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Inheritance of downy mildew resistance, β-1,3-glucanases and peroxidases in pearl millet [*Pennisetum glaucum* (L.) R. Br.] crosses

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Abstract The inheritance of resistance to downy mildew disease and the defense-related enzymes β-1,3-glucanase and peroxidase was studied in crosses of pearl millet using a generation-mean analysis. The study material comprised six generations (susceptible and resistant parents, F_1, F_2, BC_1 and BC_2) in three crosses. Seedlings from these generations were inoculated with the downy mildew pathogen *Sclerospora graminicola* and disease incidence was recorded. Analysis of constitutive levels of β-1,3-glucanase and peroxidase in the seedlings of different generations indicated that the resistant populations showed higher enzyme activities, while lower activities of the enzymes were recorded in the susceptible populations. In the generation-mean analysis, the significance of scaling tests revealed the existence of non-allelic interactions in the inheritance of resistance to downy mildew as well as with the enzymes. Among the gene effects, both additive and dominant effects were significant. All the non-allelic interaction effects were significant in the crosses. Studies on the isozyme patterns of the enzymes substantiated the results of the disease-incidence experiments in most of the generations. The results indicated that the inheritance of downy mildew disease resistance and the expression of β-1,3-glucanase and peroxidase in pearl millet is not only under the control of additive and dominant genes but are also governed by complex non-allelic interactions.

Keywords Pearl millet · Downy mildew · Resistance · Inheritance · β-1,3-Glucanase · Peroxidase

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Introduction

Downy mildew caused by *Sclerospora graminicola* (Sacc.) Schroet. is a devastating disease of pearl millet [*Pennisetum glaucum* (L.) R. Br.] in India and Africa. It causes considerable loss in grain yield and is particularly destructive in single-cross F_1 hybrids of pearl millet (Singh 1995). Use of resistant cultivars has been the most efficient strategy for the management of this disease. However, sources of durable resistance are not available and several new hybrids are being continuously developed. In such cultivar development, the primary requirement is resistance to downy mildew. But there is a lack of clear understanding of pearl millet resistance to downy mildew disease. Research on the genetics of downy mildew resistance in pearl millet has revealed the contribution of additive and non-additive gene effects in the inheritance of resistance (Basavaraju et al. 1980; Shinde et al. 1984). Variable expression of downy mildew was found in crosses of several populations of pearl millet by Pethani et al. (1980). Complete resistance to downy mildew in pearl millet genotype DMRP 292 was reported to be controlled by a single dominant gene (Singh and Talukdar 1998).

One of the important non-specific defense mechanisms of plants against pathogens is the induction of pathogenesis-related proteins, which include β-1,3-glucanases and chitinases (Van loon and Van Strien 1999), and a defense-related enzyme like peroxidase (Gonzalez et al. 1999). Several reports suggest their involvement in plant defense as antimicrobial agents and/or contributors of structural defense (Simmons 1994; Brownleader et al. 1995; Gonzalez et al. 1999). This was also shown in pearl millet resistance against downy mildew disease (Sreedhara et al. 1995; Kini et al. 2000a, b). Isozyme markers have been reported to be closely linked with resistance genes in the plant system (Medina-Filho 1980) and the properties of enzyme polymorphism have been useful in plant breeding and breeding research (Melchinger 1990; Warnke et al. 1998; Boskovic et al. 2000).

Despite the above reports, very little information is available in the literature regarding the inheritance of defense-related enzymes *vis-a-vis* downy mildew disease-resistance in pearl millet crosses. Since knowledge on the inheritance of resistance and defense-related enzymes will be valuable for developing durable resistance and predicting its effectiveness in hybrid combinations, studies were carried out with pearl millet crosses to understand the genetic effects on inheritance of resistance, as well as the expression of β-1,3-glucanase and peroxidase, in crosses of pearl millet. The results are described in this paper.

