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Biochemical analysis of cotton genotypes infected with Alternaria leaf spot disease

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ABSTRACT: An investigation was made at Regional Agricultural Research Station, Lam, Guntur to analyze cotton genotypes with varied field reaction to Alternaria leaf spot during *kharif* 2012-2013. Total sugars, reducing sugars, proteins, phenols, peroxidise and phenylalanine ammonialyase were estimated by employing standard protocols. Biochemical analysis of four cotton genotypes *viz.,,* Tulasi 144 BG II (resistant), KCH 14K 59 (moderately resistant), NCS 9605 (moderately susceptible) and NA 1325 (susceptible) revealed that the amount of reducing sugars, total sugars, total proteins, total phenols, defence enzymes *viz.,,* peroxidase and phenylalanine ammonialyase were highest in resistant genotypes followed by moderately resistant, moderately susceptible and susceptible genotypes, respectively.

Key words : Alternaria leaf spot, biochemical analysis, cotton, genotypes, resistance

Cotton is one of the most important commercial crops of the world, referred to as "King of Fibres" and also known as "White Gold". India produced 400 lakh bales of 170kg lint in 2014-2015 from an area of 126.55 lakh ha with a productivity of 537kg/ha (Anonymous, 2015). Leaf spot/blight caused by *Alternaria macrospora* Zimm is the most commonly occurring disease in Andhra Pradesh causing losses to the tune of 38.23 per cent in cotton variety LRA 5166 (Bhattiprolu and Prasada Rao, 2009).

The varieties of agricultural plants differ in their range of resistance to phytopathogens. Phenolic compounds are known to govern disease resistance in many crop plants. Activity of polyphenol oxidase (PPO), peroxidase (POX), and phenylalanine ammonialyase (PAL) increases in disease resistant plants. Biochemical analysis of different crop plants revealed differences between infected and uninfected plants. However the information in cotton *Alternaria* interaction is scanty. Hence an attempt was made to study the affect of *Alternaria* infection on cotton plants through biochemical analysis and understand the role of different compounds in determining disease reaction and their significance in disease resistance.

Cotton leaves of genotypes with different disease grades *viz.,,* resistant (Tulasi 144 BG II), moderately resistant (Jadoo BG II), moderately susceptible (NCS 9605) and susceptible (NA 1325) were subjected to biochemical analysis as detailed below :

Estimation of sugars: Total sugars in cotton leaves were estimated following anthrone method. Sugars were extracted twice with hot 80 per cent ethanol, supernatant was evaporated in a water bath at 80^0C and sugars were dissolved in water. The amount of sugars present in the extract was calculated using a standard curve prepared from glucose. The amount of reducing sugars in the leaf sample was calculated by using standard graph.

Estimation Protein : Protein estimation was carried out using Lowry's method. From the standard graph amount of protein in the sample

was calculated and expressed as mg/100 mg of sample.

Estimation of total phenols: One g of cotton leaf sample was homogenized in 10 ml of 80 per cent ethanol and centrifuged. The supernatant was reextracted and then evaporated to dryness. The residue was dissolved in distilled water and Folin Ciocalteau reagent followed by 20 per cent Na_2CO_3 was added to the aliquot. The contents were mixed thoroughly, boiled for exactly one min, cooled and absorbance at 650 nm was measured against a reagent blank. A standard curve was prepared using different concentrations of catechol and the concentration of phenols in the test sample and expressed as mg phenols/100g plant sample on fresh weight basis.

Assay of peroxidase

Enzyme extract: One g of fresh cotton leaf tissue was extracted in 3 ml of 0.1M phosphate buffer pH 7.0 by grinding with a pre cooled mortar and pestle. The homogenate was centrifuged at 18,000 rpm at 5^0C for 15 min. The supernatant was stored on ice for enzyme assay and used within 2-4 h. Enzyme activity was calculated by using the formula,

Enzyme activity units/l $= \frac{500}{\Delta t}$ where Δt is the time required in min to increase the absorbance by 0.1 preceded by an increase by 0.05.

