Theor Appl Genet (1986) 71:648–651

Biochemical genetic basis of downy mildew resistance in pearl millet

S. K. Thukral*, D. R. Satija and V. P. Gupta

Department of Genetics, Punjab Agricultural University, Ludhiana, India

Received May 10, 1985; Accepted August 12, 1985
Communicated by J. MacKey

Summary. The study of phenolic content and activities of peroxidase and polyphenoloxidase in relation to the degree of downy mildew infection of 12 pearl millet cultivars revealed that these were linearly related to the degree of resistance at both the 30 and 50 day growth stages. Useful electrophoretic differences in peroxidase and polyphenoloxidase were also observed with respect to the expression of resistance.

Key words: *Sclerospora graminicola* – Peroxidase – Polyphenoloxidase – Isozymes – *Pennisetum typhoides*

Introduction

Downy mildew of pearl millet (*Pennisetum typhoides* (Burm) S. & H.), caused by *Sclerospora graminicola* (Sacc) shroet, is a unique example of abnormal growth. The symptoms of the disease appear at the seedling stage on the folliage and end up with the development of oospores on the ears. Murty (1980) suggested that for quick screening against downy mildew, assaying resistance in terms of biochemical genetic parameters which are less influenced by the environment, would be more reliable. The present paper describes the isozyme patterns and activities of peroxidase and polyphenoloxidase together with the phenolic content of pearl millet lines having differential degrees of downy mildew resistence.

* Present address: Assistant scientist (Genetics), NARP, Haryana Agricultural University, Hissar, India

Material and methods

Twelve diverse pearl millet lines differing in their disease reaction and rated as immune (L5), highly resistant (L101, L103, L105), resistant (A5, A10, L102, L104), susceptible (L20, L106) and highly susceptible (L10, A7) were used during the present study.

The inoculum of downy mildew spores was scattered in furrows two months prior to the sowing of the test material. Secondary inoculum was also provided by infector rows (Williams et al. 1981) and sporangial inoculation (Thakur and Kanwar 1977) techniques. Downy mildew score was recorded as a percentage infection index following the scale of Williams et al. (1976). For biochemical analysis the normal and diseased samples of leaves from resistant and susceptible genotypes were collected from 30 day-(I), and 50 day-(II) old plants. Peroxidase and polyphenoloxidase activities were assayed following Shannon et al. (1966) and Bateman (1967), respectively, and expressed as ΔOD/min/mg soluble protein. Soluble protein content was estimated following the method of Lowery et al. (1951). Phenolic content was estimated by the method of Swain and Hillis (1959). Electrophoresis was conducted according to Gupta and Stebbins (1969) and peroxidases and polyphenoloxidases were localized following the method of Veech (1969) and Van Loon (1971), respectively. The zymogram patterns were recorded immediately as they appeared.

Results and discussion

Phenolic content and the activities of peroxidase and polyphenoloxidase were found to be related to degree of resistance (Table 1). The immune genotype L5 had the maximum, and the highly susceptible genotypes L10 and A7 had the minimum phenolic content, peroxidase activity and polyphenoloxidase activity. Diseased and normal leaf tissue did not differ much regarding different biochemical constituents.

It was found that the characterization of genotypes based on phenols, peroxidase and polyphenoloxidase

Table 1. Mean values of selected lines for biochemical traits in 30-(I) and 50-(II) day-old plants, and downy mildew infection index

