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RESEARCH **P**APER

Biochemical constituents of *Alternaria* blight of pathogens in pigeonpea

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The common biochemical constituents like chlorophyll and carotenes are important in imparting resistance to the crop plants. Distinct variation in chlorophyll content of pigeonpea leaves of a set of twelve genotypes which were inoculated with representatives ten isolates. In the chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotene content have recorded in higher amounts in resistant genotypes (ICP-7220, IPA-7-2) followed by moderately resistant (ICP-13174 and DA-11) and moderately susceptible (ICP-11294 and ICP-4725), whereas lower amount susceptible (BSMR-736 and ICP-7182) genotypes and highly susceptible genotypes (MAL-24, Bahar). The maximum chlorophyll and carotene content were found in resistant genotypes at early stage of plants with minimum reduction whereas, lowest content was found in susceptible genotypes old plants with highest reduction. It showed same trend in a-virulent isolates in which lowest reduction chlorophyll and carotenes content were found as compared to virulent (aggressive) isolates.

Key words : Genotypes, Resistance, Isolates, Alternaria, Chlorophyll, Carotene

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INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Mill spaugh] is one of the major food legume crops of the tropics and sub-tropics. In India, after chickpea, pigeonpea is the second most important pulse crop. India has largest acreage under pigeonpea 3.90 MH with a total production and productivity of 3.17 MT and 813 kg/ha, respectively (Anonymous, 2015). The crop suffers from Alternaria blight [*Alternaria tenuissima* (Kunze ex Pers) Wiltshire] in those areas with weather conditions favourable to the disease. The fungus attached all the aerial parts of the plant but the leaves are severely affected resulting in defoliation and reduction in yield (Kumar and Praveen, 2002 and Alka and Singh, 2004). In eastern Uttar Pradesh and adjoin western part of Bihar, severe out breaks of *Alternaria* blight was seen during disease survey on pigeonpea cultivars. Biochemical variability problem and for breeding resistant varieties, knowledge of variability in the pathogen is essential. During these processes considerable changes takes place in biochemical and physiological aspects are like changes in the concentrations of chlorophyll-a, chlorophyll-b and total chlorophyll (Sharma and Sharma, 1994) in plant tissues. Estimation of biochemical constituents help in detecting their role in the resistance mechanism. The present studies were undertaken to known the biochemical constituent's changes responsible for the resistance and the results are presented herein.

RESEARCH METHODOLOGY

Estimation of chlorophyll "a", "b", total chlorophyll and carotene :

An experiment was conducted during mid September 2011-12 at the entomological research field, BHU, Varanasi to find out chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotene were estimated. Ten representative isolates *viz*., At 17, At 24, At 30, At 44, At 45, At 60, At 71, At 83, At 98 and At 130 a set of isolates categorized on the basis of reaction to pathogen highly virulent, moderately virulent and avirulent. Which was artificially inoculated on a set of twelve pigeonpea genotypes having different degree of resistant/ susceptibility to *A. tenuissima*. Eighty genotypes of pigeonpea were screened for their reaction against pathogen along with susceptible cultivar. Out of 80 genotypes, only a set of genotypes categorized on the basis of reaction to pathogen, *viz.*, resistant (ICP- 8869, IPA-7-2, ICP-7720 and MA-98), moderately resistant (ICP-13174 and DA-11), moderately susceptible (ICP-11294 and ICP-4725), susceptible (BSMR-736 and ICP-7182) and highly susceptible (Bahar and MAL-24) were selected for detailed biochemical variability study. Twelve pigeonpea genotypes were sown in a 5.40 × 3.0 m. plot adopting RBD with spacing 45 and 20 cm, in three replications.Spore suspension of ten isolates was prepared in sterilized distilled water individually using 8 days old fungal culture and filtered through double layered sterilized cheesecloth. Spore suspension was further diluted to 1×10^4 spores/ml by adding sterilized distilled water. 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. Inoculated and un-inoculated 200 mg of the leaf sample was grounded well with mortar and pestle, separately were crushed in 80 per cent acetone. The grounded material was centrifuged at 5000 rpm for 5 minutes. The volume of supernatant of each sample was made upto 30 ml by adding 80 per cent acetone. The optical density was measured at 480, 510, 663 and 645 nm using spectrophotometer. Chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotene were calculated by using the following formulae (Arnon, 1949). The data were statistically analyzed adopting two ways FRBD.

