Int.J.Curr.Microbiol.App.Sci (2020) *9*(11): 3573-3581

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 11 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.911.428

Effect of Biotic and Abiotic Elicitors in Inducing Resistance against Cowpea Rust

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Keywords
Uredospores, Induced Resistance, Elicitors, Salicylic acid, Jasmonicacid, *Trichoderma viridae*, *Pseudomonas fluorescence*

Article Info

Accepted:
24 October 2020
Available Online:
10 November 2020

A B S T R A C T

Cowpea is important multipurpose crop which is used for the purpose of food, feed, forage, catch crop, green manuring and cover crop. The crop is prone to rust disease which causes severe yield loss. Hence to manage the disease chemicals which are expensive and hazardous are usually used by the farmers. Alternately a different strategy to manage the same was experimented with the use of elicitors which are compounds which activate chemical defence in plants and this induced defence response in susceptible variety C-152 was examined. The compounds like Salicylic acid (SA), Jasmonic Acid (JA), biocontrol agents viz., *Trichoderma viridae*, *Pseudomonas fluorescence* were used as elicitors. All elicitors were found effective in inducing resistance against rust disease by accelerating the enzyme activities like peroxidase, phenylalanine ammonia lyase and β1, 3-glucanase activity. This was higher in abiotic elicitorsthan the biotic elicitors. The highest enzyme activity of phenylalanine ammonia lyase was recorded in foliar spray of 1mM salicylic acid at 50 DAS followed by uredospores suspension after 2 days whereas, highest peroxidase and β1, 3-glucanase activity was recorded in foliar spray of 0.5mM Jasmonic acid spray at 50 DAS followed by uredospore suspension after 2 days. Hence these elicitors could be used as alternative to chemical pesticides, an important component of organic farming and integrated disease management.

Introduction

Cowpea (*Vigna unguiculata* L.) is a multipurpose crop. In India, the crop is cultivated with a total area of 654 lakh hectares, and production of 599 lakh tonnes and a productivity of 916 kg ha^{-1}(Joshi *et al.*, 2018). In Karnataka, the crop is grown in an area of 0.84 lakh hectares with a production of 0.25 lakh tonnes with low productivity 360 kg ha^{-1} as compared to the national productivity of 567 kg ha^{-1} (Prabhamani *et al.*, 2018). The overall grain yield of cowpea in the present traditional systems is low due to

several biotic and abiotic factors (Singh and Singh, 1997).

The abiotic factors that cause yield reduction include drought, temperature extremes, excessive moisture, late maturity, acidity and stress. In India, a loss due to diseases is 20% and an insect pest is 25%, Abiotic stresses reduce an average yield of crops by upto 50% (Verma and Deepthi, 2016).Among the biotic factors the crop is affected by number of fungal, bacterial, viral and nematode diseases. Among them, rust disease caused by a fungus Uromyces phaseoli var. vignae (Barclay) Arth. is one of the most important diseases that cause huge economic loss. To manage the disease there are number of strategies viz., cultural, physical, mechanical and chemical. Among them, chemical management stands as one of the important means because they are quick in action. Although fungicide can control the disease but increases the cost of production. The better understanding of plant signalling pathways has led to the discovery of natural and synthetic compounds called elicitors that induce defense responses in plants as induced by the pathogen infection. These compounds act as signal molecules at low concentrations, providing information for the plant to trigger defense.

Elicitors are the chemicals (or) bio-factors from various sources that can trigger physiological and morphological responses and also phytoalexin accumulation in the target plants and organisms. Originally elicitor was used for molecules capable of inducing the production of phytoalexins, but it is now commonly used for compounds stimulating any type of plant defense (Thakur and Sohal, 2013). Synthetic elicitors are small drug-like molecules that induce plant defense responses, but are distinct from known natural elicitors of plant immunity. Some synthetic elicitors are Polyamines, Salicylic acid, Benzothiadiazole, Jasmonic acid, Chitosan, Brassinosteroids, Tricontinol, 2,6-dichloroisonicotinic acid, Probenazole, Isotianil, Sulfanilamide, Clopamide, Butamide (Bektas and Euglem, 2015). While some of the biotic elicitors are enzymes like cellulase, pectinase and biocontrol agents includes *Trichoderma viridae, Pseudomonas flourescense etc* (Dixon, 2001)**.** As elicitors can protect crops from diseases, may also serve as promising alternatives to conventional chemical pesticides, which often are harmful and has ill effects on the environment and non-target pests.