Assay of phenylalanine ammonialyase (PAL): One g of cotton leaves were homogenized in 5 ml of 0.1 M sodium borate buffer (pH 7) containing 0.1 g insoluble polyvinyl pyrolidone (PVP). The extract was filtered through cheese cloth and the filtrate was centrifuged at 10000 rpm for 30 min at 4^0C. The supernatant was used as an enzyme source. PAL activity was determined as the rate of conversion of L-phenylalanine to transcinnamic acid at 290 nm. Samples containing 0.4 ml of enzyme extract were incubated with 0.5 ml of 0.1 M borate buffer pH 8.8 and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30^0C. In the reference cell, 0.4 ml of the inactivated enzyme extract (by boiling) was taken along with one ml of borate buffer. The amount of transcinnamic acid synthesized was calculated using the extinction coefficient of 9630/M/cm. Enzyme activity was expressed on a fresh weight basis (ç mole of transcinnamic acid/ min/g).

Biochemical analysis of different disease grades of cotton genotypes

Sugars: The amount of reducing sugars and total sugars were significantly higher in resistant genotype followed by moderately resistant, moderately susceptible and susceptible genotypes. Irrespective of the nature of reaction of genotype, the amount of reducing sugar and total sugars (Fig 1) was highest in healthy leaves compared to infected leaves.

In resistant hybrid, Tulasi 144 BG II, the amount of reducing sugars and total sugars in healthy leaves were 5.05 mg/g and 7.03 mg/g whereas the infected leaves contained 2.93 mg/g reducing sugars and 4.20 mg/g total sugars, respectively. The amount of reducing sugars and total sugars in healthy leaves of moderately resistant hybrid Jadoo BG II was 4.65 mg/g and 5.27 mg/g while infected leaves recorded 2.03 mg/g and 2.38 mg/g, respectively.

In moderately susceptible hybrid, NCS 9605, the amount of reducing sugars and total sugars in healthy leaves was 3.34 mg/g and 5.16 mg/g whereas the infected leaves contained 1.49 mg/g reducing sugars and 1.92 mg/g total sugars, respectively. The amount of reducing sugars and total sugars in healthy leaves of susceptible variety, NA 1325 was 2.21 mg/g and 4.77 mg/g while infected leaves recorded 0.91

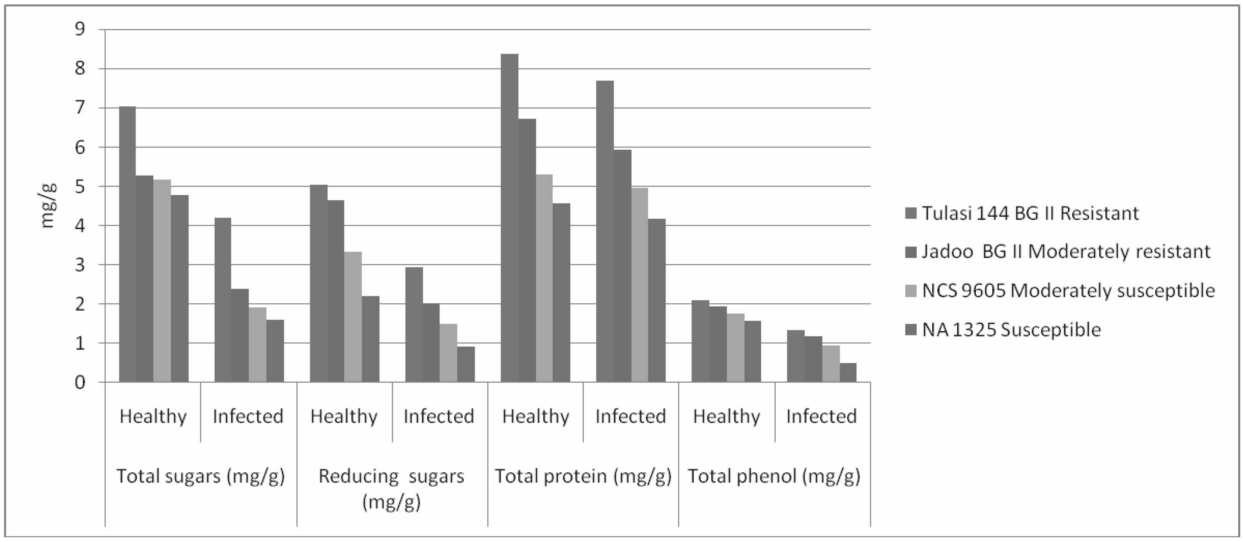

Fig 1. Effect of *Alternaria alternata* on total sugars, reducing sugar, total protein and phenol contents in cotton genotypes

mg/g and 1.59 mg/g, respectively.

