Research Article

# Biochemical Variability study in Genotype and Isolate of Alternaria Blight in Pigeonpea

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**Abstract**

Biochemical constituent's contents like, soluble proteins; enzyme activity, carbohydrate and amino acid are important in resistant to the crop plants and measured on four different days (70, 95, 120 &145 DAS) old plants. The contents are found of pigeonpea leaves of a set of twelve genotype, which were inoculated with representatives' ten isolates. In the estimation of soluble protein, nitrate reductase, nitrite reductase activity, total soluble sugar, soluble reducing sugar, non reducing sugar and amino acids content have recorded in higher amounts in resistant genotype (IPA-7-2 & ICP-7220) followed by moderately resistant (DA-11 & ICP-13174) and moderately susceptible (ICP-4725 & ICP-11294), whereas lower amount susceptible (ICP-7182 & BSMR-736) genotype and highly susceptible genotype (MAL-24 & Bahar). The maximum soluble protein, enzyme activity, carbohydrate and amino acid content were found in resistant genotype at early stage of plants with minimum reduction whereas, lowest content was found in susceptible genotype old plants with highest reduction. It showed same trend in a-virulent isolate in which, lowest reduction soluble protein, enzyme activity, carbohydrate and amino acid content were found as compared to virulent (aggressive) isolate.

**Keywords:** Alternaria blight, Pigeonpea, genotype, resistant**,** enzyme activity, protein, sugar and amino acids

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## Introduction

Pigeonpea [*Cajanus cajan* (L.) Mill spaugh] is a drought – resistant legume food crops cultivated in semi-arid regions of the world [1]. In India, pigeonpea is the second important pulse crop next to chickpea. India has largest acreage (4.65 mh) with a total production and productivity of 3.02 mt and 650 kg/ha, respectively [2]. Alternaria leaf blight caused by *Alternaria tenuissima* is an important disease of pigeonpea growing post rainy season. The common biochemical constituents like soluble proteins, enzymatic activity, carbohydrate and amino acids are important in imparting resistance to the crop plants. Plant show biochemical changes after infected by infectious agent. Sometimes, host plant is induced to synthesize these compounds upon infection. The biochemical constituents are known to play very important role in susceptibility or resistant against biotrophic, hemi-biotrophic and necrotic plant pathogens [3, 4]. Sugars are precursors for the synthesis of phytoalexins, lignin, phenolics and callose. Hence, they play an significant role in defense mechanism of plants against invade pathogens. The first step of this process is the decrease of nitrate to nitrite in the cytosol by the enzyme nitrate reductase [5]. Thus, NR is of fundamental significance in providing nitrogen to the plant. Development of resistant genotype is the most suitable approach to managemant the disease and the concept is now developing to explore the built-in plant defense mechanism in relative to pathogen attack [6]. Estimation of biochemical constituents help in detects their role in the resistance mechanism. The identification of a plant attribute that is associated with resistance to Alternaria blight could be useful for screening of resistant sources and therefore the identification of genotype resistant to Alternaria blight and the factors involved in impart resistance are significance studying. Keeping in the view of present studies were undertaken to known the biochemical changes responsible for the resistant genotype identified.

## Materials and Methods

The experiment was carryout during mid September sowing pigeonpea crop of 2011-12 at research field and laboratory work has been conducted at Hoffman's and Biological Control Laboratory, Department of Mycology and Plant Pathology, BHU, Varanasi. To find out total soluble protein content, nitrate reductase activity, nitrite reductase (NiR) activity, total soluble sugars, reducing sugar, non-reducing sugar and amino acid content were estimated. A set of twelve pigeonpea genotype plants were inoculated ten representative isolate *viz*., At30, At71, At17, At45, At24, At60, At130, At44, At83 and At98 categorized on the basis of reaction to pathogen highly virulent, moderately

virulent and a-virulent. Eighty genotype of pigeonpea were screened for their reaction against pathogen along with susceptible cultivar. Out of eighty genotype, only a set of twelve genotype categorized on the basis of reaction to pathogen, *viz.*, resistant (IPA-7-2, ICP- 8869, ICP-7720 & MA-98), moderately resistant (DA-11 &ICP-13174), moderately susceptible (ICP-4725 & ICP-11294), susceptible (ICP-7182 & BSMR-736) and highly susceptible (MAL-24 & Bahar) were selected on the basis of their reaction to the *Alternaria tenuissima* from screening nursery as per scale [7]. The genotype was of un-inoculated and inoculated for detailed biochemical variability study. Twelve-pigeonpea genotype was sown in a 5.40 × 3.0 m. plot adopting RBD with spacing 45 and 20 cm, in three replication. Attending sixty days, old plants were artificially inoculated by spraying spore-cum-mycelial suspension having 50-75 spores/ microscopic field (10X). Plots were irrigated to maintain proper moisture (80 to 100%) for 48 hrs. Another set (un-inoculated) was kept in field and the plants were watered regularly in order to maintain sufficient moisture. The leaves inoculated and un-inoculated were collected on four different dates (70, 95, 120 and 145 DAS) and brought to the laboratory and examined under microscope for preliminary examination and were properly preserved, labeled and kept in humid chamber for further studies above biochemical variability parameters were estimated applying standard and scientific protocols as mentioned given bellow. Data was statistically analyzed adopting two ways FRBD.