Materials and Methods

The analysis was carried out in Department of Plant Pathology, College of Agriculture,V. C. Farm, Mandya. To identify and to know the role of like Salicylic acid (SA), Jasmonic acid (JA), *Trichoderma viridae, Pseudomonas fluorescence* in eliciting the resistance against the pathogen an experiment was taken under greenhouse conditions. Ten treatments were taken up and replicated thrice under completely randomized design (CRD). The pots were filled with a 1:2:1 ratio of sand, soil and compost respectively. The susceptible cowpea variety C-152 was used for study. The treatments (Table 1) were imposed on the plants at 50 DAS. The leaf samples were collected before spraying and after spraying at 51, 53 and 55 DAS for analyzing the enzyme activity *viz*., peroxidase by using spectrophotometer method as described by Hartee (1955) and phenyl ammonia lyase activity by using Ross and Senderoff (1992) method and β-1, 3-*glucanase* activity was estimated using the method given by Rakshit *et al.,* (2000).

Results and Discussion

The peroxidase, phenylalanine ammonia lyase and β1,3-*glucanase*activity was recorded at 51, 53 and 55 DAS in different treatments.

Peroxidase activity

The peroxidase activity was recorded at 51, 53 and 55 DAS in different treatments. At 51 DAS the highest enzyme activity of 1.349 Δ Abs min^{-1} g^{-1} was recorded in T8 followed by T2 (1.251 Δ Abs min^{-1} g^{-1}), and T7 (1.237 Δ Abs min^{-1} g^{-1}) and there was a significant difference among them. However, the enzyme activity in T1 (1.076 Δ Abs min^{-1} g^{-1}) and T6 (1.007 Δ Abs min^{-1} g^{-1}) were on par with each other. The lowest enzyme activity of 0.640 was recorded in T10 followed by T3 (0.827 Δ Abs min^{-1} g^{-1}) and T5 (0.890 Δ Abs min^{-1} g^{-1}) and there was a significant difference among them (Table 1).

The highest enzyme activity of 1.551 Δ Abs min^{-1} g^{-1} was recorded in T8 at 53 DAS, followed by T2 (1.437 Δ Abs min^{-1} g^{-1}) and T7 (1.379 Δ Abs min^{-1} g^{-1}) and T1 (1.298 Δ Abs min^{-1} g^{-1}) and there was a significant difference among them. However, the lowest enzyme activity was recorded in T10 (0.733 Δ Abs min^{-1} g^{-1}) followed by T3 (0.905 Δ Abs min^{-1} g^{-1}) and T9 (1.041 Δ Abs min^{-1} g^{-1}) and there was a significant difference among the treatment.

Further, at 55 DAS the highest peroxidase activity was recorded in T8 (1.753 Δ Abs min^{-1} g^{-1}) followed by T2 (1.699 Δ Abs min^{-1} g^{-1}) and in T7 (1.523 Δ Abs min^{-1} g^{-1}). Whereas, the lowest enzyme activity of 0.867 Δ Abs min^{-1} g^{-1} was recorded in T10 followed by T3 (1.034 Δ Abs min^{-1} g^{-1}) and T4 (1.100 Δ Abs min^{-1} g^{-1}) and there was a significant difference among the treatments.

Thus the above results revealed that the peroxidase enzyme activity in all the treatments were significantly high than the control during all the observations recorded (51, 53 and 55 DAS). Further, among the ten treatments the highest enzyme activity was recorded (1.753 Δ Abs min^{-1} g^{-1}) with foliar spray of 0.5 mM JA followed by uredospore suspension after 2 days (T8), followed by 1.699 Δ Abs min^{-1} g^{-1}in foliar spray of SA at 1mM followed by uredospore suspension after 2 days (T2)and there was significant difference among them).