Materials and methods

Plant material

Three different crosses, 96752 × 96298 (cross I), 96770 × 96263 (cross II) and 96768 × 96282 (cross III), involving six diverse homozygous inbred lines with a varying resistant reaction to the downy mildew pathogen *S. graminicola* were chosen for study. Lines 96752, 96770 and 96768 were susceptible to pathotype 1 of *S. graminicola* under greenhouse conditions while lines 96298, 96263 and 96282 were resistant. The F_1 generation was selfed to obtain the F_2 population and the F_1 in each cross was backcrossed with both parents to obtain BC_1- and BC_2-generation seeds. Six generations (susceptible and resistant parents, F_1, F_2, BC_1 and BC_2) of all the three crosses were used as materials for this study. These materials were generated with the co-operation of Prof. S. D. Ugale, pearl millet breeder at Mahatma Phule Krishi Vidyapeet, Rahuri, Maharastra, India.

Pathogen

A virulent pathotype of *S. graminicola* isolated from the highly susceptible cultivar HB-3, and maintained on the same cultivar under greenhouse conditions, was used for all experimental work.

Inoculation of pearl millet seedlings and downy mildew disease assessment

Two-day old seedlings raised from the seeds of all the crosses were root-dip inoculated with a zoospore suspension of *S. graminicola* (40,000 zoospores/ml as estimated by a haemocytometer count) following the method of Safeeulla (1976). Seedlings dipped in sterile distilled water served as a control. The treated seedlings transplanted into earthen pots containing red loamy soil and farmyard manure were maintained for 60 days under greenhouse conditions, during which time the disease incidence (as the percentage of plants showing disease symptoms) was recorded. The experiment was repeated three times with 100 plants for each experiment.

Enzyme analysis

Extraction of enzymes

Ten 2-day old pearl millet seedlings, not inoculated with the pathogen, were macerated using 0.05 M sodium acetate buffer, pH 5.2, (1 ml g^{-1} fresh weight) and acid-washed glass beads at 4°C. The supernatant obtained after centrifugation at 9,000 g for 20 min at 4°C (HIMAC Centrifuge, HITACHI) were used as the source of enzymes. Individual plants were taken for analysis. The experiment was replicated and the replication variance and the mean of each population were used for generation-mean analysis. The protein content in the samples was estimated by the dye-binding method of Bradford (1976).

Enzyme assays

β-1,3-Glucanase was assayed following the method of Isaac and Gokhale (1982) using 0.1% Laminarin (Sigma) in 0.05 M sodium acetate buffer (pH 5.2) as substrate, and the activity was expressed as μmol of glucose formed min^{-1} for 1 mg of protein. Peroxidase activity was assayed with guaiacol as the hydrogen donor (Hammershmidt et al. 1982) and the activity was measured by recording the change in absorbance at 470 nm. One unit of specific activity (mg^{-1} protein) corresponded to an increase of 0.1 D_{470} min^{-1}.

Isozyme analysis

Isozymes of peroxidase and β-1,3-glucanase were identified after separating the proteins in 8% native polyacrylamide gels by electrophoresis (PAGE; Davis 1964) and isoelectric focusing (IEF) using the Multiphor II apparatus of LKB, Uppsala, Sweden, respectively.

Detection of isozymes

β-1,3-Glucanase isozymes were detected in the IEF gels following the method of Pan et al. (1989) as modified by Wyatt et al. (1991). The procedure of Schrauwen (1966) was used for staining peroxidase isozymes. The gels were analysed using a Bioprofil Image Analysis System (Vilber Lourmat, France). The pIs of β-1,3-glucanase isoforms were calculated using the in-built software of the Image Analysis System.

Statistical analysis

Based on the mean individual generation values determined for disease incidence, and for β-1,3-glucanase and peroxidase activities, the standard error and analysis of variance were obtained as described by Panse and Sukatme (1954). The scaling tests A, B and C were computed and the variances were calculated to test the adequacy of the additive-dominance model in each cross (Mather 1949). When the additive-dominance model was inadequate, the 6-parameter model was used to estimate the various genetic components (Hayman 1958). In scaling tests that suggested an absence of non-allelic interactions, a simple 3-parameter model was fitted (Mather 1949).

