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ISSN: 0974-4908
Res. Environ. Life Sci.
9(9) 1045-1047 (2016)

Nature of biochemical parameters in slow rusting pearl millet genotypes

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(Received: September 06, 2015; Revised received: June 08, 2016; Accepted: june 12, 2016)

Abstract: Biochemical parameters were studied to understand slow rusting resistance in relation to total free phenol content, total sugar, reducing sugar, non-reducing sugar and amino acids in both diseased and healthy leaves at 60 and 80 days after sowing. All the nine pearl millet genotypes under study indicated higher amount of total sugars and reducing sugar in rust infected leaves as compared to healthy leaves and higher amount of phenols and total amino acids in healthy leaves as compared to rust infected leaves both at 60 and 80 days after sowing.

Key words: Slow rusting resistance, Pearl millet, Genotypes and Biochemical parameters

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is the most drought and heat tolerant crop. Among the cereals it has the highest water use efficiency under drought stress.Rust caused by the fungus *Puccinia substriata* Ell. and Brath.var. *indica* (Ramchar and Cumm.) is one of the major diseases affecting both forage and grain production in pearl millet. It is reported to cause up to 51% reduction in digestible dry matter yield (Monson *et al*., 1986). Pearl millet genotypes slow rusting or partial resistance are important as they minimize the rate of spread of rust and further in checking the possible occurrence of epidemics without causing any adverse effect on the yield. However the biochemical nature in pearl millet genotypes has not been clearly understood. It is well understood that biochemicals and their oxidation products are implicated in disease resistance. Hence the present study was undertaken to understand the nature of slow rusting resistance in pearl millet genotypes in relation to biochemical parameters such as total free phenol, total sugar, reducing sugar, non-reducing sugar and amino acid content in both diseased and healthy leaves at different stages of crop growth.

Material and Methods

Nine pearl millet genotypes viz., ADMR 10, ADMR 16, ADMR 17, ADMR 27, ADMR 49, J 2496, J 2510, J 2517 and 70 SB 13 were used to study their biochemical nature in relation to rust resistance. All the nine pearl millet genotypes were sown simultaneously both in field and glass house at College of Agriculture, Vijaypura. The plants grown in the pots in glass house are used to get the healthy plants for biochemical analysis. Healthy leaves from the glass house grown plants and diseased leaves from the field trial were collected at 60 and 80 days after sowing for biochemical analysis. The leaf material was extracted in ethanol (80%) as per the procedure followed by Jaypal and Mahadevan (1968) for determination of sugars, phenols and amino acids. The reducing sugar was estimated by Nelson's modifications of Somogyi's method (Nelson, 1944). Non- reducing sugar was hydrolysed using 1 ml of 1N H_2SO_4 and then estimated as in case of reducing sugars to get the total sugars. Non- reducing sugars were calculated by subtracting the reducing sugars from that of total sugars. Total free phenols in the clarified ethanol extract of leaves from various treatments was estimated by Folin Ciocalteau Reagent (Bray and Thorpe, 1954). Total free amino acids in ethanol extracts was estimated by nin-hydrin method (Moore and Stein, 1958).

Results and Discussion

Total sugar: Total sugar content was increased in diseased leaves from 60 to 80 DAS. At 60 DAS, total sugar content was more in healthy leaves as compared to diseased leaves the genotype 70 SB 13 recorded significantly highest sugar content of 25.53 mg/g in healthy leaves and least in ADMR 49 (18.40mg/g). Where as in diseased leaves their content was more in genotype 70 SB 13 (19.17 mg), while least in J 2496 (14.07 mg/g). Further the slow rusting genotypes 70 SB 13, J 2496, J 2510 and J 2517 recorded highest sugar content, while corresponding fast rusting genotypes ADMR 10, ADMR 16, ADMR 17, ADMR 27 and ADMR 49 were recorded less sugar content. At 80 DAS, the total sugar content was more in diseased leaves compared to healthy leaves, due to synthesis of sugars by the pathogen interaction in the amylolytic activity. In the later stages the slow rusting genotypes recorded highest sugar content as compared to fast rusting genotypes. In healthy leaves maximum total sugar content was observed in ADMR 27 (21.44 mg/g) followed by ADMR 10, ADMR 16, 70 SB 13, J 2496, ADMR 49, ADMR 17 and J 2510 and these were on par with each other. Where as least total sugar content was recorded in genotype J 2517 (17.53 mg/g). In diseased leaves, the total sugar content among the genotypes was found significant. Highest sugar content was recorded in 70 SB 13 (24.15 mg/g) and it was on par with J 2510, ADMR 16, ADMR 27 and ADMR 49. Least sugar content was observed in J 2517 (18.01 mg/g) and it was on par with ADMR17, J 2496 and ADMR 10. These results are in agreement with the findings of Patil and Kulkarni (1977) also observed marked increase in reducing sugars, non-reducing sugars, total sugars and starch in the rust infected sunflower leaves caused by *P. helianthi*.