Since *Alternaria,* being favoured by low sugars, increased quantity of sugars are recorded in healthy leaves. Further, sugars act as precursor for synthesis of phenolics and reduction in sugars could be due to infection and thus lower amount of phenols in comparison to healthy leaves.

High levels of total sugars, reducing sugars and non reducing sugars in the host plant are stated to be responsible for disease resistance. Present results are in agreement with Hosagoudar *et al.*, (2008b), who recorded the highest amount of reducing sugars and total sugar in healthy leaves compared to *Alternaria* infected leaves of cotton. Chakrabarthy *et al.,* (2002) reported that the concentration of total sugar declined more rapidly in grey mildew susceptible cotton plants than in the resistant plants.

Total protein content: Irrespective of the genotype, the amount of total protein was higher in healthy leaves compared to infected leaves (Fig 1). In resistant hybrid Tulasi 144 BG II, the amount of total protein in healthy leaves was 8.38 mg/g and the infected leaves contained 7.68 mg/g. The amount of total protein in healthy leaves of moderately resistant hybrid Jadoo BG II was 6.27 mg/g and in infected leaves 5.92 mg/g.

Healthy leaves of moderately susceptible hybrid NCS 9605 contained 5.29 mg/g total protein and infected leaves contained 4.97 mg/g. In healthy leaves of susceptible variety NA 1325 (Non *Bt*) was 4.56 mg/g, while infected leaves recorded 4.17 mg/g. Irrespective of the reaction of genotype, the amount of total protein was significantly highest in resistant followed by moderately resistant, moderately susceptible and highly susceptible genotypes. It could be due to decrease in the total protein content in response to infection by foliar pathogens including *A. macrospora*. Hosagoudar *et al.*, (2008b) also found higher amount of total protein in healthy leaves compared to Alternaria infected leaves of cotton.

Total phenol content: The amount of total phenol was higher in healthy leaves compared to infected leaves irrespective of the genotype reaction to *Alternaria*, (Fig. 1). In

resistant hybrid Tulasi 144 BG II, the amount of total phenols in healthy leaves was 2.10 mg/g and the infected leaves contained 1.33 mg/g. The amount of total phenols in healthy leaves of moderately resistant hybrid Jadoo BG II was 1.95 mg/g and in infected leaves 1.19 mg/g. Healthy leaves of moderately susceptible hybrid, NCS 9605 contained 1.76 mg/g total phenols and infected leaves contained 0.95 mg/g. Total phenols in healthy leaves of susceptible variety NA 1325 was 1.58 mg/g, while infected leaves recorded 0.50 mg/g. Irrespective of the genotype, the amount of total phenols was significantly highest in resistant genotype followed by moderately resistant, moderately susceptible and highly susceptible genotypes.

Lower amount of phenols could be due to low production of the precursor *i.e.* sugars in *Alternaria* infected leaves. This could be due to rapid accumulation of phenolic compounds in the healthy leaves at 120 days than the infected leaves to defend the pathogen. Similar kind of findings were recorded by Hosagoudar *et al.*, (2008b), who observed total phenol content was highest in healthy leaves compared to *Alternaria* infected leaves of cotton. High amount of total sugars, reducing sugars, total phenols and amino acids were observed in Jayadhar cotton variety infected with *A. macrospora*. Leaves of bacterial blight resistant cotton cv. 101-102B contained 69 per cent more total phenol than the leaves of susceptible cotton cv. Acala 44 while Hosagoudar and Chattannavar (2008a) also observed decrease in total protein, total phenol, total sugar, reducing sugar and non reducing sugars in bacterial blight infected cotton plants. Biochemical factors governing grey mildew resistance in diploid cotton indicated crucial role of PAL, total phenol and gossypol in governing resistance. The magnitude of induction was invariably higher in resistant lines than in the susceptible plants (Chakrabarty *et al.*, 2002).