***Estimation of total soluble protein content***

Estimated total soluble protein content, with bind a dye reagent by the method of Bradford [8] using bovine serum albumin (BSA) as standard. 200 mg of the leaf sample was grind well with mortar and pestle in 10 mL of phosphate-buffered saline and experimental samples were prepared in 100 µl of phosphate-buffered saline. The extracts were centrifuged at 5000 rpm for 10 minutes at 25ºC. Five mL of diluted dye binding solution was added to each tube. It was mixed with the reagent and allowed to develop colour. Readings were taken at 595 nm and standard curve was plotted.

***Estimation of nitrate reductase activity (NR)***

Two hundred mg of leaf sample was taken into 15 mL culture tubes estimation of NR activity following procedure. One mL of 100 mM potassium phosphate buffer (pH 7.5) and 2 mL of 100 mM $KNO_3$ were added. 0.1 mL of n-propyl alcohol was added just before assay. The tubes were incubated at $30^0$C for 30 min for complete infiltration of the medium into the sample. After incubation the concentration of $NO_2^-$ released into the medium was estimated by adding 1 mL each of 1.0 percent sulfanilamide in 1N HCl and of 0.02 percent N-(I- Naphtyl) ethylene diamine dihydrochloride (NED) and the absorbance was recorded at 540 nm in a spectrophotometer [9] and was expressed as m moles $NO_2^-$ formed $g^{-1}$ fresh weight $hr^{-1}$.

$$\text{NAR Activity} = \text{O.D.} \times \text{X } 1000 \times \text{X } 2 / 0.5 \times 200$$

$$\mu \text{ moles } NO_2^- \text{ formed } g^{-1} \text{ fresh material } h^{-1}$$

***Estimation of nitrite reductase (NiR)***

Two hundred mg of leaves were taken into 15 mL culture tubes. Two mL of 100 mM cold potassium phosphate buffer (pH 7.5) and 1 mL of 100 mM $NaNo_3$ was added to culture tubes placed in the ice flecks. The remaining assay procedure for this enzyme was the same as that for NAR activity. The NIR activity was expressed in n moles of ammonia formed g-1 fresh material $h^{-1}$ [10]. Calculation as given for NAR.

***Estimation of total soluble sugars***

Dubois *et al.* [11] estimated the total soluble sugar using the method with anthrone reagent four mL of cold anthrone reagent was added to 1 mL of ethanolic extract. This mixture was shaken vigorously and boiled for 10 min in a boiling water bath. After cooling in running tap water, the absorbance was deduced at 620 nm in spectrophotometer.

***Estimation of reducing sugar***

Nelson [12] estimated reducing sugar using the method. To 1 mL of ethanolic extract, 1 mL of fresh copper reagent prepared by mixing copper tartarate and copper sulphate solution (25:1 vlv) was added to the above mixture. The mixture was heated for 20 min in a boiling water-bath and cooled. One mL of arsenomolybdate reagent was added to above mixture and the contents incubated for 15 min. The solution was diluted to twenty-five mL with distilled water and the colour intensity was read at 500 nm in spectrophotometer.

***Estimation of Non-reducing sugar***

Amount of non-reducing sugar was determined by following the formula suggested by Loomis and Shull [13].

$$\text{Non-reducing sugar} = \text{Total sugars - free reducing sugars} \times 0.95$$

***Estimation of Amino acid***

The ninhydrin-positive amino acids were determined by the method [14] with amino acid L-Glycine as standard. Two hundred milligram of leaves was crushed in 10 mL of 80% ethanol and boiled for 10 minutes. Supernatant (0.1 mL) was added to 0.9 mL distilled water and 1 mL ninhydrin reagent and boiled for 20 minutes. After cooling, 5 mL diluting solvent 1:1 (n-propanol: Water) was added to it. Absorbance was measured at 570 nm.

## Result and Discussion

***Estimation of total soluble protein***

A significant interaction was found among the representative ten isolate and twelve genotype based on their total soluble protein. The result is presented in **Table 1**, which indicates that total soluble protein content measured. It was recorded maximum in all stages of crop growth highly susceptible genotype MAL-24 (320.12, 2.70.36, 246.32 and 218.36 mg/g) followed by Bahar (310.12, 238.68, 225.23 and 213.33 mg/g), respectively. Whereas, the minimum content was recorded in resistant genotype ICP-7220 (218.68, 210.12, 195.32 and 175.34 mg/g) followed by IPA-7-2 (222.13, 216.32, 195.36 and 178.25 mg/g), respectively in un-inoculated genotype. In inoculated genotype maximum soluble protein was found all stages virulent isolate At17 (429.40, 369.11, 344.49 and 218.36 mg/g) followed by At45 (425.07, 365.94, 338.66 and 311.95 mg/g), respectively whereas minimum was recorded in a-virulent isolate At30 (223.12, 212.11, 198.25 and 181.12 mg/g) followed by At71 (227.55, 215.46, 204.06 and 186.45 mg/g) respectively. The data indicates that minimum soluble protein reduction was found in resistant genotype at early stage of plant whereas more content in susceptible genotype. It showed same trend in virulent isolate in which maximum reduction soluble protein as compared to a-virulent isolate. In diseased portion, although the pathogen consumed part of the plant protein but due to the addition of fungal protein to the plant tissues, the amount of total protein increased as compared to healthy leaves. In general healthy leaves of susceptible genotype contained more soluble protein than the resistant genotype.