Genotypes		Total phenols		Peroxidase activity		Polyphenoloxidase activity		Downy mildew infections index
		I	II	I	II	I	II	
L5	N	1.97	1.18	1.18	4.26	0.67	3.03	0.00
	D	–	–	–	–	–	–	
L105	N	1.41	2.89	0.97	3.40	0.56	2.23	1.01
	D	1.33	2.79	0.90	3.31	0.55	2.16	
L101	N	1.32	2.70	0.98	3.39	0.57	2.30	1.29
	D	1.23	2.65	0.92	3.30	0.57	2.17	
L103	N	1.36	2.69	0.94	3.31	0.65	2.18	1.30
	D	1.27	2.63	0.91	3.25	0.55	2.09	
A5	N	1.20	2.57	0.87	3.03	0.50	2.01	2.55
	D	1.03	2.49	0.82	2.96	0.49	1.95	
L104	N	1.30	2.63	0.78	3.12	0.49	1.89	4.79
	D	1.23	2.56	0.71	3.02	0.48	1.81	
L102	N	1.26	2.60	0.79	3.08	0.46	1.86	5.01
	D	1.12	2.53	0.77	2.99	0.46	1.78	
A10	N	1.11	1.89	0.85	3.09	0.48	1.91	5.05
	D	1.06	1.88	0.80	3.01	0.48	1.86	
L20	N	1.08	1.98	0.60	2.37	0.40	1.69	7.35
	D	1.00	1.89	0.54	2.16	0.39	1.59	
L106	N	1.17	2.02	0.53	2.44	0.40	1.70	11.48
	D	1.08	1.93	0.50	2.37	0.39	1.66	
L10	N	0.77	1.71	0.34	1.87	0.28	1.15	32.73
	D	0.72	1.63	0.30	1.78	0.26	1.11	
A7	N	0.92	1.78	0.31	1.81	0.26	1.12	40.06
	D	0.90	1.71	0.24	1.71	0.23	1.15	