Results and discussion

Downy mildew disease incidence

The reactions of different generations of three pearl millet crosses to the downy mildew pathogen are given in Table 1. The parents P_1 and P_2 used in this study were highly susceptible and highly resistant to the downy mildew pathogen *S. graminicola* as evidenced by their disease incidence. While P_1 plants inoculated with the pathogen recorded over 60% disease incidence, only about 10% of the P_2 plants showed symptoms when inoculated with the pathogen. In cross I, the F_1 showed a resistant reaction with approximately 10% disease incidence. In crosses II and III, the F_1 plants were susceptible with over 40% disease incidence. In all three crosses, BC_1 recorded a higher disease incidence and showed a susceptible reaction while BC_2 plants were resistant. Thus the results of the disease incidence experiments confirmed the susceptibility/resistance reactions of the parents.

Table 1 Mean values[a] for disease incidence, β-1,3-glucanase activity and peroxidase activity in different generations of three pearl millet crosses

Cross	P_1	P_2	F_1	F_2	BC_1	BC_2
	Disease incidence (%)[b]					
I	64.3 ± 1.65	8.0 ± 0.47	10.0 ± 0.47	49.6 ± 2.37	64.6 ± 2.13	7.6 ± 0.72
II	92.3 ± 1.66	7.3 ± 0.72	57.0 ± 1.41	60.0 ± 2.36	88.0 ± 1.89	7.0 ± 0.47
III	72.0 ± 2.16	6.0 ± 0.47	39.6 ± 2.37	39.6 ± 2.84	58.3 ± 1.66	8.0 ± 0.47
	β–1,3-Glucanase activity (μmol min^{-1} mg^{-1} protein)[c]					
I	1.204 ± 0.052	1.444 ± 0.167	1.533 ± 0.028	1.155 ± 0.114	1.173 ± 0.045	0.737 ± 0.111
II	1.110 ± 0.075	1.632 ± 0.084	1.033 ± 0.080	1.032 ± 0.135	1.121 ± 0.146	1.360 ± 0.065
III	1.837 ± 0.114	3.955 ± 0.377	2.240 ± 0.237	4.407 ± 0.295	2.125 ± 0.138	3.332 ± 0.119
	Peroxidase activity (units min^{-1} mg^{-1} protein)[c]					
I	16.71 ± 0.23	24.91 ± 2.64	35.04 ± 0.03	22.78 ± 0.11	13.40 ± 1.30	24.64 ± 4.31
II	24.50 ± 0.53	26.59 ± 0.06	18.39 ± 2.23	23.75 ± 0.52	16.14 ± 0.38	22.17 ± 0.26
III	11.76 ± 0.39	22.13 ± 0.23	11.94 ± 0.12	12.10 ± 0.11	16.96 ± 0.11	20.42 ± 0.16

[a] Results are expressed as the mean ± SE
[b] Average of three independent experiments with 100 plants per experiment
[c] Average of three independent experiments each with three replicates

Enzyme activities

β-1,3-Glucanase and peroxidase activities in plants of different generations obtained from the three crosses are described in Table 1. In cross I, the F_1 was characterized by increased peroxidase and an apparently similar β-1,3-glucanase activity to that of the resistant parent P_2. The activitites of the enzymes assayed for the F_1 generation of the other two crosses were significantly closer to the susceptible parent P_1. In all three crosses, peroxidase activity was higher in the BC_2 generation than in the BC_1. A similar trend was recorded with β-1,3-glucanase activity except in cross I where BC_1 showed higher activity than BC_2. Constitutive hydrolases and peroxidases have been associated with the resistance reaction to pathogens in various plants (Ahl Goy et al. 1992; Mozzetti et al. 1995) and genetic analysis of resistance to *Phoma tracheiphila* in three *Citrus poncirus* progenies has been reported; pathogenesis-related proteins and chitinase appear more frequently in resistant plants than in susceptible ones (Recupero et al. 1997). In pearl-millet resistance to downy mildew, higher activities of peroxidase and β-1,3-glucanase have been demonstrated (Sreedhara et al. 1995; Kumar et al. 1998; Kini et al. 2000a, b) and the results of the present study appear to suggest an association of the two enzymes, β-1,3-glucanase and peroxidase, with pearl-millet resistance to downy mildew. However, in some of the generations, the variations were limited only to a marginal increase in the activity of the enzymes assayed.