**Table 1** Effect of *Alternaria tenuissima* on total soluble proteins content (mg/g fresh tissue) of pigeonpea leaves in different genotype inoculated with different isolate at four different dates

| **Genotypes** | **70 DAS** | | **95 DAS** | | **120 DAS** | | **145 DAS** | |
|---|---|---|---|---|---|---|---|---|
| | **Control** | **Mean of ten isolates*** | **Control** | **Mean of ten isolates*** | **Control** | **Mean of ten isolates*** | **Control** | **Mean of ten isolates*** |
| ICP-8869 | 228.13 | 281.30 | 223.26 | 272.33 | 201.32 | 250.63 | 185.36 | 223.09 |
| IPA-7-2 | 222.13 | 272.71 | 216.33 | 265.79 | 195.36 | 242.13 | 178.25 | 216.54 |
| ICP-11294 | 248.56 | 300.02 | 231.22 | 279.89 | 216.32 | 262.78 | 190.45 | 235.29 |
| ICP-7220 | 218.68 | 269.43 | 210.12 | 256.61 | 195.32 | 242.10 | 175.34 | 210.03 |
| MA-98 | 227.66 | 279.07 | 222.31 | 268.14 | 198.36 | 247.81 | 184.32 | 220.95 |
| ICP-4725 | 241.33 | 294.43 | 227.32 | 276.72 | 215.63 | 260.22 | 189.36 | 228.07 |
| BSMR-736 | 261.12 | 313.19 | 238.25 | 287.39 | 221.36 | 271.32 | 192.35 | 245.37 |
| ICP-7182 | 258.32 | 308.44 | 235.36 | 284.45 | 218.36 | 266.82 | 191.45 | 237.59 |
| ICP-13174 | 230.12 | 284.89 | 224.36 | 273.69 | 208.99 | 254.66 | 186.32 | 225.45 |
| Bahar | 310.12 | 349.34 | 238.65 | 293.41 | 225.23 | 273.91 | 213.33 | 254.06 |
| MAL-24 | 320.12 | 358.09 | 270.36 | 317.66 | 246.32 | 291.31 | 218.36 | 265.52 |
| DA-11 | 240.12 | 289.27 | 225.32 | 274.76 | 213.26 | 258.39 | 189.32 | 227.69 |
| Mean | 250.53 | | 230.24 | | 212.99 | | 191.18 | |
| Factors | CD(0.05) | | CD(0.05) | | CD(0.05) | | CD(0.05) | |
| Genotype | 1.13 | | 1.53 | | 1.23 | | 1.42 | |
| Isolate | 1.08 | | 1.46 | | 1.18 | | 1.36 | |
| Genotype×Isolate | 3.74 | | 5.06 | | 4.08 | | 4.72 | |

These results concerned with the observation of changes in protein occur when the pathogen penetrates the host cells resulting in disturbances in protein and related metabolisms [15 & 16] of ginger, pigeonpea against *Alternaria*

*tenuissima* [17] and leaf spot of *Withania somnifera* [18]. There was a progressive increase in soluble protein contents of healthy and infected plants with increase in plant age.

***Estimation of nitrate reductase (NR)***

A significant interaction was found among the ten isolate and twelve genotype based on their nitrate reductase (NR). The result is presented in **Table 2**, which indicates that nitrate reductase. It was recorded maximum in all stages of resistant genotype ICP-7220 (785.59, 804.65, 828.67 and 852.23 n moles $NO_2$ formed $g^{-1}$ tissues $h^{-1}$) followed by IPA-7-2 (780.62, 796.22, 818.19 and 845.97 n moles $NO_2$ formed $g^{-1}$ tissues $h^{-1}$) respectively. Whereas, the minimum was recorded in highly susceptible genotype MAL-24 (680.27, 702.34, 728.76 and 756.13 n moles $NO_2$ formed $g^{-1}$ tissues $h^{-1}$) followed by Bahar (684.23, 708.77, 734.23 and 756.19 n moles $NO_2$ formed $g^{-1}$ tissues $h^{-1}$), respectively in un-inoculated genotype. In inoculated genotype maximum nitrate reductase (NR) was found in all stages a-virulent isolate At30 (292.47, 308.48, 333.35 and 346.36 n moles $NO_2$ formed $g^{-1}$ tissues $h^{-1}$) followed by At71 (284.24, 307.98, 331.82 and 345.97 n moles $NO_2$ formed $g^{-1}$ tissues $h^{-1}$) respectively. Whereas minimum nitrate reductase (NR) was recorded in virulent isolate At17 (173.82, 196.82, 229.28 and 254.82 n moles $NO_2$ formed $g^{-1}$ tissues $h^{-1}$) followed by At45 (177.13, 204.58, 240.25 and 261.41 n moles $NO_2$ formed $g^{-1}$ tissues $h^{-1}$), respectively. The data indicates that maximum nitrate reductase and minimum reduction was found in resistant genotype whereas, lowest nitrate reductase and highest reduction were found in susceptible genotype. It showed same trend in a-virulent isolate in which lowest reduction nitrate reductase was found as compared to virulent isolates. Because of infection in susceptible and resistant genotype, it was observed that there was a drastic reduction in nitrate reductase activity (NAR) in diseased tissue than the healthy ones. It was also noticed that health plant nitrate reductase activity (NAR) increase according age but infected (inoculated) plant reversed decrease. Similar findings pigeonpea of *Alternaria tenuissima* [17] leaves and stem tissue [19, 20] in pigeonpea genotype.

