., Mallesh, S. B. and Lingaraju, S., Cloning of *Sclerotium rolfsii* lectin gene and its nematicidal activity. *Curr. Sci.*, 2010; **98**(9): 1185 1186.
21. Lis, H. and Sharon, N., Lectins: carbohydrate-specific proteins that mediate cellular recognition. *Chem. Rev*., 1998; **98**(2): 637-674.
22. Liener, I. E. and Hill, E. G., The effect of heat treatment of the nutritive value and hemagglutinating activity of soybean oil meal: one figure . *J. Nutr*., 1953; **49**(4): 609 -620.
23. Swamy, B. M., Bhat, A. G., Hegde, G. V., Naik, R. S., Kulkarni, S. and Inamdar, S. R., Immunolocalization and functional role of *Sclerotium rolfsii* lectin in development of fungus

by interaction with its endogenous receptor. *Glycobiol*, 2004; **14**(11): 951-957

24. Swamy, B. M., Hegde, G. V., Naik, R. S. and Inamdar, S. R., T-antigen binding lectin from the phytopathogenic fungus *Sclerotium rolfsii*. *Lect. Biol. Biochem. Clin. Biochem.*,2001; **15**: 45-55.