Generation-mean analysis

The results of the generation-mean analysis and estimates of the gene effects in pearl millet are shown in Table 2. The analysis of variance revealed that the six generations differed significantly for disease incidence, as well as for β-1,3-glucanase and peroxidase activities. At least one of the scales (A, B and C) was significant for all the characters in all the crosses, except in the case of β-1,3-glucanase activity in cross II, indicating the presence of epistasis. In the case of β-1,3-glucanase in cross II, a simple additive-dominance model was adequate to explain the gene effects.

Both additive (d) and dominance (h) effects were significant in the expression of all the characters. But only additive activity was significant for β-1,3-glucanase and peroxidase activities in cross I. The magnitude of d was, however, more than that of h for disease incidence. This indicated the preponderance of an additive gene effect.

Among the epistatic gene effects, all the three types of interaction, i, j and l, were significant in crosses II and III for disease incidence and peroxidase activity. In the case of cross I, j and l effects were significant for disease incidence and the l effect alone for peroxidase activity. For β-1,3-glucanase activity, the l effect was significant in cross I, and the i and l effects in cross III.

The predominantly additive action for disease resistance and the high heritability estimate (> 99%) may help to select for disease-resistant types in the subsequent generations. However, the large magnitude of dominance (h) in some cases and the presence of duplicate epistatis in these traits would tend to hinder progress. Recurrent selection followed by pedigree breeding or biparental mating or a selective dialle mating system may prove effective in improving disease resistance.

Isozyme analysis

The inheritance of isozymes has been documented in almond (Vezvaei et al. 1995) and *Brassica* (Simonsen and Heneen 1995) and the present study describes the inheritance pattern of β-1,3-glucanase and peroxidase isozymes in three crosses of pearl millet (Table 3).

Table 2 Scaling test and estimate of genetic parameters for downy mildew resistance and activities of glucanase and peroxidase in pearl millet crosses

Cross	A	B	C	m	d	h	i	j	l
% Disease incidence									
I	35.35 ** ± 3.39	– 2.78 ± 2.10	72.64** ± 6.89	44.81 ** ± 1.67	37.53 ** ± 1.84	– 56.54 ** ± 7.67	– 40.08 ± 7.62	19.07 ** ± 1.96	7.51** ± 10.08
II	16.56** ± 4.82	– 34.00** ± 1.90	15.24* ± 7.50	50.80 ** ± 1.70	54.59 ** ± 2.17	–28.65 * ± 8.21	– 32.74 ** ± 8.05	25.31 ** ± 2.50	50.24 ** ± 11.46
III	0.32 ± 3.24	– 20.34** ± 2.20	3.60 ± 8.98	39.01 ** ± 2.04	33.39 ** ± 1.33	– 21.80 ** ± 8.79	– 23.61 ** ± 8.58	10.34 ** ± 1.55	43.63 ** ± 10.44
β–1,3-Glucanase activity									
I	– 0.38** ± 0.13	– 1.50** ± 0.34	– 1.09 ± 0.60	1.16 ** ± 0.14	0.44 ** ± 0.15	– 0.59 ± 0.64	– 0.79 ± 0.63	0.56 ± 0.18	2.68** ± 0.85
II	– 0.10 ± 0.38	0.05 ± 0.21	– 0.63 ± 0.70	1.03 ** ± 0.17	– 0.24 ** ± 0.20	0.50 ** ± 0.78	–	–	–
III	0.17 ± 0.47	0.47 ± 0.62	7.36** ± 1.63	4.41 ** ± 0.36	– 1.21 ** ± 0.22	– 7.37 ** ± 1.56	– 6.72** ± 1.52	– 0.15 ± 0.33	6.07 ** ± 1.86
Peroxidase activity									
I	– 24.96** ± 2.61	–10.68 ± 9.02	–20.58** ± 2.69	22.78** ± 0.11	– 11.24* ± 4.50	– 0.82 ± 9.12	– 15.05 ± 9.02	7.14 ± 4.7	50.69** ± 18.22
II	10.22** ± 0.47	6.78** ± 0.41	– 9.36** ± 0.67	12.10** ± 0.11	– 3.46** ± 0.20	21.36** ± 0.63	26.36** ± 0.58	1.72** ± 0.30	– 43.36** ± 1.03
III	– 10.61** ± 2.41	– 0.64 ± 2.29	7.11 ± 4.94	23.75** ± 0.52	– 6.03** ± 0.45	– 25.52** ± 3.19	– 18.37** ± 2.26	– 4.99** ± 0.53	29.62** ± 5.27

