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Estimation of biochemical factors responsible for resistance to root and collar rot (*Macrophomina phaseolina* (Tassi) Goid.) in okra

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Abstract

Twenty okra genotypes were screened for their resistance to root and collar rot incited by *Macrophomina phaseolina* in the experimental fields of Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University. Based on their disease reaction, they were grouped into four categories *i.e.*, resistant, moderately resistant, susceptible and highly susceptible. The biochemical analysis of the resistant and susceptible genotypes revealed that the total soluble sugar, reducing sugar and non reducing sugar decreased following inoculation by the pathogen. The decrease in total soluble sugar, reducing sugar and non reducing sugar were 21.54, 21.73 and 21.42 per cent in resistant variety, whereas in highly susceptible variety it was 6.06, 6.10 and 3.03 per cent, respectively. The total phenol content, soluble protein and peroxidase activity increased on inoculation with the pathogen. In the resistant variety, the increase in phenol content, soluble protein and peroxidase activity was 23.4, 32.56 and 63.86 per cent whereas in moderately resistant variety this was 19.85, 21.63 and 55.29 per cent, respectively. Susceptible and highly susceptible genotypes had low to moderate increase in the three parameters. The experimental results confirmed the importance of these biochemical factors in imparting the host resistance to root and collar rot.

Keywords: Okra, *Macrophomina phaseolina*, Phenol, Sugar, Protein, Peroxidase

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INTRODUCTION

Okra (*Abelmoshus esculentus* (L.) Moench) is an important annual vegetable belonging to *Malvacea* family. Okra is known by different names *viz.*, ladies finger, bhindi, bamia, okro or gumbo in different parts of the world. It is mainly grown in India, Nigeria, Sudan, Pakistan, Ghana, Egypt, Saudi Arabia, Mexico and Cameroon. India rank first in area and production followed by Nigeria. In India, it is grown in an area of 0.528 million ha with an annual production of 6.14 million tonnes. West Bengal is the leading producer followed by Gujarat (Anonymous, 2017). There is a great scope of increasing the okra production in the country, but some biotic factors like diseases hamper the successful cultivation. The major diseases affecting the crop include yellow vein mosaic, powdery mildew, *Cercospora* leaf spot, etc. Root and collar rot incited by *M. phaseolina* is emerging as a major threat to okra cultivation especially in areas having low rainfall and prolonged dry spell. *Macrophomina*

phaseolina is one of the most damaging primarily soil borne pathogens having heterogeneous host specificity. It infects about 500 plant species in more than 100 families throughout the world (Singh *et al.*, 1990). The present study was undertaken to evaluate the biochemical factors like total soluble sugar, total phenol, soluble proteins and peroxidase responsible for imparting resistance to root and collar rot in okra against *M. phaseolina*. This would help in further validation of field generated data on disease resistance of different okra genotypes. Resistant germplasm may further be used in breeding programmes for development of disease resistant commercial cultivars.

MATERIALS AND METHODS

Twenty okra genotypes were screened for resistance to root and collar rot incited by *Macrophomina phaseolina* for two seasons (Summer and *Kharif*, 2016) at the experimental field of Department of Pathology, B. A. College of Agriculture, Anand Agriculture University, Anand.

Based on the disease reactions, the genotypes were categorized into resistant, moderately resistant, moderately susceptible and susceptible as per Raj and Prasad (1975). One genotype from each category [(AOL-15-23– Resistant; AOL-15-21– Moderately resistant; AOL-13-96– Susceptible; AOL-12-55– Highly susceptible)] were selected for biochemical analysis and two replications were maintained for each genotype. The okra plants for the biochemical tests were raised in the pots contaminated with the pathogen mass multiplied in sand-maize medium. Collar portion from 30 days old seedlings was used for the various assays.

Total soluble sugar: Total soluble sugars from the resistant and susceptible okra varieties were determined by phenol sulphuric acid method as described by Bhatnagar *et. al.* (2005). Two hundred mg sample was digested in five ml 80 per cent alcohol and incubated for two hours on shaker. Then it was centrifuged and 1 ml of the clear solution was taken in test tube and evaporated to dryness in boiling water bath. One ml distilled water was added and used as sample. Working standards (0.2, 0.4, 0.6, 0.8 and 1 ml) were pipetted into series of test tubes. 0.1 and 0.2 ml of the sample solution was pipetted into two separate test tubes and volume was made upto 1 ml with distilled water. A blank of 1 ml of distilled water was also prepared. Then one ml of phenol was added to each test tube followed by five ml 96 per cent sulphuric acid to each test tube and shaken well for 10 min. The test tubes were then placed in a water bath at 25-30 °C for 20 min. The colour development was measured at 490 nm. TSS present in the samples were calculated from the standard graph.