**Table 2** Effect of *Alternaria tenuissima* on nitrate reductase (NR) activity (n moles $NO_2^-$ formed $g^{-1}$ tissues $h^{-1}$) of pigeonpea leaves in different genotype inoculated with isolate at four different dates.

| **Genotypes** | **70 DAS** | | **95 DAS** | | **120 DAS** | | **145 DAS** | |
|---|---|---|---|---|---|---|---|---|
| | **Control** | **Mean of ten isolates*** | **Control** | **Mean of ten isolates*** | **Control** | **Mean of ten isolates*** | **Control** | **Mean of ten isolates*** |
| ICP-8869 | 755.23 | 290.22 | 778.23 | 312.20 | 804.98 | 340.10 | 832.78 | 359.22 |
| IPA-7-2 | 780.62 | 299.61 | 796.22 | 321.65 | 818.19 | 348.73 | 845.97 | 368.46 |
| ICP-11294 | 701.24 | 274.83 | 724.23 | 296.46 | 748.34 | 325.07 | 778.98 | 342.12 |
| ICP-7220 | 785.59 | 307.05 | 804.65 | 331.75 | 828.67 | 358.41 | 852.23 | 374.58 |
| MA-98 | 768.29 | 295.80 | 791.23 | 316.92 | 812.43 | 344.39 | 834.32 | 362.53 |
| ICP-4725 | 712.13 | 277.88 | 738.97 | 299.62 | 765.85 | 330.34 | 791.67 | 345.83 |
| BSMR-736 | 690.86 | 269.71 | 712.44 | 291.60 | 734.78 | 318.57 | 764.65 | 334.76 |
| ICP-7182 | 695.34 | 272.01 | 718.18 | 294.30 | 744.67 | 322.31 | 771.78 | 338.27 |
| ICP-13174 | 748.76 | 288.12 | 769.29 | 309.63 | 791.45 | 336.89 | 817.13 | 355.89 |
| Bahar | 684.23 | 264.90 | 708.77 | 287.30 | 734.23 | 313.04 | 756.19 | 331.96 |
| MAL-24 | 680.27 | 263.35 | 702.34 | 283.83 | 728.76 | 310.99 | 756.13 | 329.87 |
| DA-11 | 734.23 | 285.92 | 758.78 | 307.90 | 784.27 | 334.94 | 808.33 | 353.47 |
| Mean | 728.07 | | 750.28 | | 774.72 | | 800.85 | |
| Factors | CD(0.05) | | CD(0.05) | | CD(0.05) | | CD(0.05) | |
| Genotype | 0.97 | | 0.94 | | 1.00 | | 1.312 | |
| Isolate | 0.93 | | 0.90 | | 0.96 | | 1.256 | |
| Genotype×Isolate | 3.22 | | 3.10 | | 3.32 | | 4.352 | |

***Estimation of nitrite reductase activity (NIR)***

A significant interaction was found among the ten isolate and twelve genotype based on their nitrite reductase activity. The result is presented in **Table 3**, which indicates that nitrite reductase activity content. It was maximum recorded in all stages of resistant genotype ICP-7220 (658.16, 684.36, 708.36 and 734.35 n moles ammonia formed $g^{-1}$ tissues $h^{-1}$) followed by IPA-7-2 (589.34, 612.64, 638.72 and 668.46 n moles ammonia formed $g^{-1}$ tissues $h^{-1}$), respectively. Whereas the minimum was recorded in highly susceptible genotype MAL-24 (411.68, 429.67, 445.67 and 462.37 n moles ammonia formed $g^{-1}$ tissues $h^{-1}$) followed by Bahar (446.32, 471.28, 488.34 and 506.63 n moles ammonia formed $g^{-1}$ tissues $h^{-1}$), respectively in un-inoculated genotype. In inoculated genotype maximum nitrite reductase activity (NIR) was recorded in all stages virulent isolate At17 (729.94, 759.26, 789.87 and 817.34 n moles

ammonia formed g-$^{1}$ tissues h-$^{1}$) followed by At45 (726.94, 756.26, 781.87 and 812.34 n moles ammonia formed g-$^{1}$ tissues h-$^{1}$) respectively. Whereas the minimum nitrite reductase was recorded in all stages a-virulent isolate At30 (413.13, 441.35, 484.69 and 513.53 n moles ammonia formed g-$^{1}$ tissues h-$^{1}$) followed by At71 (415.53, 446.65, 488.89 and 518.35 n moles ammonia formed g-$^{1}$ tissues h-$^{1}$) respectively. The data indicates that maximum nitrite reductase activity was found in resistant genotype whereas minimum in susceptible genotype. It showed same trend in a-virulent isolates in which lowest reduction of activity was found as compared to virulent isolate.

Present investigation corroborated [17] pigeonpea of *Alternaria tenuissima*, [21] grapevine of virus-infected and [22] Phytophthora leaf blight of taro. In comparison with the uninoculated leaves, blight infected leaves showed reduction in activity of nitrate reductase genotype. Changes in biochemical contain under induced blight stress as compared with uninoculated control were less in resistant genotype than that in susceptible genotype. The deviations in biochemical contents were highest in susceptible genotype.

