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Changes of Antioxidant Defense Related Enzymes of *Vigna radiata* (L.) R. Wilczek in Response to Salicyclic Acid and Jasmonic Acid Against *M. phaseolina*

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ABSTRACT

Charcoal rot is a severe problem on mungbean, and its control is mostly dependent on the use of chemical pesticides. However, this is not a long-term solution due to environmental concerns and the possibility of residues. Induced resistance is one of the most important mechanisms for treating disease under these circumstances, since it increases the activity of different defense-related enzymes and non-enzymatic antioxidants. The effects of inducers such as salicyclic acid (SA) and jasmonic acid (JA) at low, medium, and high concentrations, viz. JA (1mM, 2.5 mM, 4 mM) and SA (0.5 mM, 1 mM, 2 mM), on the induction of resistance to manage In a net house, the illness of mungbean charcoal rot was researched in three different varieties: resistant, moderately sensitive, and vulnerable. When compared to the water sprayed control on un-inoculated plants, elicitor treatments had the highest levels catalase and phenylalanine ammonia lyasecontent SA@2 mM was the most effective treatment for raising catalase and phenylalanine ammonia lyasecontent content, followed by JA for disease resistance. These modifications can be traced to the work of inducers, and it is a well-known fact that elicitors were utilised to help plants improve their defence mechanisms.

Key words: *Salicyclic acid, Jasmonic acid, Antioxidant enzymes, Charcoal rot, Mungbean,*

Introduction

Mungbean is a major pulse crop in our country. Mungbean is farmed in India throughout three seasons. The three seasons are Kharif, Rabi, and Summer. It is also grown in rainfed situations during kharif and residual moisture during rabi in the eastern and southern areas of India. The seed rate of mungbean in the kharif season is 10-15 kg/ha, whereas the seed rate in the spring season is 20-3 kg/ha (Chadha, 2010). Polyphenolic compounds found in mungbean include simple phenols, flavonoids, and tannins, all of which are natural antioxidants (Prior and Gu, 2005; Sanos Bulega and Scalbert, 2000; Amarowicz *et al.,* 2004 and Troszunska and Cisa, 2002). However, biotic factors are responsible for up to 44% – 60% of pulse crop losses (Deshkar *et al.,* 1974; Bashir and Malik, 1988). Mungbean charcoal rot is caused by *Macrophomina phaseolina*, which decreases crop output, especially in arid settings (Charles 1978; Hoes., 1985).

Mungbean is the world's most important legume crop (*Vigna radiata* L. Wilzeck). Vigna is a member of the Leguminoseae family and the Papilinoidae subfamily. It is mostly grown in Asia, although it has recently become popular in Africa and the Americas. *Vigna radiata*, on the other hand, has a high protein content and is eaten as sprouts and dry seeds. Due to

its rapid growth and early maturity features, as well as its ability to replace soil nutrients, mungbean is an useful crop that is often grown in dry and semi-arid areas. Mungbean's main feature is that it reduces fertiliser use and provides nitrogen fertiliser to agriculture fields in short supply, while also strengthening soil structure and providing plant protein. However, under stress, flowering and maturity times are shortened compared to well-watered conditions. Due to erratic annual rainfall and a lack of source management, crop yields are substantially affected.

Plants are affected by *Macrophomina phaseolina* in the root, stem, branches, petiole, leaves, pods, and seeds. Furthermore, *Macrophomina phaseolina* seed infection ranges from 2.2-15.7 percent, resulting in a 10.8% reduction in grain yield and a 12.3% reduction in protein content in urdbean (Kaushik *et al*., 1987). In mature plants, *Macrophomina phaseolina* creates red to brown lesions on the roots and stems. As a result of the dark mycelia and black microscelerotia, plants got defoliated and wilted (Abawl and Pastor- Corrales, 1990). *Macrophomina phaseolina* is a heat tolerant pathogen in the 60-65°C temperature range (Bega and Smith, 1962: Milhail and Acron, 1984). In its imperfect form, *Macrophomina phaseolina* (Whittaker, 1969) causes charcoal rot, and in its perfect form, *Sclerotium bataticulum* Taub causes charcoal rot (Butl.).

