Plant Archives Volume 20 No. 2, 2020 pp. 3799-3802 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

STUDIES ON THE INDUCTION OF DEFENCE RELATED ENZYMES PEROXIDASE AND POLYPHENOL OXIDASE AGAINST *SCLEROTIUM ROLFSII* IN GROUNDNUT

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Abstract

Groundnut (*Arachis hypogaea L.*) is an important oil seed crop. The low productivity in groundnut is attributed to many production constraints. Among these, biotic factors particularly diseases play a major role in limiting the yield of groundnut. Soil born diseases, stem rot caused by *Sclerotium rolfsii Sacc.* is an important pathogen which is attacking different crops. This disease is wide spread and causes serious losses in groundnut. The hypersensitive reaction (HR), one of the most efficient and visible parts of the defense mechanisms in nature against invading pathogens, is associated with a coordinated and integrated set of metabolic alterations which are instrumental in impeding further pathogen ingress or alleviating stress. It includes a variety of novel proteins and secondary metabolites. This study aimed to examine the induction of different stress related enzymes like peroxidases (POD) and poly phenol oxidases (PPO). The maximum activity of Peroxidase (2.89) and poly phenol oxidase (1.42) observed in the *Ulva lactuca* @10g (T_1) treated groundnut plants on 5th day. Further, the enzyme activity was decreased on 7th day

key words: Groundnut, *Sclerotium rolfsii*, Peroxidise, Polyphenol oxidase

Introduction

Groundnut (*Arachis hypogaea L.*) is an important oil seed crop belonging to *Fabaceae* family and it is grown in all over the world. It is called as king of oil seed. In India it is one of the most important food and cash crops and plays a vital role in the oil seed economy of India. It is fourth most important source of edible oil and third most important source of vegetable protein. Globally, the crop is raised on 26.4 million hectares with a total production of 37.1 million MT. The average productivity is 1400 kg/ha (IOPEPC, 2017). The soil borne pathogen, *Sclerotium rolfsii* Sacc. is an important pathogen of groundnut. The sclerotia of the pathogen are the principal means of survival in soil and debris (Garren, 1961). The disease is distributed throughout the world and prevalent particularly in warm dry climates. In India, stem rot of groundnut was first recorded by Butler and Bisby (1931) and causes yield losses of over 25 per cent (Mayee and Datar, 1988). An ailment of stem rot of groundnut causes 13 to 59 per cent yield loss during both the rainy and summer seasons (Nautiyal, 2002).

Inducing the plant's own defense mechanisms by prior application of a biological inducer is thought to be a novel plant protection strategy. *Trichoderma* spp. was found to be effective biological inducers to induce plants own defense mechanism in various crop plants against plant pathogenic fungi (Sureka *et al.*, 2013). The enhanced induction of Peroxidase (PO) and Polyphenoloxidase (PPO) might have contributed for the induced systemic resistance triggered by various biotic and abiotic inducers (Barilli *et al.*, 2010). The use of *Trichoderma* spp. and *Pseudomonas* spp. as bioagents induced the accumulation of some enzymes such as chitinase, peroxidase and polyphenol oxidase, which play an important role in plant defence mechanisms against pathogens infection in treated bean plants, which increased in them more than in untreated one (Abd-El-Khair *et al.,* 2011).

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Materials and Methods

Virulence in pot culture

The stem rot incidence was assessed at 25th, 50th and 75th DAS. The isolates collected from different parts of Cuddalore districts were designated as follows Sr_1 (Poovanur), Sr_2 (Sivapuri), Sr_3 (Killai), Sr_4 (Palakollai), Sr_5 (Abatharanapuram), Sr_6 (Vadalur), Sr_7 (T.S. Pillai), Sr_8 (Kurinjipadi), Sr_9 (Nadiyappattu), Sr_{10} (Kammapuram).

Collection of plant samples

The groundnut seeds were pre-treated with different seaweed extract and the biocontrol agents separately then they were sown in pots with challenge inoculation of pathogen .The seeds without treatment serves as control. The plant samples were collected starting from zero to seven days after challenge inoculation of the pathogen. Four plant samples were collected from each replication of the treatment separately and used for analysis.