Peroxidase activity: PO activity was higher in infected leaves compared to healthy leaves of all the genotypes (Fig. 2). In resistant

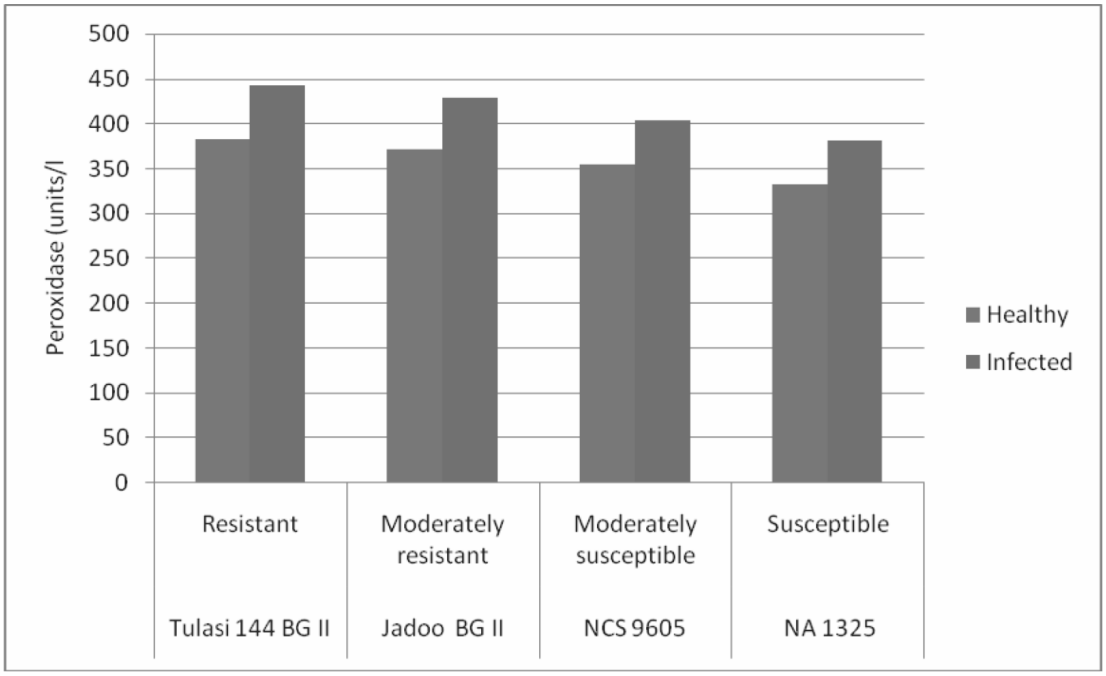

Fig. 2. Effect of *Alternaria alternata* on peroxidise content in cotton genotypes

hybrid Tulasi 144 BG II, the PO activity in healthy leaves was 382.84 units/l and the infected leaves contained 442.48 units/l. The PO activity in healthy leaves of moderately resistant hybrid Jadoo BG II was 371.46units/l and in infected leaves 428.12 units/l. Healthy leaves of moderately susceptible hybrid NCS 9605 contained 354.10 units/l PO and infected leaves contained 403.94units/l. In healthy leaves of susceptible variety NA 1325 was 332.88units/l, while infected leaves recorded 381.04 units/l. Irrespective of the genotype, the amount of PO activity was significantly highest in resistant genotype followed by moderately resistant, moderately susceptible and highly susceptible genotypes. While studying the dynamics of peroxidise activity in *Verticillium* resistant and susceptible varieties of cotton, Egor Pshenichnov *et al.,* (2011) observed increased peroxidise activity in resistant variety. They also recorded higher peroxidae activity in the control resistant variety than the control susceptible variety. Present studies also confirm correlation of peroxidise activity with the level of resistance in cotton genotypes infected with *Alternaria alternata*.