Hyaline hyphae with thin walls to light brown or dark brown hyphae with septa describe *M. phaseolina*. On parent hyphae, branches from the main hyphae are usually generated at a right angle, with constriction at the point of origin. Microsclerotia are spherical, oval, or oblong masses of hardened fungal mycelium that are light brown when young and darken (brown to black) as they age. Pycnidia are larger than microsclerotia, dark brown to black, rough, globose or irregular, beaked, and ostiolated, and are rarely seen in nature (Lakhran *et al*., 2018). On synthetic media, the fungus can demonstrate wide variation in mycelium colour, microsclerotia distribution, pycnidia production, and chlorate phenotypes. Despite this, amplification of the internal transcribed spacers (ITS) revealed that isolates were all from the same species (Almomani *et al*., 2013). With the consistent supply of highly potent and newer broad spectrum fungicides in recent decades, indiscriminate constant use of diverse pesticides has become the most essential component of mungbean disease management strategy. The use of environmentally appropriate elicitors in plant disease management has gained importance and attention as a result of these situations. Elicitors are molecules that activate chemical defence in plants at low concentrations; they work as signal substances that tell the plant when it's time to use chemical defence. Boller, 1993; Ebel and Cosio, 1994). The current work examines phenolic accumulation, ortho-dihydroxy phenols, and the action of a putative oxidant scavenger: ascorbic acid against *Macrophomina phaseolina* in mungbean genotypes. The plant's resistance, moderately susceptible, and susceptibility reactions may be related to the plant's variable metabolomics responses, according to the experiment. Where the resistance reaction was mostly caused by increased defence metabolites in plant.

Materials and Methods

Plant materials and pathogen inoculation

As done by Biswas *et al*., the seeds of three genotypes of mungbean (*Vigna radiata*) viz., resistant, moderately susceptible, and susceptible viz., Bireswar, 2-sukumar 3, and Samrat were surface sterilised by 1.0 percent sodium hypochlorite and sown post seed treatment with various elicitors with different concentrations *viz*. JA (1mM, 2.5 mM, 4 mM) and SA (0.5 mM, 1 mM, 2 mM), The infected stems and leaves were isolated and symptoms were observed. The stems and leaves were properly cleaned with tap water to remove dirt. Infected plant parts were cut into small pieces (0.5-1.0 mm) and surface sterilised with a 0.1 percent mercuric chloride solution for 2 minutes, then rinsed three times with sterilised glass distilled water and blot dried. Aseptically, one piece of potato dextrose agar (PDA) was given to each slant. The pathogen was then cultured for four to five days at 30 °C in culture tubes. For the pot experiment, mungbean plants of a specific genotype (15 days old) were infected with a mycelial suspension of 3 days old culture generated on potato dextrose broth, filtered and homogenised to give $1X10^3$ viable propagules per millilitre. With a hand atomizer, this suspension was sprayed on the mungbean plants' foliage until it ran off. Diseased samples were taken after retaining the inoculated seedlings at high humidity for 150 hours to assess catalase and phenylalanine ammonia lyasecontent. Plants in the control group were

grown and sprayed with distilled water.

Treatment details

T1: Seed treatment with elicitors, with pathogen inoculation
T2: Seed treatment with elicitors, without pathogen inoculation
T3: Without seed treatment (water), with pathogen inoculation
T4: Without seed treatment (water), without pathogen inoculation

Biochemical studies

Mungbean seedlings were collected from different treatments and the catalase and phenylalanine ammonia lyasecontent of were estimated at 15 DAS, 18 DAS and 5 DAI.

Determination of Catalase

The drop in absorbency (decomposition of H_2O_2) at 240 nm was used to evaluate CAT activity. In 970 l phosphate buffer, 20 l H_2O_2 (500 mM) and 10 l enzyme extract were added to the reaction mixture (50 mM, pH 7). The extinction coefficient of 36 M^{-1} cm1 for H_2O_2 was used to compute CAT activity, which was represented as mol min1 mg1 protein.

Determination of phenylalanine ammonia lyase (PAL)

Total phenylalanine ammonia lyase activities were estimated from the samples collected thrice as per Sadasivam and Manickam, 1992). Pipette out 1.9 ml 0.1 M buffer, 1 ml of 10mM phenylalanine ammonia lyase and 0.1 ml of enzyme extract in a cuvette. Use 2 ml of buffer for preparation of blank. Mix well. place the cuvette in the spectrophotometer and adjust the absorbance at 250 nm. Record the observations every 15 minutes for upto 7 readings.