N = normal tissue; D = diseased tissue

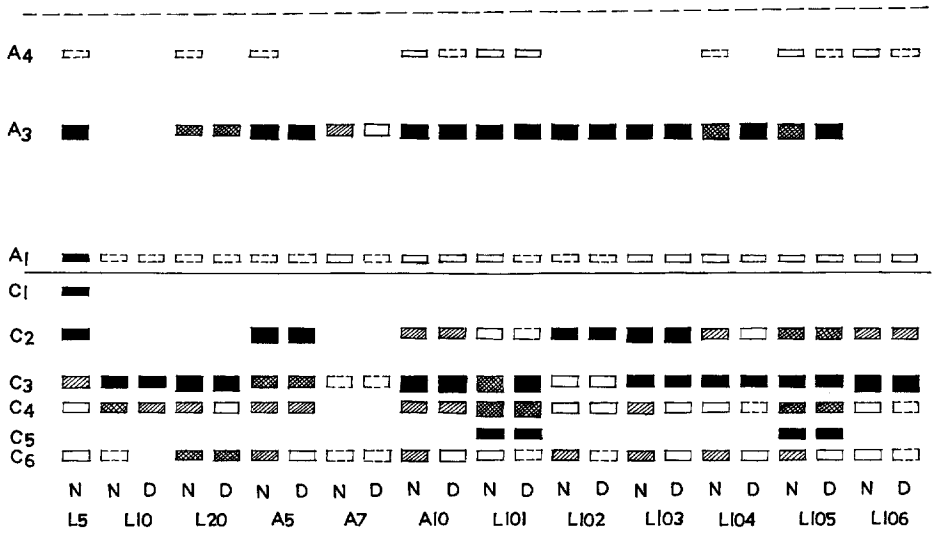

Fig. 1. Isozyme pattern of peroxidases at 30-day stage

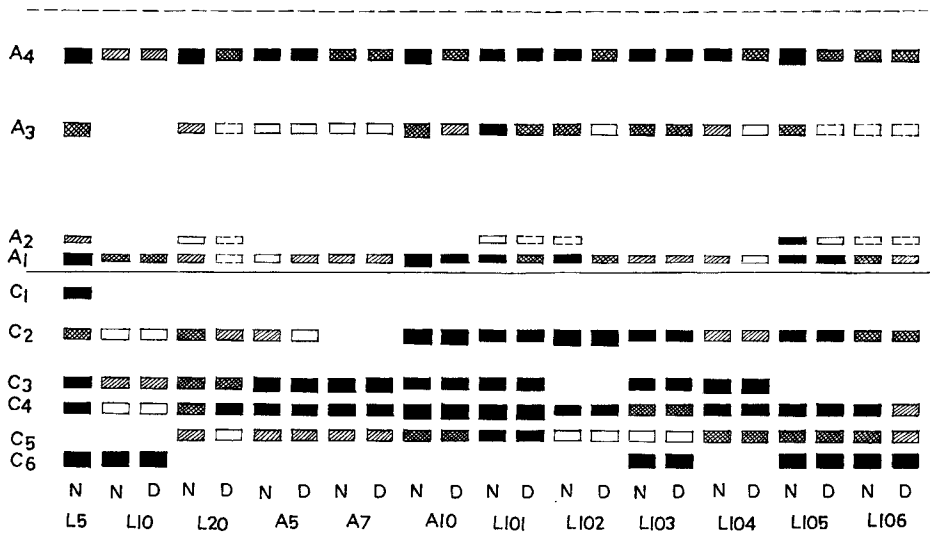

Fig. 2. Isozyme pattern of peroxidases at 50-day stage

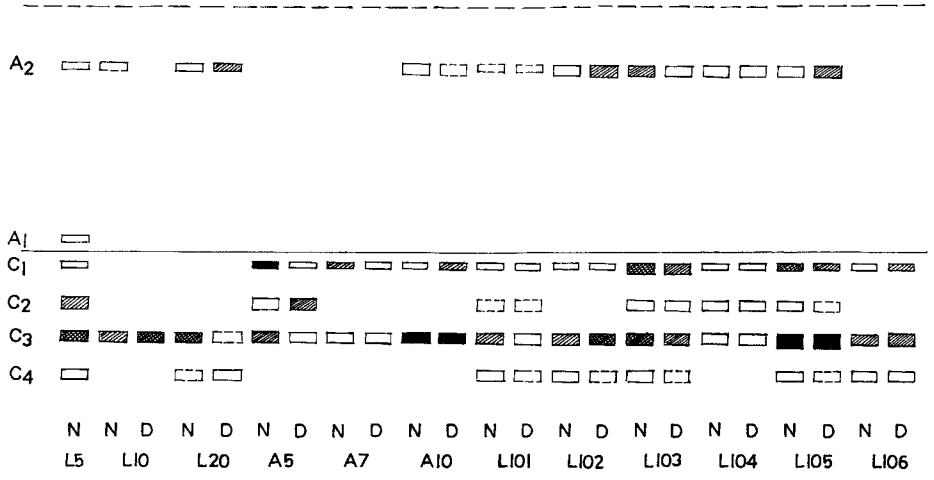

Fig. 3. Isozyme pattern of polyphenoloxidases at 30-day stage

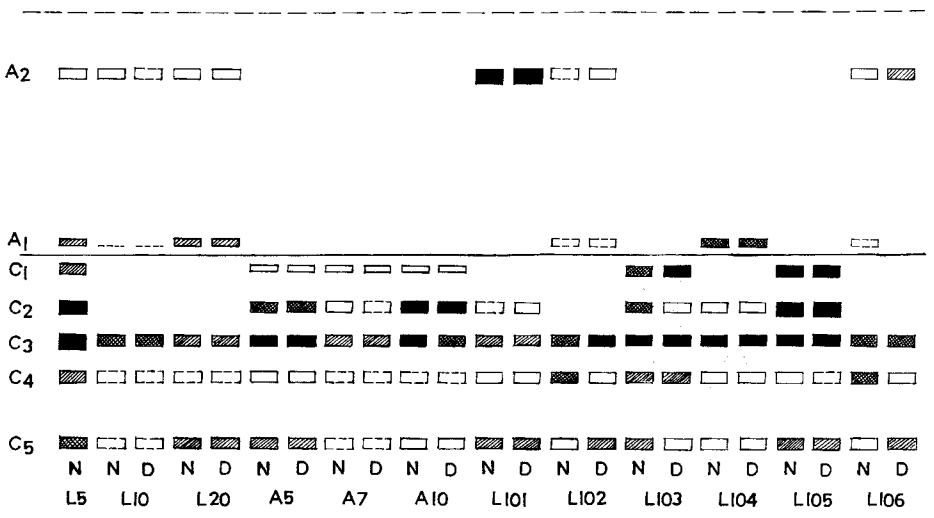

Fig. 4. Isozyme pattern of polyphenoloxidases at 50-day stage

remained similar at the two stages, though their concentrations increased with the growth of the plant, being higher at the 50 day stage than at the 30 day stage. This implied that resistant genotypes have an inherent capacity to produce high phenols and phenoloxidizing enzymes independent of growth stage. Banding patterns observed in normal leaf tissue were used for the characterization of the lines (Figs. 1–4). The band C_1 of peroxidase at the 30 and 50 day stage as well as A_1 of polyphenoloxidase at the 30 day stage was characteristic of the immune genotype L5. Highly susceptible genotypes L10 and A7 were characterised by the absence of C_2 and A_3 of peroxidase and C_2 and C_4 of polyphenoloxidase at the 30 day stage. C_5 of peroxidase was present only in the highly resistant genotypes L101 and L105 at the 30 day stage. A high intensity of polyphenoloxidase was observed in band C_1 in L103 and L105 at the 30 day stage and for A_2 in L101 at the 50 day stage.

The present study suggests that screening for resistance can be done irrespective of disease development (since only negligible within line variation was observed for normal and diseased plants). The reliability of such a technique will depend upon how close the relationship is between a particular banding pattern and disease resistance. Even if there should not be a complete correlation, this technique can still be helpful for quick preliminary screening of large array of genotypes.