Phenylalanine Ammonia Lyase (PAL) Activity

(The phenylalanine ammonia lyase (PAL) enzyme activity was recorded at 51, 53 and 55 DAS. At 51 DAS the highest enzyme activity of 124 µmol of trans cinnamic acid min^{-1} g^{-1} was recorded in T2 followed by T1 (121 µmol of trans cinnamic acid min^{-1} g^{-1}) and T8 (118 µmol of trans cinnamic acid min^{-1} g^{-1}), which were on par with each other. The lowest enzyme activity was noticed in T10 (81 µmol of trans cinnamic acid min^{-1} g^{-1}) followed by T9 (88 µmol of trans cinnamic acid min^{-1} g^{-1}) and T5 (95 µmol of trans cinnamic acid min^{-1} g^{-1}) and there was a significant difference among them (Table 2).

Further, at 53 DAS the highest enzyme activity of 127 µmol of trans cinnamic acid min^{-1} g^{-1} was recorded in T2 followed by treatment T1 (122 µmol of trans cinnamic acid min^{-1} g^{-1}) and T8 (121 µmol of trans cinnamic acid min^{-1} g^{-1}) and were on par with each other. The lowest enzyme activity was recorded in T10 (82 µmol of trans cinnamic acid min^{-1} g^{-1}) followed by T9 (90 µmol of trans cinnamic acid $min^{-1}g^{-1}$) and T5 (96 µmol of trans cinnamic acid min^{-1} g^{-1}) and there was a significant difference among them.

However, the highest enzyme activity was recorded in T2 (130 µmol of trans cinnamic acid min^{-1} g^{-1}) at 55 DAS, followed by T1 (125 µmol of trans cinnamic acid min^{-1} g^{-1}) and T8 (124 µmol of trans cinnamic acid min^{-1} g^{-1}) which were on par with each other. Whereas, the lowest enzyme activity of 83 µmol of trans cinnamic acid min^{-1} g^{-1} was

recorded T10 followed by T9 (91 µmol of trans cinnamic acid min^{-1} g^{-1}) and T5 (97 µmol of trans cinnamic acid min^{-1} g^{-1}) and there was a significant difference among them.

The above results indicated that the PAL activity in all the treatments were significantly high than the control during all the observations recorded (51, 53 and 55 DAS). Further, among the ten treatments highest PAL activity was recorded with foliar spray of 1 mM salicylic acid followed by uredospore suspension after 2 days (T2) (130 µmol of trans cinnamic acid min^{-1} g^{-1}), followed by foliar spray of 1 mM salicylic acid at 50 DAS (T1) (125 µmol of trans cinnamic acid min^{-1} g^{-1}) which were significantly difference with each other.

The least enzyme activity of 124 µmol of trans cinnamic acid min^{-1} g^{-1} was recorded in control (T10) followed by 91 µmol of trans cinnamic acid min^{-1} g^{-1} with spray of uredospore suspension).

Table.1 Effect of different elicitors on peroxidase enzyme activity in the cowpea leaves

Treatments	Treatment details	Peroxidase activity (Δ Abs min^{-1} g^{-1})		
		51 DAS	53 DAS	55 DAS
T1	Salicylic acid is sprayed at 1mM concentration at 50 DAS	1.076	1.298	1.493
T2	Foliar spray of 1mM salicylic acid at 50 DAS followed by uredospores suspension after 2 days	1.251	1.437	1.699
T3	*Trichoderma viride*0.2% is sprayed at 50 DAS	0.827	0.905	1.034
T4	Foliar spray of 0.2% *Trichoderma viride* spray at 50 DAS followed by uredospores suspension after 2 days	0.927	1.005	1.100
T5	*Pseudomonas fluorescence* 0.1% is sprayed at 50 DAS	0.890	1.078	1.168
T6	Foliar spray of 0.1% *Pseudomonas fluorescence* spray at 50 DAS followed by uredospores suspension after 2 days	1.007	1.180	1.248
T7	Jasmonic acid 0.5mM spray at 50 DAS	1.237	1.379	1.523
T8	Foliar spray of 0.5mM Jasmonic acid spray at 50 DAS followed by uredospore suspension after 2 days	1.349	1.551	1.753
T9	Uredospore suspension sprayed at 50 DAS	0.901	1.041	1.127
T10	Control	0.640	0.733	0.867
F		**	**	**
S.Em±		0.0134	0.0235	0.0240
CD @ 1%		0.0537	0.0947	0.0967