Reducing sugars: Reducing sugars of plant samples was estimated by Nelson's modification of Somogyi's method (Nelson, 1944). Hundred mg sample was weighed and sugars were extracted with hot 80 per cent ethanol twice (5 ml each time). Supernatant was collected and evaporated by keeping over water bath at 80 °C. 10 ml water was added to dissolve the sugars. 0.1 and 0.2 ml of the aliquot and working standard (0.2, 0.4, 0.6, 0.8 and 1 ml) were pipetted into test tubes and volume was made up to two ml with distilled water. Two ml distilled water served as blank. One ml of alkaline copper tartrate was added into each tube and kept in boiling water for 10 min. One ml of arsenomolybdate acid reagent was added to test tubes after cooling. The volume of the test tubes were made up to 10 ml and absorbance was measured at 620 nm after 10 min. The amount of reducing sugar was calculated from standard graph. The results were expressed as mg/g fresh weight.

Total phenol content: Total phenol content from the resistant and susceptible okra plants was

estimated by Folin ciocalteau method as described by Malik and Singh (1980). The dilute extracts of different concentrations were taken in 10 ml glass tubes and total volume made to three ml with distilled water. These were then mixed with 0.5 ml Folin – Ciocalteau reagent (1:1 with water) and 2 ml Na₂CO₃ (20 %). The tubes containing the blue solutions were warmed for one minute, cooled and absorbance was measured at 650 nm against the reagent blank. The standard curve was prepared using known concentrations of catechol at 650 nm. The total phenol content in the test samples was calculated from the standard curve and expressed as mg/g fresh weight.

Soluble protein: Soluble protein was estimated using Lowry method (Lowry *et al.* 1951). Forty mg collar region of okra plants were weighed and transferred to a digestion flask. One gram catalyst mixture and two ml concentrated sulphuric acid were added and the mixture was digested until the solution turn colourless (approx. 40 min. at 370 °C). After cooling minimum quantity of water was added to dissolve the solids and allowed to cool. Ten ml of boric acid was pipetted into 100 ml Erlenmeyer flask and two to three drops of the indicator was added. The flask was then placed under the condenser extending below the surface of the solution. The digest was transferred to distillation apparatus and the flask was rinsed four times with two to three ml of distilled water. Ten ml of sodium hydroxide-sodium thiosulphate solution was added to still and stem distilled until 20 ml of distillate collected (20 min.). The receiving flask was lowered and distillation was continued for one more time tip of the condenser was washed with few of drops water and receiving flask was lowered. The contents of the receiving flask were titrated to grey endpoint/ first appearance of violet colour. A blank titration was determined (without sample) using the same quantity of reagents and same digestion and distillation period as for sample determination. A standard check of tyrosine was also made (10 mg/l).

Percentage of nitrogen (%N) = $\frac{(\text{ml of HCl in determination} - \text{ml blank}) \times \text{normality} \times 100}{14.007 \times \text{mg sample}}$ Eq.1

Protein (%) = % N x 6.25Eq.2

Peroxidase: The peroxidase activity was estimated as per the method suggested by Costa *et al.* (2002). Exactly, 300 mg of plant tissue was taken and homogenised with one ml of protein extraction buffer (SDS 0.02%, Tris buffer 0.5M and β-Mercaptoethanol 1%) and vortexed in two ml centrifuge tubes. These samples were centrifuged at 13,000 rpm for 10 minutes. Clear supernatant was collected and used for analysis. The reaction mixture contained 2.90 ml of 0.03 per cent H₂O₂ (substrate) in 0.01 M phosphate buffer (pH 6.0), 25 µl of one per cent ortho-dianisidine in methanol and 100 µl of enzyme extract. The

reaction was initiated by adding the enzyme. The change in colour of oxidized dye was read at 460 nm up to three minutes, at an interval of 15 seconds. Blank was run without the addition of substrate. The enzyme activity was expressed as change in OD/min/fresh tissue.

RESULTS AND DISCUSSION

Total soluble sugar, total phenol content, soluble protein and peroxidase activity was determined before and six days after the inoculation by the pathogen and results are presented in table 1 and 2.