Reducing sugar: The results revealed that, the reducing sugar content (Table 1) increased in diseased leaves from 60 to 80 DAS, due to higher amylase activity in infected leaves. At 60DAS, the reducing sugar content was more in healthy leaves as compared to diseased leaves. In diseased leaves, the highest reducing sugar content was recorded in genotype J 2496 (13.25 mg/g) followed by 70 SB 13 and J 2510 and were on par with each other. Least reducing sugar content was noticed in ADMR 17 (10.03 mg/g) and it was on par with ADMR 49, ADMR 27, ADMR 16 and ADMR 10. In healthy leaves, maximum reducing sugar content was observed in 70 SB 13 (13.80 mg/g) followed by J 2496, J 2517, J 2510, ADMR 27 and ADMR 16 and were on par with each other. Least reducing sugar content was observed in ADMR 49 (10.40 mg/g) and it was on par with the reducing sugar content of ADMR 17 and ADMR 10. Further when we compared to slow and fast rusting genotypes, the reducing sugar content was more in slow rusters as compare to fast rusters. At 80 DAS, the reducing sugar content in both healthy and diseased leaves differed significantly among the genotypes. The reducing sugar was more in diseased leaves compared to healthy leaves. Maximum reducing sugar content was recorded in diseased leaves of J 2496 (14.63 mg/g) and least in healthy leaves of ADMR 49(10.12 mg/g). In healthy leaves, maximum reducing sugar content was recorded in J 2510 (12.28 mg/g) and it was on par with J 2496, ADMR 16, ADMR 27 and J 2517. The genotype ADMR 49 recorded least reducing sugar content of 10.12 mg/g and it was on par with ADMR 17, 70 SB 13 and ADMR 10. In diseased leaves, highest reducing sugar content was observed in J 2496 (14.63 mg/g) and least in ADMR 17 (10.40 mg/g). The reducing sugar content in J 2596 was on par with the reducing sugar content of J 2510. Next genotype which has recorded highest reducing sugar content was J 2517 (12.95 mg/g) followed by ADMR 10, ADMR 16 and 70 SB 13 and were on par with each other. Further the slow rusting genotypes was recorded more sugar content as compared to fast rusting genotypes. Many earlier workers reported higher content of reducing sugars in resistant genotypes compared to moderately resistant and susceptible genotypes (Sunkad and Kulkarni, 2006).

Non-reducing sugars: The results revealed at 60 DAS, the non-reducing sugar content (Table 1) was more in healthy leaves compared to diseased leaves, due to diversion of sugars by pathogen in infected leaves. In healthy leaves, the maximum non- reducing sugar content was observed in genotype 70 SB 13 (11.73 mg/g) followed by J 2510 ADMR 10 and ADMR 16 and the least in genotype ADMR 17 (7.78 mg/g). In diseased leaves, the non- reducing sugar content varied among genotypes. Maximum non-reducing sugar content was recorded in genotype ADMR10 (8.28 mg/g) followed by ADMR 16 and ADMR 27 and the least in J 2496 (0.82 mg/g). Further when we compared to slow and fast rusting genotypes, the non reducing sugar content was less in slow rusters as compare to fast rusters at both 60 and 80 days after sowing. At 80 DAS, the non-reducing sugar content was less in healthy leaves compared to diseased leaves. In healthy leaves the non-reducing sugar content was maximum in genotype ADMR 10 and ADMR 27(9.4 mg/g) followed by ADMR 49 and ADMR 17 and the genotype J 2510 was recorded least non reducing sugar of 6.04 mg/g. In rust infected leaves, the highest non- reducing content was recorded in genotype 70 SB 13 (11.95 mg/g) followed by ADMR 16 and J 2510 and the least in J 2517 (5.06 mg/g). Patil and Kulkarni (1977) also observed marked increase in reducing sugars, non-reducing sugars, total sugars and starch in the rust infected sunflower leaves caused by *P. helianthi* Schw.