**Table 3** Effect of *Alternaria tenuissima* on nitrite reductase (NiR) activity (n moles ammonia formed $g^{-1}$ tissues $h^{-1}$) of pigeonpea leaves in different genotype inoculated with different isolate at four different dates.

| **Genotypes** | **70 DAS** | | **95 DAS** | | **120 DAS** | | **145 DAS** | |
|---|---|---|---|---|---|---|---|---|
| | **Control** | **Mean of ten isolates*** | **Control** | **Mean of ten isolates*** | **Control** | **Mean of ten isolates*** | **Control** | **Mean of ten isolates*** |
| ICP-8869 | 548.67 | 573.70 | 574.34 | 602.01 | 596.41 | 633.29 | 613.29 | 660.64 |
| IPA-7-2 | 589.34 | 637.57 | 612.64 | 662.47 | 638.72 | 682.58 | 668.46 | 710.74 |
| ICP-11294 | 459.34 | 498.15 | 481.64 | 529.22 | 503.64 | 553.98 | 534.32 | 589.56 |
| ICP-7220 | 658.16 | 703.53 | 684.36 | 727.33 | 708.36 | 755.31 | 735.34 | 780.97 |
| MA-98 | 586.18 | 630.28 | 609.37 | 654.87 | 631.34 | 667.48 | 664.31 | 705.70 |
| ICP-4725 | 468.16 | 506.55 | 494.32 | 535.87 | 517.82 | 562.43 | 536.13 | 626.62 |
| BSMR-736 | 449.15 | 481.56 | 471.51 | 507.86 | 495.61 | 543.73 | 521.38 | 577.62 |
| ICP-7182 | 452.34 | 487.28 | 475.38 | 512.57 | 498.19 | 547.11 | 526.67 | 585.72 |
| ICP-13174 | 539.56 | 564.57 | 568.12 | 597.82 | 584.19 | 627.21 | 608.32 | 653.19 |
| Bahar | 446.32 | 478.73 | 471.28 | 504.52 | 488.34 | 526.30 | 506.53 | 547.17 |
| MAL-24 | 411.68 | 429.09 | 429.67 | 464.18 | 445.67 | 503.08 | 462.37 | 535.82 |
| DA-11 | 506.97 | 545.45 | 538.48 | 594.12 | 571.64 | 613.00 | 596.34 | 643.00 |
| Mean | 509.66 | | 534.26 | | 556.66 | | 581.12 | |
| Factors | CD(0.05) | | CD(0.05) | | CD(0.05) | | CD(0.05) | |
| Genotype | 0.90 | | 0.69 | | 0.88 | | 0.67 | |
| Isolates | 0.86 | | 0.66 | | 0.84 | | 0.64 | |
| Genotype×Isolate | 2.99 | | 2.28 | | 2.92 | | 2.22 | |

***Estimation of total soluble sugar***

The result is presented in **Table 4**, which indicates that total soluble sugar measured, it was found maximum in all stages of resistant genotype ICP-7220 (49.20, 49.86, 50.19 and 50.46 mg/g) followed by IPA-7-2 (48.30, 48.97, 49.62 and 49.82 mg/g), respectively in both inoculated and un-inoculated leaves. Whereas, the minimum was recorded in highly susceptible genotype MAL-24 (28.76, 29.16, 29.73 and 29.96 mg/g) followed by Bahar (28.86, 29.43, 29.80 and 30.06 mg/g), respectively in un-inoculated genotype. Inoculated genotype maximum total soluble sugar was found in all stages of a-virulent isolate At30 (46.80, 46.16, 45.81 and 45.60 mg/g) followed by At71 (46.75, 46.08 45.73 and 45.53 mg/g), respectively. Whereas the minimum sugar was recorded in all stages virulent isolate At17 (24.98, 24.71, 24.30 and 23.82 mg/g) followed by At45 (25.08, 24.81, 24.38 and 23.90 mg/g) respectively. The data indicates that maximum total soluble sugar and its minimum reduction were found in resistant genotype with early stage of plant whereas lowest sugar and highest sugar reduction were found in susceptible genotype old plants. It showed same trend in a-virulent isolate lowest sugar reduction was found as compared to virulent isolate. It was also notice total sugar increase in un-inoculated plants according stage but infected (inoculated) plant reversed decrease. It may be the cause of biochemical chemical change occurred due to infection by pathogen. Therefore, it can be clear that low amount of carbohydrates in resistant genotype may be responsible for resistant of genotype against test pathogen. Similarly, [23] reported that the plant infected with leaf spot of rose caused by *Alternaria alternata* showed a decrease in the quantity of total sugar and reducing sugar compared to the healthy plant. Therefore, present investigation revealed that the low amount of carbohydrates in resistant genotype imparts resistance against test pathogen.