Total soluble sugar: The total soluble sugar was found to be high in the resistant genotype followed by the moderately resistant ones. Total soluble sugar before inoculation by the pathogen was found in range of 2.59 mg/g in highly susceptible genotype (AOL-12-55) to 6.64 mg/g in resistant genotype (AOL-15-23). The moderately resistant (AOL-15-21) and susceptible genotype (AOL-13-96) had TSS of 5.41 and 3.78 mg/g respectively. After inoculation with the pathogen, the TSS was found to decrease in all the genotypes with highest percentage decrease recorded in the resistant genotype (21.54%) followed by the moderately resistant genotype (17.71%). In susceptible and highly susceptible genotype TSS decreased by 13.41 and 6.06 per cent respectively.

The reducing and non reducing sugar also had a similar trend as that of total soluble sugar. Reducing sugar was found to be highest in resistant genotype both before (2.58 mg/g) and after (2.03 mg/g) inoculation and showed a reduction by 21.73 per cent. Initial reducing sugar content in moderately resistant, susceptible and highly susceptible genotypes were 2.04, 1.45 and 1.20 mg/g which decreased by 15.57, 12.05 and 6.10 per cent, respectively after inoculation.

Non reducing sugar content in the plants was found to be higher than reducing sugar content. The non reducing sugar content in resistant geno-

type decreased from 4.04 to 3.18 mg/g (21.42%) after inoculation by the pathogen. The non reducing sugar content decreased by 19.01, 14.25 and 6.03 per cent in moderately resistant, susceptible and highly susceptible genotypes, respectively. The decrease in sugar content after inoculation by pathogen may be attributed to increased respiration which leads to breakdown of sugar for the synthesis of defence related compounds like proteins and phenols. Since the respiration increase at a higher rate in the resistant genotype than the susceptible genotype the decrease in sugar content is high in resistant genotype compared to susceptible ones.

The similar conclusion were reported by El- Fikri *et al.* (2004) who reported that the sesamum varieties resistant to charcoal rot had higher soluble sugar content (3.22-8.38%) compared to the susceptible varieties (1.18-3.97%). Sandhu *et al.* (1998) has reported that the total soluble sugar and reducing sugar content decreased upon infection by the pathogen. According to Shahabuddin and Anwar (1992) there was a decrease in total sugar and reducing sugar in jute following inoculation with *M. phaseolina*. Patil *et al.* (1985) reported that in tolerant genotypes the sugar level was two to three times higher than the susceptible ones.

Total phenol content: The total phenol content was found to be higher in the resistant genotype followed by the moderately resistant ones and increased upon inoculation with the pathogen (Table 2). In the resistant genotype, the total phenolics increased by 23.40 per cent from 1.41 mg/g in control plants to 1.74 mg/g after inoculation with the pathogen. The second highest percentage increase of total phenolics was recorded in the moderately resistant genotype (19.85%). The susceptible and highly susceptible genotypes had only a minor increase in total phenol content viz., 8.62 and 5.68 per cent respectively.

Phenolics are major antifungal and antibacterial defence molecule in many plant species including

Table 1. Effect of *M. phaseolina* infection on total soluble sugar, reducing sugar and non reducing sugar.

Disease Reaction	Before Inoculation (mg/g)			After Inoculation (mg/g)			Decrease (%)		
	Total Soluble Sugar	Reducing Sugar	Non Reducing Sugar	Total Soluble Sugar	Reducing Sugar	Non Reducing Sugar	Total Soluble Sugar	Reducing Sugar	Non Reducing Sugar
Resistant	6.64	2.58	4.04	5.21	2.03	3.18	21.54	21.73	21.42
Moderately Resistant	5.41	2.04	3.36	4.45	1.73	3.36	17.71	15.57	19.01
Susceptible	3.78	1.45	2.33	3.27	1.28	2.33	13.41	12.05	14.25
Highly Susceptible	2.59	1.20	1.39	2.43	1.13	1.31	6.06	6.10	6.03

Table 2. Effect of *M. phaseolina* infection on total phenol, soluble protein and peroxidase activity

Disease Reaction	Before Inoculation			After Inoculation			Increase (%)		
	Phenol (mg/g)	Protein (%)	Peroxidase (Δ OD/min/g of fresh tissue)	Phenol (mg/g)	Protein (%)	Peroxidase (Δ OD/min/g of fresh tissue)	Phenol	Protein	Peroxidase
Resistant	1.41	6.35	2.83	1.74	9.41	4.64	23.40	32.56	63.86
Moderately Resistant	1.24	5.47	2.77	1.49	6.98	4.30	19.85	21.63	55.29
Susceptible	1.08	5.03	2.48	1.17	5.58	3.35	8.62	9.80	34.79
Highly Susceptible	1.01	4.37	2.46	1.06	4.59	3.25	5.68	4.76	32.07

okra. Many of the phenolics are directly toxic to the pathogens, while others strengthen the cell wall and resist the hydrolytic enzymes of the pathogen (Singh, 2009). The results of the present study further signifies importance of faster accumulation of phenolics in imparting resistance to the pathogen in the resistant genotype.