* *P* = 0.05–0.01, ** *P* = 0.01

Table 3 Schematic representation of β-1,3-glucanase isozymes of the three crosses of pearl millet. – absence of band, + low intensity, ++ moderate intensity, +++ high intensity

Isoforms	Cross I						Cross II						Cross III					
pI	**P1**	**P2**	**F1**	**F2**	**BC1**	**BC2**	**P1**	**P2**	**F1**	**F2**	**BC1**	**BC2**	**P1**	**P2**	**F1**	**F2**	**BC1**	**BC2**
9.6	++	++	++	++	++	++	++	++	++	++	++	++	++	+++	+++	+++	++	+++
9.4	–	–	–	–	–	–	+	++	++	+	+	+	–	++	–	–	–	++
9.2	–	–	–	–	–	–	–	++	+	+	+	++	–	++	–	–	–	+++
9.0	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+
8.6	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+
8.2	+	++	+	+	+	++	+	+	++	++	++	+++	++	++	++	++	++	++
7.5	–	++	+	–	–	++	–	++	+	+	+	++	–	–	–	–	–	–
6.2	–	–	–	–	–	–	–	–	–	–	–	–	++	++	++	++	++	++
3.0	+	++	++	++	++	++	+	++	+	++	+	++	++	++	++	++	++	++

In isoelectric focusing gels, nine isoforms (seven basic and two acidic) of β-1–3-glucanase were identified. Of these, the isoform corresponding to pI 9.6 was the most prominent in the samples of all three crosses. In cross I, a total of four isoforms were detected. Though different in intensity, the two basic isoforms (pIs 9.6 and 8.2) and the acidic isoform (pI 3.0) were present in all samples. The isoform of pI 7.5 was more prominent in the resistant parent P_2 and was also inherited in the F_1 and BC_2 .

Six isoforms (four basic and two acidic) of the β-1,3-glucanase were detected in cross II. Isoforms of pIs 9.6, 9.4, 8.2 and 3.0 were identified in all samples, and the isoforms of pI 9.2 and 7.5 present in all samples appeared to be absent in P_1. These results suggested that the F_1, F_2, BC_1 and BC_2 generations inherited the isoform pI 7.5 from the resistant parent P_2. The pI 3.0 isoform of β-1,3-glucanase apparently occurred at low concentrations in the P_1, F_1 and BC_1 as evidenced by the lower staining intensity of this protein in enzyme zymograms.

Of the six basic and two acidic isoforms of β-1,3-glucanase in cross III, enzymes corresponding to pIs of 9.6, 8.2, 6.2 and 3.0 were detected in all the samples. The resistant parent P_2 and its backcross BC_2 showed the additional two basic isoforms of pI 9.2 and 9.4, which were not detected in the P_1, F_1, F_2 and BC_1. P_2 and BC_2 also showed two basic isoforms of pI 9.0 and 8.6, although with low intensity, but were not detected in any of the other samples.