Infection by pathogens bring about various changes in amino acid metabolism of the host plant. Amino acids act as inhibitory to the activity of pathogen or precursors of various fungal toxic compounds, particularly phenolics. In the present investigation, the total amino acids content (Table 2) was decreased in both healthy and diseased leaves from 60 to 80 DAS, due to utilization of amino acids and synthesis of phenolics by the pathogen. At 60 DAS, in healthy leaves, highest amino acid content was observed in genotype 70 SB 13 (16.38 mg/g) followed by J 2496, ADMR 16, ADMR 27 and ADMR 17 and they were on par with each other. Least amino acid content was recorded in genotype J 2517 (10.88mg/g) and it was on par with ADMR 49 and J 2510. In diseased leaves, the amino acids content significantly differed among the genotypes. The maximum amino acids content was recorded in genotype ADMR 16 (11.78 mg/g) followed by ADMR 49, J 2510, J 2496 and 70 SB 13 and they were on par with each other and the least amino acids content was observed in ADMR 27 (9.96 mg/g). Further the amino acid content was more in slow rusting genotypes as compared to fast rusting genotypes.

At 80 DAS, the amino acids content in both healthy and diseased leaves differed significantly among the genotypes. In healthy leaves, the maximum amino acids content was recorded in genotype J 2517 and J 2496 (11.73 mg/g) followed by J 2510, ADMR 10, ADMR 17 and 70 SB 13 and they were on par with each other. Least amino acids content was found in ADMR 27 (9.84 mg/g). In rust

Table-1: Total, reducing and non-reducing sugar content (mg/g of dry leaf weight) in healthy and diseased leaves of different pearl millet genotypes

Genotypes	60DAS Healthy			60DAS Diseased			80DAS Healthy			80DAS Diseased		
No.	Total sugar	Reducing sugar	Non-reducing sugar	Total sugar	Reducing sugar	Non-reducing sugar	Total sugar	Reducing sugar	Non-reducing sugar	Total sugar	Reducing sugar	Non reducing sugar
Fast Rusters												
ADMR 10	21.96	11.40	10.55	19.05	10.77	8.28	20.79	10.86	9.94	20.02	12.65	7.38
ADMR 16	22.33	12.08	10.25	18.03	10.58	7.45	20.61	11.59	9.02	23.50	12.49	11.01
ADMR 17	19.00	11.22	7.78	16.56	10.03	6.53	18.66	10.21	8.45	19.11	10.40	8.71
ADMR 27	22.10	12.49	9.60	17.81	10.49	7.32	21.44	11.50	9.94	22.08	11.67	10.41
ADMR 49	18.40	10.40	8.00	15.20	10.40	4.80	19.32	10.12	9.20	21.16	11.04	10.12
Slow Rusters												
J 2496	21.88	13.63	8.25	14.07	13.25	1.82	19.46	11.73	7.73	20.01	14.63	5.38
J 2510	23.92	13.01	10.91	16.12	12.30	3.82	18.32	12.28	6.04	23.82	13.05	10.76
J 2517	23.02	13.10	9.92	14.77	11.18	3.59	17.53	11.45	6.07	18.01	12.95	5.06
70 SB 13	25.53	13.80	11.73	19.17	12.87	6.30	20.29	10.76	9.52	24.15	12.20	11.95
Mean	22.01	12.35	9.67	16.75	11.32	5.55	19.60	11.17	8.43	21.32	12.34	8.98
S.Em±	1.53	0.41	-	1.51	0.46	-	0.88	0.33	-	0.96	0.30	-
CD (0.01)	6.33	1.70	-	NS	1.89	-	3.62	1.36	-	3.96	1.23	-

DAS = Days after sowing NS= Non SignificantAmino acids

Table-2. Amino acids and total phenols content (mg/g of dry leaf weight) in healthy and diseased leaves of different pearl millet genotypes

Genotypes No.	60 DAS Healthy		60 DAS Diseased		80 DAS Healthy		80 DAS Diseased	
	Amino acids	Total Phenols	Amino acids	Total Phenols	Amino acids	Total Phenols	Amino acids	Total Phenols
Fast Rusters								
ADMR 10	12.44	19.76	10.58	15.27	11.22	18.77	10.49	15.25
ADMR 16	13.62	18.00	11.78	16.75	10.85	19.97	9.75	16.46
ADMR 17	12.20	18.71	11.13	17.48	11.13	19.10	9.01	17.39
ADMR 27	12.89	18.53	9.66	17.02	9.84	17.11	8.92	17.66
ADMR 49	11.01	19.32	11.59	18.22	10.39	20.24	10.21	15.45
Slow Rusters								
J 2496	14.50	23.13	11.32	18.36	11.73	20.15	9.25	18.87
J 2510	11.88	21.88	11.32	19.60	11.59	20.15	10.35	18.77
J 2517	10.88	24.63	10.07	18.95	11.73	18.77	9.80	17.80
70 SB 13	16.38	23.88	11.18	19.88	11.04	18.08	9.66	20.01
Mean	12.86	20.87	10.95	18.06	11.06	19.15	9.72	17.52
S.Em±	0.47	0.79	0.45	1.07	0.22	0.38	0.20	0.82
CD (0.01)	1.92	3.28	1.86	NS	0.90	1.52	0.81	3.38