**Table 4** Effect of *Alternaria tenuissima* on total soluble sugar (mg/g fresh tissue) of pigeonpea leaves in different genotype inoculated with different isolate at four different dates

| Genotypes | 70 DAS | | 95 DAS | | 120 DAS | | 145 DAS | |
|---|---|---|---|---|---|---|---|---|
| | Control | Mean of ten isolates* | Control | Mean of ten isolates* | Control | Mean of ten isolates* | Control | Mean of ten isolates* |
| ICP-8869 | 43.40 | 40.97 | 43.67 | 39.68 | 43.96 | 38.69 | 44.29 | 37.05 |
| IPA-7-2 | 48.30 | 45.67 | 48.97 | 45.13 | 49.62 | 44.93 | 49.85 | 44.80 |
| ICP-11294 | 35.09 | 32.89 | 35.34 | 32.62 | 35.89 | 32.20 | 36.23 | 30.51 |
| ICP-7220 | 49.20 | 46.70 | 49.86 | 46.16 | 50.19 | 45.95 | 50.46 | 45.78 |
| MA-98 | 43.60 | 41.21 | 44.06 | 40.41 | 44.49 | 39.90 | 44.82 | 37.42 |
| ICP-4725 | 35.51 | 33.64 | 36.84 | 33.46 | 37.17 | 32.86 | 37.52 | 31.61 |
| BSMR-736 | 29.41 | 26.83 | 30.19 | 26.02 | 30.52 | 25.75 | 30.78 | 25.19 |
| ICP-7182 | 29.90 | 26.95 | 30.38 | 26.33 | 30.71 | 26.00 | 30.91 | 25.50 |
| ICP-13174 | 40.38 | 37.96 | 40.89 | 37.36 | 41.26 | 37.00 | 41.59 | 36.34 |
| Bahar | 28.86 | 26.36 | 29.43 | 25.78 | 29.80 | 25.48 | 30.06 | 25.23 |
| MAL-24 | 28.76 | 26.05 | 29.19 | 25.57 | 29.73 | 25.21 | 29.96 | 24.95 |
| DA-11 | 39.65 | 37.33 | 40.02 | 36.67 | 40.57 | 36.42 | 40.90 | 35.99 |
| Mean | 37.67 | | 38.24 | | 38.66 | | 38.95 | |
| Factors | CD(0.05) | | CD(0.05) | | CD(0.05) | | CD(0.05) | |
| Genotype | 0.88 | | 0.89 | | 0.88 | | 0.86 | |
| Isolates | 0.85 | | 0.85 | | 0.85 | | 0.83 | |
| Genotype×Isolate | NS | | NS | | NS | | NS | |

***Estimation of soluble reducing sugar***

The result is presented in **Table 5**, which indicates that soluble reducing sugar. It was recorded maximum in all stages of resistant genotype ICP-7220 (19.64, 20.32, 20.65 and 20.98 mg/g) followed by IPA-7-2 (19.55, 20.21, 20.61 and 20.91 mg/g), respectively whereas, the minimum was recorded in highly susceptible genotype MAL-24 (11.52, 12.16, 12.36 and 12.69 mg/g) followed by Bahar (11.56, 12.21, 12.54 and 12.87 mg/g) respectively in un-inoculated genotype. In inoculated genotype maximum soluble reducing sugar was found in all stages a-virulent isolate At30 (18.12, 17.81, 17.27 and 16.65 mg/g) followed by At71 (18.06, 17.76, 17.22 and 16.62 mg/g), respectively. Whereas the minimum sugar was found in all stages of virulent isolate At17 (8.59, 8.43, 8.21 and 8.07 mg/g) followed by At45 (8.71, 8.46, 8.23 and 8.11 mg/g), respectively. The data indicates that maximum soluble reducing sugar and its minimum reduction was found in resistant genotype at early stage of plant whereas lowest sugar and highest reduction were found in susceptible genotype old plants. It showed same trend in a-virulent isolate in which lowest reduction of soluble reducing sugar was found as compared to virulent isolate. It was also notice that soluble reducing sugar increase in un-inoculated plants according stage but infected (inoculated) plant reversed decrease.

Sugars may be due to a stimulated respiration in Alternaria blight infected plant. This increased rate of respiration demanding large amount of substrate is responsible for breakdown of carbohydrate into simple sugar by increase activity. Which will convert starch into simple sugar resulting in increased level of sugar in Alternaria blight infected plant and the accumulation of more reducing sugar in infected plants may be due to the response of pathogen stress as reported [24 & 25].

***Estimation of non reducing sugar***

The result is presented in **Table 6**, which indicates that non reducing sugar measured, it was maximum recorded in resistant genotype ICP-7220 (30.55, 30.56, 30.58 and 30.53 mg/g) followed by IPA-7-2 (29.73, 29.77, 30.04 and 29.99 mg/g), respectively. Whereas the minimum was recorded in highly susceptible genotype MAL-24 (17.82, 17.64), Bahar (17.89) and moderately susceptible ICP-7182 (17.82) mg/g) followed by in Bahar (17.88, 17.83 and 19.84 mg/g in 70, 95 and 145 days), MAL-24 (17.99 in 120 days) in un inoculated genotype. In inoculated genotype maximum non reducing sugar was found in virulent isolate At17 (29.63 mg/g), At130 (29.30, 30.34 and 30.65), and followed by At45 (29.61) 60 (29.27), 71(29.37) and (29.79) mg/g) respectively. Whereas the minimum non reducing sugar was found in virulent isolate At17 (16.82, 16.59, 16.15 in 70, 120 and 145 DAS) and At24 (16.59 in 95 DAS mg/g) followed by At45 (16.82, 16.58 and 16.20 in 70, 120 and 145 DAS) At60 (16.60 95 DAS) mg/g) respectively. The data indicates that maximum non reducing sugar and its minimum reduction was found in resistant genotype with early stage of plant whereas, lowest sugar and highest reduction were found in susceptible genotype. It showed same

trend in a-virulent isolate in which lowest reduction of non reducing sugar was found as compared to virulent isolate. Non-reducing sugar was also in the same trend as it is cumulative effect of total sugar and soluble reducing sugar.