Results and Discussion

The effect of elicitors on phenol accumulation and total ascorbic acid content of three genotypes of mungbean (*Vigna radiata*) viz., resistant, moderately susceptible, and susceptible viz., Bireswar, 2-sukumar 3, and Samrat against charcoal rot caused by *Macrophomina phaseolina* was investigated. Elicitors used at various concentrations reduced disease incidence and demonstrated a significant difference between the observed defence related compounds (Table 1-2 and Fig. 1-2). It was discovered that increasing the concentration of the different elicitors resulted in a significant increase in defence related compounds in both pathogen and nonpathogen inoculated plants, and the differences were significant in all three genotypes.

It is evident from the, (Table 1 and Fig. 1) however, the accumulation of Catalase was maximum inBireswarmungbean genotype when compared to the other two genotypes *i.e.*, JA@2.5 Mm 5.64 µmol of H_2O_2/min/mg of protein, JA@4 Mm 7.88 µmol of H_2O_2/min/mg of protein and JA@4 Mm 6.90 ìmol of H_2O_2/min/mg of protein, when compared to SA@1 Mm 4.09 µmol of H_2O_2/min/mg of protein, JA@4mM 5.00 µmol of H_2O_2/min/mg of protein JA@2.5 Mm 4.59 µmol of H_2O_2/min/mg of protein and JA @2.5Mm 2.98 µmol of H_2O_2/min/mg of protein, JA @4 Mm 4.05 µmol of H_2O_2/min/mg of protein, JA@2.5 Mm 3.64 µmol of H_2O_2/min/mg of pro-

Table 1. Impact of various elicitors on mungbean genotypes on Catalase

Treatments	Catalase (µmol of H_2O_2/min/mg of protein)								
	Bireswar (Resistant)			2- sukumar 3 (Moderately susceptible)			Samrat (Susceptible)		
	15 DAS	18 DAS	5 DAI	15 DAS	18 DAS	5 DAI	15 DAS	18 DAS	5 DAI
JA @ 1 mM	5.64	7.53	6.54	3.53	4.65	4.29	2.69	3.56	3.35
JA @2.5 mM	5.93	7.82	6.84	3.82	4.94	4.59	2.98	3.86	3.64
JA @ 4 mM	5.66	7.88	6.90	3.55	5.00	4.31	2.71	4.05	3.37
SA @ 0.5 mM	5.52	7.41	6.75	3.40	4.53	4.37	2.90	3.64	3.49
SA @ 1 mM	5.53	7.42	6.44	4.09	4.54	4.52	2.58	3.57	3.51
SA @ 2 mM	5.54	7.43	6.44	3.42	4.57	4.39	2.92	3.46	3.25
CONTROL	5.35	7.24	6.25	3.24	4.36	4.01	2.40	3.28	3.06
SEM	0.10	0.13	0.20	0.16	0.13	0.18	0.20	0.17	0.16
CD	0.30	0.39	0.60	0.47	0.40	0.53	0.60	0.52	0.49
CV	3.07	2.95	5.15	7.39	4.80	6.87	12.25	7.98	8.08

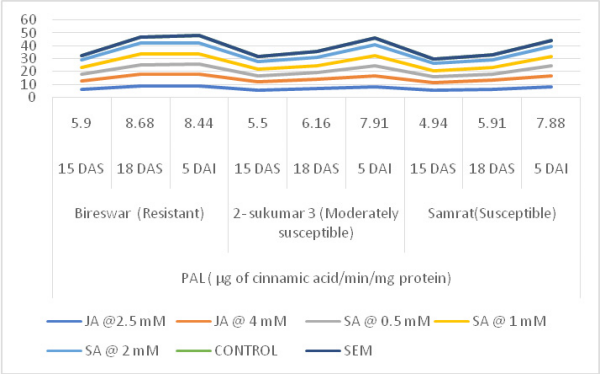

Fig. 1. Graphical representation of impact of elicitors on catalase in selected genotypes

tein of 2-sukumar 3 and Samrat genotypes at 15 DAS, 18 DAS and 5 DAI respectively.