Enhanced PO activity in infected leaves in other pathosystems is well known. PO oxidizes the phenol into quinones, which was not only antimicrobial, but also releases highly reactive free radicals and in that way increases the rate of polymerization of phenolic compounds into lignin like substances. This lignin is deposited in cell wall and helps to prevent further spread of the pathogen.

Phenylalanine ammonialyase (PAL) activity: Irrespective of the reaction of genotype, PAL activity was higher in infected leaves than in healthy leaves (Fig 3). Lowest PAL activity of 1100.7 ç moles of transcinnamic acid/min/g was recorded in healthy leaves of susceptible variety NA 1325 while resistant hybrid Tulasi 144 BG II recorded highest of 1952.2 ç moles transcinnamic acid/min/g which was statistically significant. Similar trend was observed with respect to

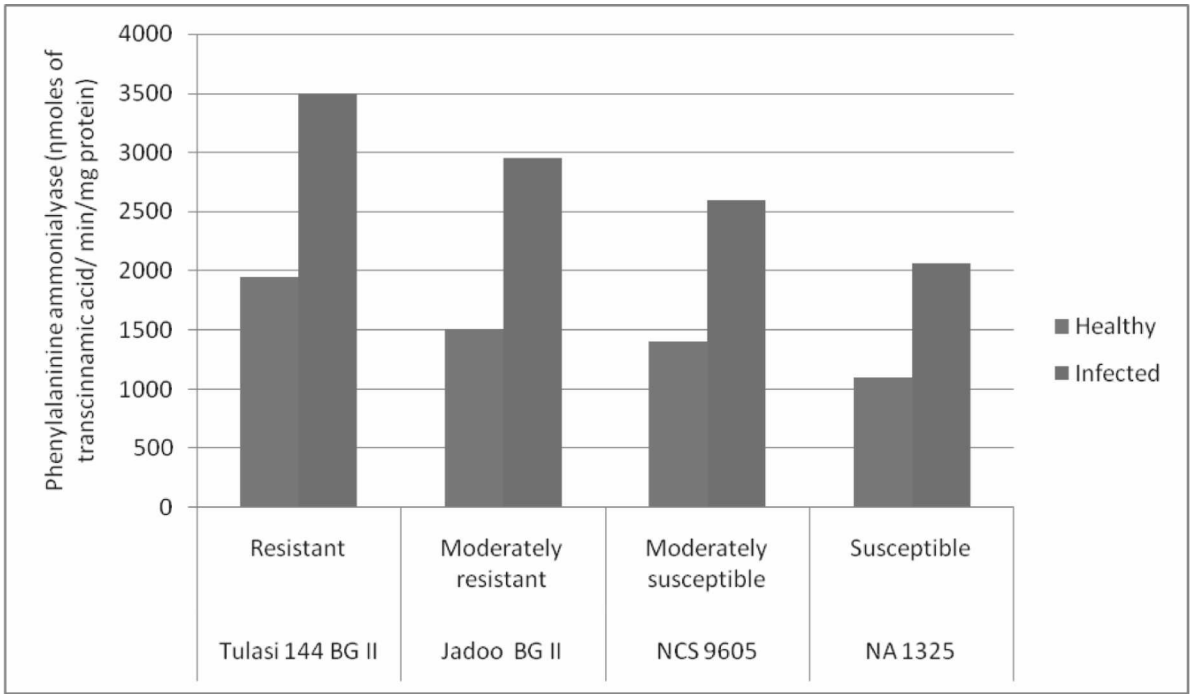

Fig. 3. Effect of *Alternaria alternata* on phenylalaninine ammonialyase content in cotton genotypes

infected leaves of different grades. Comparable results were obtained in other systems. PAL leads to lignin precursors and several secondary plant metabolites, derived from phenylpropanoid pathways involved in resistance, like phytoalexins and salicylic acid (SA) and is induced in many defence reactions.

Based on the present studies it is concluded that the amount of reducing sugars, total sugars, total proteins, total phenols, defence enzymes *viz.,,* peroxidase and phenylalanine ammonialyase were highest and significant in resistant genotypes followed by moderately resistant, moderately susceptible and susceptible genotypes indicating their role in disease reaction of cotton genotypes.

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