DAS =days after sowing NS= Non Significant

infected leaves, the highest amino acids content was found in genotype ADMR 10 (10.49mg/g) followed by J 2510, ADMR 49, J 2517, ADMR 16 and 70 SB 13 and were on par with each other. Least amino acids content was recorded in genotype ADMR 27 (8.92mg/g). Further the amino acids content was found similar in both slow and fast rusting genotypes (Table2).These results are in agreement with findings of Ahmed *et al*.(1985) who studied the effect of leaf rust infection on amino acid and amide content of wheat (*T.aestivum*) var. Pak-70. They detected 19 ninhydrin positive compounds from the leaf and 15 from the stem of Pak-70 cultivar in both infected and healthy leaves. In leaves glutamine, histidine, arginine and tryptophan occurred only in diseased samples and cysteine and tyrosine only in healthy samples. Glycine, leucine, alanine, phenylalanine and asparagine were found in both healthy and diseased samples.

Total free phenols : Among all biochemical compounds, phenols have been found to play an important role in determining resistance or susceptibility of a host to parasitic infection. Many earlier workers reported higher content of phenolics in resistant genotypes than the susceptible (Rubin and Aksenova, 1957; Raghunathan *et al.,* 1958; Anahosur and Naik, 1985). The results revealed that, the total phenol content (Table2) decreased in both healthy and diseased leaves from 60 to 80 DAS, due to decrease inactivity of enzymes like phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL). At 60 DAS, the total phenol content was more in healthy leaves as compared to diseased leaves. In healthy leaves the phenol content was found significant among the genotypes. Maximum phenol content was found in genotype J 2517 (24.63mg/g) followed by the genotype 70 SB 13, J 2496, J 2510 and they were on par with each other. Least phenol content was found in ADMR 16 (18.0 mg/g) and it was on par with ADMR 27, ADMR 17 and ADMR 49. In rust infected leaves the phenol content was found non significant among the genotypes, however the maximum phenol content was observed in genotypes 70 SB 13 (19.88 mg/g) and J 2510 (19.60 mg/g)and least phenol content was recorded in ADMR 27 (17.02mg/g).

At 80 DAS, the phenol content was more in healthy leaves compared to diseased leaves irrespective of the genotypes, due to decrease enzyme activity in diseased leaves. The phenol content differed significantly among genotypes. In healthy leaves, the genotype ADMR 49 recorded maximum phenol content (20.254 mg/g) followed by J 2510, ADMR 16, ADMR 17, ADMR 10 and J 2517 and were on par with each other. Significantly least phenol content was observed in ADMR 27 (17.11 mg/g). In diseased leaves, the maximum phenol content was recorded in genotype 70 SB 13 (20.01 mg/g) followed by J 2496, J 2510, ADMR 27 and ADMR 17 and were on par with each other and least phenol content was found in genotype ADMR 10 (15.25 mg/g). Further when we compared to slow and fast rusting genotypes, the phenol content was more in slow rusters and less in fast rusters at both 60 and 80 days after sowing. These results are in conformity with findings of Saxena *et al*. (1987) noted the changes in total phenolics in wheat in relation to infection by *P. recondita*. The resistant varieties, HD 2009 and HD 2285 showed higher levels of phenols than susceptible varieties WG 377 and WG 357 in response to infection at vegetative and reproductive stages. Sunkad and Kulkarni (2006) studied biochemical changes in resistant (GPBD-4 and DH-22), moderately resistant (K-134 and R-8808) and susceptible (KRG-1 and TMV-2) genotypes of groundnut. Resistant and moderately resistant genotypes recorded more sugars, phenol, ortho-dihydroxy phenol and protein contents than susceptible ones.

The overall study indicated that, higher amounts of total sugars, reducing sugars were found in rust infected leaves as compared to healthy leaves of different genotypes and higher amounts of total phenols and total amino acids values were found in healthy leaves as compared to diseased leaves at both the stages of crop growth.

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