Enzyme Extraction

1 g of root samples was homogenized with 2 ml of 0.1 M sodium citrate buffer (pH 5.0) at 4ºC. The homogenate was centrifuged for 20 minutes at 1000 rpm. The supernatant was used as crude enzyme extract for enzyme activity. Enzyme extracted in 0.1 M sodium phosphate buffer (pH 7.0) was used for the estimation of peroxidase (PO), polyphenol oxidase (PPO) and Peroxidase activity was assayed as per the procedure described by Hamerschmidt *et al.* (1995). The reaction mixture consisted of 2.5 ml of a mixture containing 0.25 per cent (v/v) guaiacol in 0.01 M sodium phosphate buffer, pH 6.0 and 0.1 M hydrogen peroxide. Enzyme extract (0.1ml) was added to initiate the reaction, which was followed calorimetrically at 470 nm. Crude enzyme preparations were diluted to given changes in absorbance at 470 nm of 0.1 to 0.2 absorbance units/min. The boiled enzyme preparation served as blank. Activity was expressed as changes in absorbance at 470 nm/min/g of fresh tissue.

Polyphenol oxidase (PPO)

PPO activity was determined as per the procedure given by Mayer *et al.* (1965). The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 0.2 ml of the enzyme extract. To start the reaction, 0.01 M catechol was added and the activity was expressed as changes in absorbance at 470 nm/min of fresh tissue.

Pot culture experiment

At the time of sowing, seeds were treated with bio control agents *viz. T. asperellum* @4g/kg and *Pseudomonas fluorescens* @10g/kg of seeds then the treated seeds were sowed in the earthen pots filled with 5kg of sick soil (50g of inoculums /kg of soil) followed by soil application of *Ulva lactuca*, *Trichoderma asperellum* and *Pseudomonas fluorescencs* @ 10g/pot on 30th, 45th and 60th DAS respectively.

Treatment schedule

T_1 - *Ulva lactuca* @ 10g/kg
T_2 - *Trichoderma asperellum* (Ta_2) @ 10g /kg SA
T_3 - *Pseudomonas fluorescens* (Pf_7) @ 10g /kg SA
T_4 - TNAU *Trichoderma* @ 10g /kg SA
T_5 - TNAU *Pseudomonas fluorescens* @ 10g /kg SA
T_6 - Carbendazim @ 0.1% ST
T_7 - Inoculated control
SA - Soil application
ST - Seed treatment

Result and Discussion

Pathogenicity of *Sclerotium rolfsii* under pot culture condition

A pot culture experiment was conducted to test the virulence of isolates collected from different villages of

Table 1: Pathogenicity of *Sclerotium rolfsii* isolates under pot culture condition

S. No	Isolates	Stem rot incidence (%)				Mean
		30 DAS	60 DAS	90 DAS	At harvest (105 DAI)	
1	Sr_1	14.35^{g} (22.26)	27.69^{g} (31.75)	35.81^{g} (36.76)	39.62^{g} (39.01)	29.37
2	Sr_2	18.99^{d} (25.83)	36.47^{d} (37.15)	40.08^{d} (39.28)	44.19^{d} (41.66)	34.93
3	Sr_3	16.37^{e} (23.86)	34.19^{e} (35.78)	39.89^{e} (39.17)	43.23^{e} (41.11)	33.42
4	Sr_4	12.41^{h} (20.63)	25.89^{h} (30.58)	34.51^{h} (35.98)	36.93^{h} (37.42)	27.44
5	Sr_5	27.56^{a} (31.67)	49.61^{a} (44.78)	51.66^{a} (45.95)	57.68^{a} (49.42)	46.63
6	Sr_6	20.19^{b} (26.70)	38.76^{b} (38.50)	43.27^{b} (41.13)	48.88^{b} (44.36)	37.78
7	Sr_7	10.11^{j} (18.54)	20.89^{j} (27.20)	28.35^{j} (32.17)	32.16^{j} (34.55)	22.88
8	Sr_8	15.79^{f} (23.41)	32.01^{f} (34.46)	37.47^{f} (37.74)	41.18^{f} (39.92)	31.61
9	Sr_9	19.23^{e} (26.01)	36.42^{e} (37.12)	41.19^{e} (39.93)	46.89^{e} (43.22)	35.93
10	Sr_{10}	11.99^{i} (20.26)	22.87^{i} (28.57)	31.15^{i} (33.93)	33.47^{i} (35.35)	24.87

*Mean of three replication, *Values in the column followed by common letters do not differ significantly at 5% level by Duncan's multiple range test (DMRT)

Table 2: Changes in peroxidase (PO) activity in *Sclerotium rolfsii* challenged groundnut plants treated with different formulations