Chl *a* (mg g^{1} leaf fresh weight) = [12.3(A663) – 0.86 (A545)] × V/1000 × W

Chl b (mg g leaf fresh weight) = [19.3(A645) – 3.60 (A663)] × V/1000 × W

Total Chl (mg g^{-1} leaf fresh weight) = [20.2(A645) +8.02(A663)] × V/1000 × W

Carotene = [17.6(A480) -1.49(A510)]×V/1000 × W

where,

A= Optical density at respective nm

V= Volume of extracted solution made upto 30 ml

W=Weight of sample (0.1 g).

RESEARCH FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Estimation of chlorophyll 'a':

The common biochemical constituents like chlorophyll and carotenes are important in imparting resistance to the crop plants. Sometimes host plant is induced to synthesize these compounds upon infection. The chlorophyll contents were estimated of twelve pigeonpea genotypes having different degree of resistant/ susceptibility leaves which were inoculated and un-inoculated of the ten isolate fungus *A. tenuissima*. The result is presented in Table (1) which indicates that chlorophyll 'a' content measured on four different dates old plants, maximum content was recorded in all stages of resistant genotypes ICP-7220 (2.32, 2.08, 1.96 and 1.82 mg/g) followed by IPA-7-2 (2.22, 2.00, 1.91 and 1.78 mg/g), respectively whereas, the minimum content was recorded in highly susceptible genotypes MAL-24 (1.75, 1.65, 1.40 and 1.24 mg/g) followed by Bahar (1.87, 1.67, 1.49 and 1.36 mg/g), respectively in un-inoculated genotypes. In inoculated genotypes maximum chlorophyll 'a' content was found in all stages of a-virulent isolates At 30 (2.24, 1.99, 1.82 and 1.64 mg/g) followed by At71 (2.21, 1.97, 1.80 and 1.62 mg/g), respectively whereas, minimum content was found in virulent isolates At17 (1.48, 1.33, 1.17 and 1.05 mg/g) followed by At 45 (1.48, 1.35,

1.18 and 1.07 mg/g), respectively. The data indicates that maximum chlorophyll 'a' content was found in resistant genotypes at early stage of plants with minimum reduction whereas, lowest content were found in susceptible genotypes old plants with highest reduction. It showed same trend in a-virulent isolates in which lowest reduction of chlorophyll content was found as compared to virulent isolates.

Estimation of chlorophyll 'b':

The result is presented in Table 2 which indicate that chlorophyll 'b' content was measured on four different dates olds plants, maximum content was recorded in all stages of highly susceptible genotypes MAL-24 (0.72, 0.83, 0.93 and 0.99 mg/g) followed by Bahar (0.70, 0.81, 0.92 and 0.98 mg/g), respectively whereas, minimum recorded in resistant genotypes ICP-7220 (0.42, 0.55, 0.73 and 0.78 mg/g) followed by IPA-7-2 (0.44, 0.57, 0.74 and 0.79 mg/g), respectively in un-inoculated. In inoculated genotypes maximum chlorophyll content 'b' was found in all stages of a-virulent isolates At30 (0.59, 0.74, 0.87 and 0.96 mg/g) followed by At71 (0.57, 0.70, 0.84 and 0.94 mg/g,), respectively whereas, minimum content was recorded in virulent isolates At17 (0.12, 0.26, 0.39 and 0.47 mg/g) followed by At45 (0.14, 0.29, 0.41 and 0.49 mg/g), respectively. The data indicate that maximum chlorophyll 'b' content was found in resistant genotypes at early stage of plant with minimum reduction whereas, lowest content were found in susceptible genotypes old plants with highest reduction. It showed same trends in a-virulent isolates in which lowest reduction of chlorophyll content was found as compared to virulent isolates.