DAS- Days After Sowing
**- significant

Table.2 Role of elicitors on phenylalanine ammonia lyase (PAL) activity in the cowpea leaves

Treatments	Treatment details	PAL (µ moles of trans cinnamic acid min^{-1} g^{-1})		
		51 DAS	53 DAS	55 DAS
T1	Salicylic acid is sprayed at 1mM concentration at 50 DAS	121	122	125
T2	Foliar spray of 1mM salicylic acid at 50 DAS followed by uredospores suspension after 2 days	124	127	130
T3	*Trichoderma viride* 0.2% is sprayed at 50 DAS	105	107	109
T4	Foliar spray of 0.2% *Trichodermaviride* spray at 50 DAS followed by uredospores suspension after 2 days	112	116	118
T5	*Pseudomonas fluorescence* 0.1% is sprayed at 50 DAS	95	96	97
T6	Foliar spray of 0.1% *Pseudomonas fluorescence* spray at 50 DAS followed by uredospores suspension after 2 days	98	99	101
T7	Jasmonic acid 0.5mM spray at 50 DAS	115	118	120
T8	Foliar spray of 0.5mM Jasmonic acid spray at 50 DAS followed by uredospore suspension after 2 days	118	121	124
T9	Uredospore suspension sprayed at 50 DAS	88	90	91
T10	Control	81	82	83
F		**	**	**
SEm±		0.8563	0.4944	0.4944
CD @ 1%		3.4459	1.9895	1.9895

DAS- Days After Sowing **- significant

Table.3 Estimation of the β1,3-glucanase activity in cowpea leaves due to different elicitors

Treatments	Treatment details	β1,3-glucanase (µg of glucanase released g^{-1} fresh wt)		
		51 DAS	53 DAS	55 DAS
T1	Salicylic acid is sprayed at 1mM concentration at 50 DAS	14	16	18
T2	Foliar spray of 1mM salicylic acid at 50 DAS followed by uredospores suspension after 2 days	17	20	23
T3	*Trichoderma viride* 0.2% is sprayed at 50 DAS	9	9	10
T4	Foliar spray of 0.2% *Trichodermaviride* spray at 50 DAS followed by uredospores suspension after 2 days	10	12	14
T5	*Pseudomonas fluorescence* 0.1% is sprayed at 50 DAS	7	8	9
T6	Foliar spray of 0.1% *Pseudomonas fluorescence* spray at 50 DAS followed by uredospores suspension after 2 days	9	10	12
T7	Jasmonic acid 0.5mM spray	15	18	21
T8	Foliar spray of 0.5mM Jasmonic acid spray at 50 DAS followed by uredospore suspension after 2 days	20	24	27
T9	Uredospore suspension sprayed at 50 DAS	13	14	15
T10	Control	7	8	9
F		**	**	**
S.Em±		0.3496	0.3651	0.2108
CD @ 1%		1.4068	1.4693	0.8483

DAS- Days After Sowing **- significant

Plate.1 Experimental setup to identify effective elicitors against rust disease in C-152

β1,3-*glucanase* Activity

Where β1,3-*glucanase* enzyme activity was determined at 51, 53 and 55 DAS.