These finding collaborated [26] in groundnut genotype against late leaf spot, in the *Alternaria sp*. of sunflower [27, 28] of *A. brassicae* in mustard, [29] in pigeonpea of leaf blight (*A. tenuissima*), [21] in disease leaf spot of *Withania somnifera* (*A. alternata*), [30] Chrysanthemum leaf blight disease (*A. chalmydospora*) and [31] of Ivy gourd fruit (*A. pluriseptata*).

**Table 5** Effect of *Alternaria tenuissima* on soluble reducing sugar (mg/g fresh tissue) of pigeonpea leaves in different genotype inoculated with different isolate at four different dates

| **Genotypes** | **70 DAS** | | **95 DAS** | | **120 DAS** | | **145 DAS** | |
|---|---|---|---|---|---|---|---|---|
| | **Control** | **Mean of ten isolates*** | **Control** | **Mean of ten isolates*** | **Control** | **Mean of ten isolates*** | **Control** | **Mean of ten isolates*** |
| ICP-8869 | 17.04 | 15.51 | 17.72 | 15.17 | 18.06 | 14.89 | 18.39 | 14.57 |
| IPA-7-2 | 19.55 | 17.84 | 20.21 | 17.65 | 20.61 | 17.27 | 20.91 | 16.83 |
| ICP-11294 | 13.30 | 11.40 | 13.99 | 11.25 | 14.32 | 10.94 | 14.66 | 10.60 |
| ICP-7220 | 19.64 | 17.95 | 20.32 | 17.71 | 20.65 | 17.35 | 20.98 | 16.88 |
| MA-98 | 17.36 | 15.60 | 18.05 | 15.32 | 18.38 | 15.01 | 18.72 | 14.66 |
| ICP-4725 | 13.45 | 11.63 | 14.14 | 11.34 | 14.47 | 11.10 | 14.80 | 10.67 |
| BSMR-736 | 11.67 | 9.55 | 12.45 | 9.45 | 13.12 | 9.29 | 13.60 | 9.06 |
| ICP-7182 | 11.82 | 9.69 | 12.52 | 9.56 | 13.38 | 9.41 | 13.78 | 9.15 |
| ICP-13174 | 15.61 | 13.56 | 16.28 | 13.34 | 16.62 | 13.19 | 16.95 | 12.85 |
| Bahar | 11.56 | 9.48 | 12.21 | 9.28 | 12.54 | 9.03 | 12.87 | 8.75 |
| MAL-24 | 11.52 | 9.37 | 12.16 | 9.22 | 12.36 | 8.90 | 12.69 | 8.71 |
| DA-11 | 15.38 | 13.45 | 16.06 | 13.22 | 16.39 | 12.95 | 16.73 | 12.62 |
| Mean | 14.83 | | 15.51 | | 15.91 | | 16.26 | |
| Factors | CD(0.05) | | CD(0.05) | | CD(0.05) | | CD(0.05) | |
| Genotype | 0.22 | | 0.27 | | 0.28 | | 0.285 | |
| Isolates | 0.21 | | 0.25 | | 0.27 | | 0.273 | |
| Genotype×Isolate | NS | | NS | | NS | | NS | |

**Table 6** Effect of *Alternaria tenuissima* on non reducing sugar (mg/g fresh tissue) of pigeonpea leaves in different genotype inoculated with different isolate at four different dates

| **Genotypes** | **70 DAS** | | **95 DAS** | | **120 DAS** | | **145 DAS** | |
|---|---|---|---|---|---|---|---|---|
| | **Control** | **Mean of ten isolates*** | **Control** | **Mean of ten isolates*** | **Control** | **Mean of ten isolates*** | **Control** | **Mean of ten isolates*** |
| ICP-8869 | 27.21 | 26.24 | 26.83 | 25.28 | 26.80 | 24.55 | 26.82 | 23.21 |
| IPA-7-2 | 29.73 | 28.73 | 29.77 | 28.36 | 30.04 | 28.53 | 29.99 | 28.81 |
| ICP-11294 | 22.45 | 22.07 | 22.04 | 21.93 | 22.28 | 21.81 | 22.30 | 20.43 |
| ICP-7220 | 30.55 | 29.65 | 30.56 | 29.34 | 30.58 | 29.48 | 30.53 | 29.74 |
| MA-98 | 27.11 | 26.39 | 26.91 | 25.86 | 27.03 | 25.65 | 27.04 | 23.50 |
| ICP-4725 | 22.73 | 22.60 | 23.41 | 22.69 | 23.43 | 22.32 | 23.46 | 21.48 |
| BSMR-736 | 18.32 | 17.76 | 18.36 | 17.05 | 18.06 | 16.92 | 17.86 | 16.59 |
| ICP-7182 | 18.67 | 17.74 | 18.49 | 17.24 | 18.00 | 17.06 | 17.82 | 16.81 |
| ICP-13174 | 25.55 | 25.07 | 25.42 | 24.69 | 25.47 | 24.47 | 25.49 | 24.13 |
| Bahar | 17.88 | 17.36 | 17.83 | 16.96 | 17.89 | 16.90 | 17.84 | 16.92 |
| MAL-24 | 17.82 | 17.15 | 17.64 | 16.82 | 17.99 | 16.75 | 17.90 | 16.68 |
| DA-11 | 25.03 | 24.55 | 24.76 | 24.11 | 24.99 | 24.12 | 25.01 | 24.00 |
| Mean | 23.59 | | 23.50 | | 23.55 | | 23.50 | |
| Factors | CD(0.05) | | CD(0.05) | | CD(0.05) | | CD(0.05) | |
| Genotype | 0.89 | | 0.91 | | 0.93 | | 0.88 | |
| Isolates | NS | | NS | | NS | | 0.84 | |
| Genotype×Isolate | NS | | NS | | NS | | NS | |