The analysis of the zymograms of the peroxidase isozymes revealed a total of seven proteins in cross I and II, and six in III. A schematic representation of the peroxidase isozymes of the three crosses is shown in Table 4.

In cross I, of the seven isoforms, PER 3 was apparently not detected in the susceptible parent P_1 and in the

Table 4 Schematic representation of peroxidase isozymes of the three crosses in pearl millet. – absence of band, + low intensity, ++ moderate intensity, +++ high intensity

Isoforms	Cross I						Cross II						Cross III					
	P1	P2	F1	F2	BC1	BC2	P1	P2	F1	F2	BC1	BC2	P1	P2	F1	F2	BC1	BC2
PER 1	+	++	+	+	+	+++	++	+	+	+	++	+	+	++	+	+	+	++
PER 2	++	++	+	+	+	++	++	+	+	+	++	+	+	++	+	+	+	++
PER 3	–	++	+	+	–	++	+	–	+	+	+	–	+	+++	+	++	+	++
PER 4	++	++	+	++	++	++	+	–	+	+	+	–	+	++	+	+	+	++
PER 5	++	+++	+	++	+	+++	++	+	+	+	++	+	+	++	+	+	+	++
PER 6	++	++	+	+	++	++	++	+	+	+	++	+	+	++	+	+	++	+++
PER 7	++	+++	+	+	++	+++	++	+	+	+	++	+	-	-	-	-	-	-

backcross BC_1 due to low intensity reactions. However, the occurrence of different concentrations of this isozyme in the other generations was evidenced by the different enzyme reaction intensity on zymogram gels. The isoforms PER 1, 5 and 7 were of higher intensity in the resistant parent P_2 and its backcross BC_2 and these isoforms of low intensity were features of the susceptible parent P_1 and its backcross BC_1. In the F_1 and F_2, these isoforms were of moderate intensity. In cross II, the isoforms PER 3 and 4 were not detected in the resistant parent P_2 and its backcross BC_2. The isoforms PER 1, 2, 5 and 6 showed a higher intensity in the susceptible parent P_1 and its backcross BC_1. A total of six isoforms occurred in cross III. All of them showed higher intensity in the resistant parent P_2 and its backcross BC_2. In the F_1 and F_2 these isoforms were of moderate intensity.

The results of the present study suggested a similar inheritance pattern for β-1,3-glucanase and peroxidase and the operation of both additive and non-additive gene inheritance. This provides additional evidence for the involvement of these enzymes in the resistance of pearl millet to downy mildew. The nature of the gene action involved in resistance is vital for the development of an efficient breeding strategy for cultivar development. The involvement of additive × dominance and dominance × dominance interactions indicate the necessity to postpone selection for the trait to later generations, when sufficient epistatic interactions would have become fixed. Diallel selective mating as used in *Pennisetum* (Jonsan 1970) and biparental matings in segregating generations may also be useful in maintaining pearl millet genetic variability.

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References

Ahl Goy P, Felix G, Metraux JP, Meins Jr F (1992) Resistance to disease in the hybrid *Nicotiana glutinosa* × *Nicotiana debneyi* is associated with high constitutive levels of β-1,3-glucanase, chitinase, peroxidase and polyphenol oxidase. Physiol Mol Plant Pathol 41: 11–21

Basavaraju R, Safeeulla KM and Murhty BR (1980) The role of gene effects and heterosis for resistance to downy mildew in pearl millet. Indian J Genet Plant Dis 40: 537–548

Boskovic R, Russell K, Tobutt KR, Ridont MS (2000) An isozyme marker linked to the incompatibility locus in cherry. Theor Appl Genet 100: 512–518

Bradford MM (1976) A rapid and sensitive method for the quantitation on microgram quantities of protein utlizing the principle of protein-dye binding. Anal Biochem 72: 248–254