Estimation of total chlorophyll :

The result is presented in Table 3 which indicates that total chlorophyll content was recorded maximum in all stages of resistant genotypes in ICP-7220 (2.85, 2.62, 2.38 and 2.22 mg/g) followed by IPA-7-2 (2.82, 2.60, 2.37 and 2.18 mg/g), respectively, whereas the minimum was recorded in highly susceptible genotypes in MAL-24 (2.34, 2.23, 1.98 and 1.81 mg/g) followed by Bahar (2.43, 2.25, 2.08 and 1.86 mg/g), respectively in un-inoculated genotypes. In inoculated genotypes maximum total chlorophyll content was found in all stages of a-virulent isolates At30 (2.77, 2.52 2.32 and 2.19 mg/g) followed by At71 (2.76, 2.50, 2.24 and 2.17mg/g), respectively whereas the minimum content was recorded

Table 1: Effect of *Alternaria tenuissima* on chlorophyll' a' content (mg/g fresh tissue) of pigeonpea leaves in different genotypes inoculated with different isolates at 70, 95, 120 and 145 days after sowing

Genotypes	70 DAS		95 DAS		120 DAS		145 DAS	
	Control	Mean of 10 isolates*	Control	Mean of 10 isolates*	Control	Mean of 10 isolates*	Control	Mean of 10 isolates*
ICP-8869	2.13	1.91	1.94	1.76	1.72	1.52	1.63	1.40
IPA-7-2	2.22	1.98	2.00	1.81	1.91	1.68	1.78	1.44
ICP-11294	1.98	1.78	1.74	1.56	1.63	1.44	1.49	1.28
ICP-7220	2.32	2.14	2.08	1.90	1.96	1.72	1.82	1.53
MA-98	2.16	1.91	1.98	1.78	1.77	1.54	1.64	1.42
ICP-4725	2.03	1.81	1.80	1.60	1.64	1.46	1.52	1.30
BSMR-736	1.95	1.67	1.70	1.53	1.54	1.41	1.38	1.20
ICP-7182	1.96	1.70	1.71	1.54	1.58	1.42	1.40	1.26
ICP-13174	2.08	1.86	1.86	1.71	1.70	1.50	1.54	1.36
Bahar	1.87	1.63	1.67	1.49	1.49	1.33	1.36	1.16
MAL-24	1.75	1.58	1.65	1.46	1.40	1.25	1.24	1.13
DA-11	2.05	1.84	1.84	1.64	1.65	1.47	1.53	1.35
Mean	2.04	-	1.83	-	1.67	-	1.53	-
Factors		C.D.(P=0.05)		C.D.(P=0.05)		C.D.(P=0.05)		C.D.(P=0.05)
Genotype		0.04		0.03		0.05		0.02
Isolates		0.03		0.03		0.03		0.02
Genotype×Isolates		NS		NS		NS		0.05

*Mean of three replication NS= Non-signficant

Table 2: Effect of *Alternaria tenuissima* on chlorophyll 'B' content (mg/g fresh tissue) of pigeonpea leaves in different genotypes inoculated with different isolates at 70, 95, 120 and 145 days after sowing

Genotypes	70 DAS		95 DAS		120 DAS		145 DAS	
	Control	Mean of 10 isolates*	Control	Mean of 10 isolates*	Control	Mean of 10 isolates*	Control	Mean of 10 isolates*
ICP-8869	0.51	0.33	0.63	0.46	0.77	0.59	0.87	0.69
IPA-7-2	0.44	0.27	0.57	0.43	0.74	0.56	0.79	0.62
ICP-11294	0.63	0.42	0.75	0.53	0.88	0.68	0.94	0.77
ICP-7220	0.42	0.25	0.55	0.40	0.73	0.55	0.78	0.61
MA-98	0.49	0.30	0.60	0.45	0.75	0.57	0.81	0.64
ICP-4725	0.62	0.40	0.73	0.51	0.85	0.66	0.92	0.74
BSMR-736	0.67	0.46	0.79	0.58	0.91	0.71	0.97	0.82
ICP-7182	0.65	0.44	0.77	0.56	0.90	0.70	0.96	0.80
ICP-13174	0.58	0.36	0.67	0.48	0.83	0.62	0.89	0.71
Bahar	0.70	0.47	0.81	0.61	0.92	0.72	0.98	0.85
MAL-24	0.72	0.49	0.83	0.63	0.93	0.74	0.99	0.88
DA-11	0.60	0.38	0.69	0.49	0.84	0.64	0.91	0.73
Mean	0.59	-	0.70	-	0.84	-	0.90	-
Factors		C.D.(P=0.05)		C.D.(P=0.05)		C.D.(P=0.05)		C.D.(P=0.05)
Genotype		0.04		0.02		0.02		0.02
Isolates		0.03		0.02		0.02		0.01
Genotype×Isolates		NS		NS		NS		0.04