The results showed that at 51 DAS, the highest enzyme activity of 20 was recorded in T8 followed by T2 (17 µg of glucanase released g^{-1} fresh wt.) and T7 (15 µg of glucanase released g^{-1} fresh wt.) and there was a significant difference among them. Whereas, the lowest enzyme activity of 7 µg of glucanase released g^{-1} fresh wt. was recorded in T10, followed by T5 (7 µg of glucanase released g^{-1} fresh wt.) and in T3 (9 µg of glucanase released g^{-1} fresh wt.) which were on par with each other.

At 53 DAS, the highest enzyme activity was recorded in T8 (24 µg of glucanase released g^{-1} fresh wt.) followed by T2 (20 µg of glucanase released g^{-1} fresh wt.) and T7 (18 µg of glucanase released g^{-1} fresh wt.) and there was a significant difference among them. The lowest enzyme activity was reported in T10 (8 µg of glucanase released g^{-1} fresh wt.) followed by T5 (8 µg of glucanase released g^{-1} fresh wt.) and in T3 (9 µg of glucanase released g^{-1} fresh wt.) and were on par with each other.

The highest enzyme activity of 27 µg of glucanase released g^{-1} fresh wt. was recorded in T8 at 55 DAS followed by T2 (23 µg of glucanase released g^{-1} fresh wt.) and T7 (21 µg of glucanase released g^{-1} fresh wt.) and there was a significant difference among them. Whereas, the least enzyme activity was recorded in T10 (9 µg of glucanase released g^{-1} fresh wt.) followed by T5 (9 µg of glucanase released g^{-1} fresh wt.) and in T3 (10 µg of glucanase released g^{-1} fresh wt.) and were on par with each other.

The above findings revealed that there was increase in activity of β1, 3-*glucanase*in all treatments significantly over control. Further, highest enzymatic activity of 27 was observed with foliar spray of JA at 0.5 mM followed by spraying of uredospore suspension after 2 days (T8) followed by foliar spray of 1mM salicylic acid followed by uredospore suspension after 2 days (T2) 23 µg of glucanase released g^{-1} fresh wt which were significantly differ with each other.

The least enzyme activity of 9 µg of glucanase released g^{-1} fresh wt. was recorded in control (T10) followed by 9 µg of glucanase released g^{-1} fresh wt with foliar spray of 0.1% *Pseudomonas fluorescence* (T5), and 10 µg of glucanase released g^{-1}

fresh wt with foliar spray of 0.1% *Trichoderma viridae* (T3)and were on par with each other) (Table 3).

In the above results it was found that there was increase in the activity of enzymes and less pustules were observed as the days increases when we treated the plants with biotic and abiotic elicitors and the similar reports were observed by Sathyabama and Balasubramanian (1999) reported that pre-treatment with SA reduced the number of rust pustules. PAL is the first enzyme of phenylpropanoid metabolism in higher plants and it has been suggested it plays a significant role in regulating the accumulation of phenolics, phytoalexins and lignins, the three key factors responsible for disease resistance (Vidhyasekaran 1988). In the present study β1,3-*glucanase*was higher in Pf1-treated plants challenge-inoculated with pathogen indicating that it might have resulted in the lysis of invading pathogen. Similar reports was found by Samia and Khallal (2007) where Peroxidase (POX), Polyphenols oxidase (PPO) and PAL significantly increased in tomato plants in response to *Fusarium oxysporum*. However, JA-treated plants (alone or combined with AM fungi) recorded the highest POX and PPX activities than the other infected plants. Similar results were investigated where in a higher peroxidase activity of 1.43 was observed in plants after application of 1 mM SA followed by the inoculation of *Alternaria alternata* pathogen in peanut (Chitra *et al.*, 2008).

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How to cite this article:

Suryakanth, Venkatesh, N. S. Pankaja, N. Umashankar Kumar, J. Mahadeva and Sugeetha, G. 2020. Effect of Biotic and Abiotic Elicitors in Inducing Resistance against Cowpea Rust. *Int.J.Curr.Microbiol.App.Sci.* 9(11): 3573-3581. doi: https://doi.org/10.20546/ijcmas.2020.911.428