***Estimation of amino acid content***

The result is presented in **Table 7**, which indicates that amino acid was maximum recorded in all stages of resistant genotype ICP-7220 (9.46, 8.03, 6.26 and 5.96 mg/g) followed by IPA-7-2 (9.43, 7.56, 6.11 and 5.52 mg/g), respectively. Whereas the minimum was recorded in highly susceptible genotype MAL-24 (5.35, 4.95, 3.23 and 2.90 mg/g) followed by Bahar (5.64, 4.97, 3.39 and 2.95 mg/g) in un-inoculated genotype. In inoculated genotype maximum amino acid was found in all stages of a-virulent isolate At30 (9.34, 7.83, 6.12 and 4.01 mg/g) followed by At71 (9.24, 7.80, 6.06 and 3.96 mg/g), respectively. Whereas the minimum amino acid was recorded in all stages of virulent isolate At17 (4.95, 4.26, 2.83 and 1.31 mg/g) followed by At45 (5.20, 4.28, 2.85 and 1.35 mg/g), respectively. The data indicates that maximum amino acid and its minimum reduction were found in resistant genotype at early stage of plant whereas lowest amino acid and highest reduction in susceptible genotype. It showed same trend in a-virulent isolate in which lowest reduction of amino acid was recorded as compared to virulent isolate. The present investigation corroborated [16] in ginger, [21] *Withania somnifera* of *Alternaria alternata*, [32] mulberry to leaf blight, [33] in *Beet mosaic virus*.

**Table 7** Effect of *Alternaria tenuissima* on free amino acid content (mg/g fresh tissue) of pigeonpea leaves in different genotype inoculated with different isolate at four different dates

| **Genotypes** | **70 DAS** | | **95 DAS** | | **120 DAS** | | **145 DAS** | |
|---|---|---|---|---|---|---|---|---|
| | **Control** | **Mean of ten isolates*** | **Control** | **Mean of ten isolates*** | **Control** | **Mean of ten isolates*** | **Control** | **Mean of ten isolates*** |
| ICP-8869 | 8.54 | 7.82 | 6.58 | 6.17 | 4.84 | 4.40 | 4.25 | 2.99 |
| IPA-7-2 | 9.43 | 8.96 | 7.56 | 6.99 | 6.11 | 5.71 | 5.52 | 3.49 |
| ICP-11294 | 7.31 | 6.27 | 5.39 | 5.07 | 3.96 | 3.47 | 3.29 | 2.61 |
| ICP-7220 | 9.46 | 9.16 | 8.03 | 7.49 | 6.26 | 5.77 | 5.96 | 3.86 |
| MA-98 | 8.59 | 7.72 | 6.84 | 6.35 | 5.44 | 4.51 | 5.28 | 3.34 |
| ICP-4725 | 7.60 | 6.85 | 5.44 | 5.10 | 3.99 | 3.56 | 3.33 | 2.65 |
| BSMR-736 | 6.33 | 5.99 | 5.16 | 4.72 | 3.67 | 3.30 | 2.97 | 2.37 |
| ICP-7182 | 6.43 | 6.06 | 5.36 | 4.94 | 3.78 | 3.41 | 3.11 | 2.53 |
| ICP-13174 | 8.26 | 7.25 | 5.90 | 5.46 | 4.73 | 4.31 | 4.08 | 2.82 |
| Bahar | 5.64 | 5.35 | 4.97 | 4.58 | 3.39 | 3.16 | 2.95 | 2.20 |
| MAL-24 | 5.35 | 5.15 | 4.95 | 4.55 | 3.23 | 3.02 | 2.90 | 1.91 |
| DA-11 | 7.64 | 6.93 | 5.49 | 5.18 | 4.34 | 3.92 | 4.05 | 2.73 |
| Mean | 7.55 | | 5.97 | | 4.48 | | 3.97 | |
| Factors | CD(0.05) | | CD(0.05) | | CD(0.05) | | CD(0.05) | |
| Genotype | 0.21 | | 0.22 | | 0.19 | | 0.223 | |
| Isolates | 0.20 | | 0.21 | | 0.19 | | 0.213 | |
| Genotype×Isolate | NS | | NS | | NS | | NS | |

## Conclusion

The maximum soluble protein, enzyme activity, carbohydrate and amino acid content were found in resistant genotype at early stage of plants with minimum reduction whereas, lowest content was found in susceptible genotype old plants with highest reduction. It also showed same trend in a-virulent isolate in which, lowest reduction soluble protein, enzyme activity, carbohydrate and amino acid content were found as compared to virulent (aggressive) isolate in present investigation.

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