*Mean of three replication NS= Non-significant

Table 3: Effect of *Alternaria tenuissima* on total chlorophyll content (mg/g fresh tissue) of pigeonpea leaves in different genotypes inoculated with different isolates at 70, 95, 120 and 145 days after sowing

Genotypes	70 DAS		95 DAS		120 DAS		145 DAS	
	Control	Mean of 10 isolates*	Control	Mean of 10 isolates*	Control	Mean of 10 isolates*	Control	Mean of 10 isolates*
ICP-8869	2.77	2.48	2.34	2.19	2.24	2.04	2.14	1.87
IPA-7-2	2.82	2.52	2.60	2.29	2.37	2.18	2.18	1.99
ICP-11294	2.55	2.28	2.28	2.11	2.12	1.95	1.92	1.74
ICP-7220	2.85	2.60	2.62	2.37	2.38	2.21	2.22	2.09
MA-98	2.79	2.50	2.57	2.28	2.36	2.11	2.16	1.92
ICP-4725	2.62	2.39	2.30	2.14	2.13	1.96	1.95	1.77
BSMR-736	2.50	2.22	2.26	2.09	2.09	1.91	1.88	1.69
ICP-7182	2.52	2.25	2.27	2.10	2.11	1.94	1.90	1.71
ICP-13174	2.74	2.45	2.33	2.18	2.17	2.01	2.05	1.83
Bahar	2.43	2.18	2.25	2.06	2.08	1.87	1.86	1.67
MAL-24	2.34	2.14	2.23	2.02	1.98	1.83	1.81	1.64
DA-11	2.66	2.42	2.32	2.17	2.15	1.98	2.04	1.81
Mean	2.64	-	2.36	-	2.18	-	2.01	-
Factors		C.D. (P=0.05)		C.D. (P=0.05)		C.D. (P=0.05)		C.D. (P=0.05)
Genotype		0.02		0.04		0.02		0.04
Isolates		0.02		0.04		0.02		0.03
Genotype×Isolates		NS		0.13		0.05		NS

*Mean of three replication NS= Non-significant

virulent isolates At17 (1.84, 1.73, 1.55 and 1.40 mg/g) followed by At45 (1.92, 1.81, 1.61 and 1.45 mg/g), respectively. The data indicate that maximum total chlorophyll content was recorded in resistant genotypes at early stage of plants with minimum reduction whereas, lowest content were found in susceptible genotypes old plants with highest reduction. It showed same trend in a-virulent isolates in which lowest reduction of total chlorophyll content was found as compared to virulent isolates. Total chlorophyll content was also in the same trend as it is cumulative effect of chlorophyll 'a' and 'b'.

In the present study, analysis of biochemical constituents chlorophyll 'a', chlorophyll 'b' and total chlorophyll have recorded in higher amounts in resistant cultivars followed by moderately resistant and moderately susceptible, whereas lower amount in susceptible genotypes in both un-inoculated and inoculated leaves. Their relative concentration was also found decreased as a result of disease, but the rate of decrease was higher in susceptible genotypes than resistant genotypes. Chlorophyll content significantly reduced under inoculated condition in all the genotypes compared to their corresponding values in un-inoculated. The maximum chlorophyll content was found in resistant genotypes at early stage of plants with minimum reduction whereas, lowest content were found in susceptible genotypes old plants with highest reduction. It showed same trend in a-virulent isolates in which lowest reduction chlorophyll content was found as compared to virulent isolates. Abnormalities in the form and destruction of chloroplasts are common features of diseased tissue in plants infected with pathogens, which usually exhibit reduced photosynthetic rate, phosphorylation, hill reaction and carbon dioxide assimilation (Bawden, 1999). These changes may be partially or completely accounted by a reduction in chlorophyll content. In the susceptible cultivars more reduction in chlorophyll content may be due to death of more leaf tissues due to infection of *Alternaria*. Also in the present study, chlorophyll 'b' was found more sensitive than chlorophyll 'a' as it recorded higher reduction percentage after inoculation of *A. tenuissima*. Many researchers reported similar findings reduction of synthetic pigment like Singh and Singh (1999) on pigeonpea pathogen *A. tenuissima*, Bhaskaran and Kandaswamy (1978); Amaresh (2000) and Mesta (2006) on alternaria blight of sunflower, Theertha and Shambulingappa (1986) in rust sunflower. Bhavani *et al.* (1998) on sunflower mosaic virus, Pati *et al.* (2007)