S. No	Treatments	PO activity in plants (changes in absorbance min^{-1} mg $protein^{-1}$)			
		Time interval (days)			
		0	3	5	7
1	*Ulva lactuca* @ 10g/kg SA	2.63	2.75	2.89	2.79
2	*Trichoderma asperellum* (Ta_2) @ 10 g/ kg SA	2.45	2.64	2.78	2.73
3	*Pseudomonas fluorescens* (Pf_7) @ 10 g/ kg SA	2.22	2.52	2.61	2.60
4	TNAU Tv 10 g/ kg SA	2.17	2.38	2.58	2.56
5	TNAU Pf 10 g/ kg SA	2.06	2.19	2.46	2.43
6	Carbendazim 0.1% ST	1.85	2.02	2.27	2.20
7	Control	1.72	1.97	2.03	1.91

groundnut growing areas of Cuddalore district. Among the ten isolates collected, the isolate Sr_5 collected from Abatharanapuram recorded the maximum disease incidence of 46.63 per cent under artificial inoculation and was identified as a virulent culture followed by Sr_6 which recorded 37.78 per cent from Vadalur. The least disease incidence was observed in T.S. Pillai isolate Sr_7 which recorded 22.88 per cent (Table 1).

Yrlania *et al.* (2015) proved pathogenicity of *S. rolfsii* on peanut plants. The first symptoms were verified 48 h after inoculation, which evolved to stem bottleneck and plant wilting with the presence of white mycelium under green house condition. Pathogenicity proved on 15 days old groundnut plants (cv. GJG-9) under pot conditions. The initial symptoms were observed as water soaked brown to dark brown spots at basal portion of plants and the leaves of infected plants gradually yellowing and dry up 4 days after of inoculation (Bekriwala TH *et al.*, 2016). Pathogenicity reactions were observed for all the 10 isolates *S. rolfsii*, the isolate S.r-9 exhibited maximum disease incidence (100%) followed by S.r-7 (90.67%). The lowest disease incidence (46.33%) was recorded in S.r-6 isolate under pot culture experiments (Chandra Sekhar *et al.*, 2017). The disease intensity of different isolates upon artificial inoculation showed the virulence nature of isolates determines the disease intensity in their host.

Changes in Peroxidase (PO) activity in *Sclerotium rolfsii* challenged groundnut plants treated with different formulations

The results revealed that increased activity of peroxides in all treatment when compared with pathogen alone inoculated. Among the various treatments, the maximum activity of peroxidase (2.89) observed in the *Ulva lactuca* @10g (T_1) treated groundnut plants on 5th day. Further, the enzyme activity was decreased on 7th day (Table 2).

Inoculation of *Erysiphe graminis* and Potassium Phosphate treatment in barley leaves led to significant increase in activities of peroxidase and lipoxygenase enzymes on 5th days after inoculation (Mitchell and Walters, 2004). Yu *et al.* (2011) proved that foliar application of silicon in cucumber plants inoculated with *Pseudoperonospora cunbensis* enhanced the defense related enzymes Peroxidase (PO). Sangeetha *et al.* (2010) reported that the banana fruits treated with antagonistic mixture Pf_3 + Bs_1 recorded maximum induction of PO activity.

Changes in Polyphenol oxidase (PPO) activity in *Sclerotium rolfsii* challenged groundnut plants treated with different formulations

The increased activity of PPO was observed in plants challenge inoculated with Sr_5 in groundnut. Application of *Ulva lactuca* @10g led to increased PPO activity (1.42) up to 5th day and thereafter a gradual decline was observed (Table 3). Plants inoculated with pathogens alone recorded comparatively less PPO activity and the activity was very low on 5th day after inoculation, also PPO activity decreased with age in control.

Durga devi *et al.* (2014) reported that bulb treatment

Table 3: Changes in Polyphenol oxidase (PPO) activity in *Sclerotium rolfsii* challenged groundnut plants treated with different formulations

S. No	Treatments	PPO activity in plants (changes in absorbance min^{-1} mg $protein^{-1}$)			
		Time interval (days)			
		0	3	5	7
1	*Ulva lactuca* @ 10g/kg SA	1.13	1.35	1.42	1.40
2	*Trichoderma asperellum* (Ta_2) @ 10 g/ kg SA	1.09	1.29	1.38	1.36
3	*Pseudomonas fluorescens* (Pf_7) @ 10 g/ kg SA	1.02	1.27	1.32	1.30
4	TNAU Tv 10 g/ kg SA	0.98	1.19	1.28	1.25
5	TNAU Pf 10 g/ kg SA	0.90	1.13	1.24	1.21
6	Carbendazim 0.1% ST	0.82	1.08	1.20	1.18
7	Control	0.75	1.00	1.16	1.10

with consortial formulation of *T. viride* and *B. subtilis* challenged with the *Lasidiplodia therobromae* recorded maximum induction of PPO activities in tubrose plants. Biocontrol agent *T. harzianum* followed by inoculation of *A. sesami* enhanced induction of defence related enzyme PPO which may be effective defence mechanism shown by the plants against *Alternaria* leaf spot of sesame (Lubaina and murugan, 2015).

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