References

Bateman DF (1967) Increase in polyphenoloxidase in diseased plant tissue. In: Source book of laboratory excercises in plant pathology. Am Phytopathol Soc Freeman, San Francisco, pp 208–209

Gupta VK, Stebbins GL (1969) Peroxidase activity in hooded and awned barley at successive stages of development. Biochem Genet 3:5–24

Lowry OH, Rosenbrough NJ, Farr A, Randall RJ (1951) Protein measurement with the folinphenol reagent. J Biol Chem 193:265–275

Murty BR (1980) Developments and lacunae in the genetic improvement of *Pennisetums.* In: Gupta VP, Minocha JL (eds) Trends in genetical research on Pennisetums. Punjab Agricultural University, Ludhiana, pp 9–18

Shannon L, Key E, Lew J (1966) Peroxidase isozymes from horseradish roots. 1. Isolation and physical properties. J Biol Chem 241:2166–2175

Swain T, Hillis WC (1959) The phenolic constituents of *Prunus domestica* (I). The quantitative analysis of phenolic constituents. J Sci Food Agric 10:63–68

Thakur DP, Kanwar JS (1977) Infectivity of sporangia of *Sclerospora graminicola* in pearl millet downy mildew. Indian J Mycol Plant Pathol 7:104–105

Vanloon LC (1971) Tobacco polyphenoloxidases: A specific staining method indicating non-identity with peroxidase. Phytochemistry 10:503–507

Veech JA (1969) Localization of peroxidase in infected tobaccos, susceptible and resistant to black shank. Phytopathology 59:560–571

Williams RJ, Singh SD, Thakur RP (1976) Rating scales and standard drawings for incidence and severity assessment of pearl millet diseases. 1. Downy mildew, ergot and smut. ICRISAT, Hyderabad, India

Williams RJ, Singh SD, Pawar MN (1981) An improved field screening technique for downy mildew resistance in pearl millet. Plant Dis 65:239–241