Table 4: Effect of *Alternaria tenuissima* on carotene content (mg/g fresh tissue) of pigeonpea leaves in different genotypes inoculated with different isolates at 70, 95, 120 and 145 days after sowing

Genotypes	70 DAS		95 DAS		120 DAS		145 DAS	
	Control	Mean of 10 isolates*	Control	Mean of 10 isolates*	Control	Mean of 10 isolates*	Control	Mean of 10 isolates*
ICP-8869	1.94	1.82	2.04	1.65	2.07	1.51	2.15	1.40
IPA-7-2	1.97	1.85	2.07	1.68	2.15	1.55	2.22	1.47
ICP-11294	1.81	1.69	1.87	1.55	1.95	1.45	1.98	1.32
ICP-7220	2.00	1.87	2.08	1.69	2.19	1.57	2.26	1.49
MA-98	1.95	1.83	2.06	1.65	2.11	1.53	2.16	1.41
ICP-4725	1.82	1.70	1.91	1.57	1.96	1.46	2.01	1.34
BSMR-736	1.76	1.63	1.84	1.50	1.89	1.41	1.95	1.29
ICP-7182	1.80	1.68	1.86	1.53	1.93	1.43	1.97	1.31
ICP-13174	1.89	1.76	1.94	1.62	2.04	1.49	2.08	1.39
Bahar	1.75	1.61	1.80	1.49	1.87	1.39	1.91	1.26
MAL-24	1.74	1.60	1.79	1.47	1.83	1.37	1.89	1.24
DA-11	1.86	1.72	1.93	1.61	1.99	1.47	2.04	1.37
Mean	1.86	-	1.93	-	2.00	-	2.05	-
Factors		C.D.(P=0.05)		C.D.(P=0.05)		C.D.(P=0.05)		C.D.(P=0.05)
Genotype		0.01		0.01		0.02		0.02
Isolates		0.02		0.01		0.01		0.02
Genotype×Isolates		NS		0.04		0.04		0.05

*Mean of three replication NS= Non-significant

Withania somnifera (*Alternaria alternata*), Ravi and Sreeramulu (2009) in teak plants of powdery mildew and Ghose *et al.* (2010) mulberry leaves on leaf blight.

Estimation of total carotene :

A significant interaction was found among the ten isolates and twelve genotypes except 70 DAS on the basis of their carotene content. The result is presented in Table 4 which indicates that carotene content measured on four different dates old plants, maximum content in was recorded all stages of resistant genotypes ICP-7220 (2.00, 2.08, 2.19 and 2.26 mg/g) followed by IPA-7-2 (1.97, 2.07, 2.15 and 2.22 mg/g), respectively whereas, the minimum content was recorded in highly susceptible genotypes MAL-24 (1.74, 1.79, 1.83 and 1.89 mg/g) followed by Bahar (1.75, 1.80, 1.87 and 1.91 mg/g), respectively in un-inoculated genotypes. The maximum carotene content was recorded in inoculated genotypes in all stages of a-virulent isolates At 30 (1.94, 1.77, 1.62 and 1.52 mg/g) followed by At 71 (1.92, 1.77, 1.58 and 1.48 mg/g), respectively whereas, the minimum content was recorded in virulent isolates At 17 (1.50, 1.38 1.22 and 1.02 mg/g) followed by At 45 (1.51, 1.39, 1.23 and 1. 04 mg/g), respectively. The data indicate that maximum carotene content was found in resistant genotypes at early stage of plants with minimum reduction whereas, lowest content were recorded in susceptible genotypes old plants with highest reduction. It showed same trend in a-virulent isolates in which lowest reduction of carotene content was found as compared to virulent isolates. It was also noticed that in healthy plants carotene increase according age but in infected (inoculated) plants reversed decrease. Similar results were reports by Singh and Singh (1999) in pigeonpea leaves (*A. tenuissima*) and Ghose *et al.* (2010) in mulberry leaf blight.

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