It is evident from the, (Table 2 and Fig. 2) however, the PAL was maximum in Bireswarmungbean genotype when compared to the other two genotypes *i.e.*, JA@2.5 Mm 6.19 µg of cinnamic acid/min/mg protein, JA@4 Mm 9.38 µg of cinnamic acid/min/mg protein and JA@4 Mm 9.41 µg of cinnamic acid/min/mg protein, when compared to JA@4 Mm 6.33 µg of cinnamic acid/min/mg protein, JA@4mM 7.31 µg of cinnamic acid/min/mg protein JA@4 Mm 8.88 µg of cinnamic acid/min/mg protein and JA @4Mm 5.75 µg of cinnamic acid/min/mg protein, JA @4Mm 6.81 µg of cinnamic acid/min/mg protein, JA@4 Mm 8.57 µg of cinnamic acid/min/mg protein of 2-sukumar 3 and Samrat genotypes at 15 DAS, 18 DAS and 5 DAI respectively.

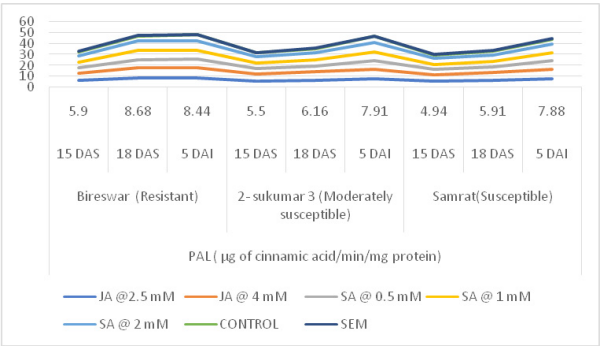

Fig. 2. Graphical representation of impact of elicitors on ascorbic acid content in selected genotypes of mungbean

From the above findings, The production of ROS such as singlet oxygen (O_2), superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) is one of the first responses of plants to an attempted infection. Hypersensitive response (HR) (Lamb and Dixon 1997), phytoalexin production (Daudi *et al.* 2012), callose deposition (O'Brien *et al*. 2012), and systemic acquired resistance (SAR) have all been linked to reactive oxygen species (ROS) (Alvarez *et al*. 1998). ROS molecules also play important roles in cell signalling, some physiological processes, and as second messengers for the activation of genes encoding protective proteins (Mendoza, 2011). It has been demonstrated that ROS accumulation plays a critical role in a variety of non-host interactions such as barley/*Blumeriagraminisf. sp. tritici* (Hückelhoven *et al*. 2001), cowpea/*Erysiphe cichoracearum* (Mellersh *et al*. 2002), and pepper/*Blumeriagraminis* f. sp. *tritici* pathosystems (Hao *et al*. 2011).

Conflict of Interest

There are no conflict of interests to declare to publish this article.

Table 2. Impact of various elicitors on mungbean genotypes on PAL content

Treatments	PAL (µg of cinnamic acid/min/mg protein)								
	Bireswar (Resistant)			2- sukumar 3 (Moderately susceptible)			Samrat (Susceptible)		
	15 DAS	18 DAS	5 DAI	15 DAS	18 DAS	5 DAI	15 DAS	18 DAS	5 DAI
JA @ 1 mM	5.90	8.68	8.44	5.50	6.16	7.91	4.94	5.91	7.88
JA @2.5 mM	6.19	8.72	8.73	5.74	6.71	8.11	5.61	6.47	7.96
JA @ 4 mM	6.71	9.38	9.41	6.33	7.31	8.88	5.75	6.81	8.57
SA @ 0.5 mM	5.05	7.21	7.76	4.98	5.25	7.78	4.80	5.08	7.82
SA @ 1 mM	5.34	8.28	7.96	5.20	5.72	7.89	4.72	5.33	7.33
SA @ 2 mM	5.56	8.39	8.38	5.67	6.25	8.07	5.55	5.67	7.62
CONTROL	3.58	4.89	5.86	3.54	4.40	5.61	3.17	3.76	4.72
SEM	0.26	0.49	0.30	0.25	0.28	0.21	0.27	0.33	0.42
CD	0.80	1.49	0.92	0.75	0.85	0.63	0.81	1.01	1.28
CV	7.87	10.09	6.21	7.70	7.76	4.44	8.86	9.83	9.28

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