Kumari *et al.* (2015) has reported a positive correlation between polyphenol content and resistance to the charcoal rot resistance in sorghum cultivars. Similarly, Doley and Jite (2013) has reported an increase in total phenolics in groundnut plants upon infection with *M. phaseolina*. According to the report of Sharma (2011), *Macrophomina* infection induced a significant increase in the polyphenols, polyphenol oxidases and PAL activity in resistant sesamum cultivars. Similar conclusions on increase in polyphenol content in jute upon infection by *Macrophomina* were made by Shahabuddin and Anwar (1992).

Soluble protein: The soluble protein content in the uninoculated plants ranged from 4.37 per cent in highly susceptible genotype to 6.35 per cent resistant genotype. After inoculation with the pathogen, there was an increase in protein content by 32.56, 21.63, 9.80 and 4.76 per cent in resistant, moderately resistant, susceptible and highly susceptible genotypes respectively. In resistant genotype the protein content rose from 6.35 to 9.41 per cent whereas in moderately resistant genotype it rose from 5.47 to 6.98 per cent. The susceptible and highly susceptible genotypes recorded only minor increase of 9.80 and 4.76 per cent respectively.

The different types of protein play a major role in defence mechanisms of plant. It may be due to this reason that there was an increase in protein content in the infected plants. Since the respiration and synthesis of defence related metabolites in resistant varieties rise rapidly, there is a faster accumulation of protein and thereby increase in protein percentage in plant system following infection by the pathogen.

Kaur *et al.* (2008) has reported that the protein content in maize cultivars moderately resistant to charcoal rot was higher (10.78%) compared to the moderately susceptible cultivars (10.19%). According to Doley and Jite (2013) the protein content in the groundnut plants inoculated with *Macrophomina* was higher compared to the control plants. Sharma (2011) has reported an increase in all defence related protein in sesamum to varying levels of infection by *M. phaseolina*.

Peroxidase: Initially the peroxidase activity was 2.83 in resistant genotype while it was 2.46 in the highly susceptible genotype and it increased after inoculation. The activity in susceptible and moderately resistant genotypes ranged from 2.48 to 2.77 units. The increase in activity was highest

in case of resistant varieties *i.e.* 63.86 percent followed by the moderately resistant genotype (55.29%). In susceptible genotype, the activity increased from 2.48 to 3.35 *i.e.* by 34.79 per cent, whereas this increase was only 32.07 per cent in highly susceptible genotype.

Peroxidase is one of the key enzymes in plant defence mechanisms. It breaks down the hydrogen peroxide in plant system and the increase in peroxidase activity positively correlated to the level of disease resistance in plants. Kumari *et al.* (2015) has reported higher peroxidase activity in cultivars of sorghum resistant to charcoal rot thereby sustaining lesser oxidative damage. According to Abhoshasha *et al.* (2008) the inoculation of sunflower with *M. phaseolina* resulted in a higher peroxidase activity in the resistant cultivars. Ashraf *et al.* (2005) has reported that in chickpea, infection with *M. phaseolina* increased the peroxidase activity and this increase was highest in the resistant varieties (45.24%) followed by the moderately resistant varieties (39.62-42.52%). While in susceptible increase was only 13.51 to 25.54 per cent over control.

Conclusion

It can be concluded that the various biochemical parameters namely sugar, soluble protein, total phenol and peroxidase activity play a major role in host resistance against *M. phaseolina* in okra. Moreover, these biochemical parameters will help us in further validation of field generated data on evaluation of disease resistance various pests and diseases. However, more detailed investigation on the exact role played by these biomolecules in the host resistance would help us in manipulating them for improving the crop resistance to various biotic agents. The genotypes (AOL-15-23 & AOL-15-21) which showed resistant/ moderately resistant reaction may be used for breeding programmes for the development of disease resistant commercial cultivars to effectively tackle the root and collar rot of okra.

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