Brownleader MD, Ahmed N, Traevan M, Chaplin MF, Dey PM (1995) Purification and partial characterization of tomato extensin peroxidase. Plant Physiol 109: 1115–1123

Davis BJ (1964) Disc electrohoresis. II. Method and application to human serum proteins. Ann NY Acad Sci 121: 404–427

Gonzalez LF, Rojas MC, Perez FJ (1999) Diferulate and lignin formation is related to biochemical differences of wall-bound peroxidases. Phytochemistry 50: 711–717

Hammerschimdt R, Nuckles E, Kuc J (1982) Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol Plant Pathol 20: 73–82

Hayman BI (1958) The separation of epistatis from additive and dominance variation in generation means. Heredity 12: 371–390

Isaac S, Gokhale AV (1982) Autolysis: a tool for protoplast production from *Aspergillus nidulans*. Trans Br Mycol Soc 78: 389–394

Jonsan NF (1970) A diallel selective mating for cereal breeding. Crop Sci 10: 629–635

Kini RK, Vasanthi NS, Umesh-Kumar S, Shetty HS (2000a) Purification and properties of a major isoform of β-1,3-glucanase from pearl millet seedlings. Plant Sci 150: 139–145

Kini RK, Vasanthi NS, Umesh-Kumar S, Shetty HS (2000b) Induction of β-1,3-glucanase in seedlings of pearl millet in response to infection by *Sclerospora graminicola*. European J Plant Pathol 106: 267–274

Kumar VK, Hindumathi, CK, Kini RK, Shetty HS (1998) Prior inoculation inducing resistance to downy mildew (*Sclerospora graminicola*) enhances growth, peroxidase, and β-1,3-glucanase activity in pearl millet (*Pennisetum glaucum*). J Plant Pathol 80: 203–209

Mather K (1949) Biometrical Genetics. Dover Publication Incorporated New York

Medina-Filho HP (1980) Linkage of *Aps-1*, *Mi* and other markers on chromosome 6. Rep Tomato Genet Coop 30: 26–28

Melchinger AE (1990) Use of molecular markers in breeding for oligogenic disease resistance. Plant Breed 104: 1–19

Mozzetti C, Ferraris L, Tamietti G, Matta A (1995) Variation in enzyme activities in leaves and cell suspensions as markers of

incompatibility in different *Phytophthora* - pepper interactions. Physiol Mol Plant Pathol 46: 95–107
Pan SQ, Ye XS, Kuc J (1989) Direct detection of β-1,3-glucanase isozymes on polyacrylamide electrophoresis and isoelectrofocusing. Anal Biochem 182: 136–140
Panse VG, Sukatme PV (1954) Statistical methods for agriculture workers. Indian Council of Agricultural Research, New Delhi
Pethani KV, Kapoor RL, Chadra S (1980) Gene action and phenotypic stability for incidence of downy mildew disease in pearl millet. Indian J Agric Res 14: 217–223
Recupero GR, Gentile A, Russo MP, Domina F (1997) Genetic analysis of resistance to *Phoma tracheiphila* in three *Citrus* and *Poncirus* progenies. Theor Appl Genet 116: 198–200
Safeeulla KM (1976) Biology and control of the downy mildew of pearl millet, sorghum and finger millet. Wesley Press, Mysore, India
Schrauwen J (1966) Nachweis von Enzymen nach elektrophoretischer Trennung an Polyacrylamidsauren. J Chromatogr 23: 177–180
Shinde RB, Patil FB, Sangve RA (1984) Resistance to downy mildew in pearl millet. J Maharastra Agric Univ 9: 337–338
Simmons CR (1994) The physiology and molecular biology of plant 1,3-β-D-glucanases and 1,3;1,4-β-D-glucanases. Crit Rev Plant Sci 13: 325–387
Simonsen V, Heneen WK (1995) Inheritance of isozymes in *Brassica campestris* L. and genetic divergence among different species of *Brassiceae*. Theor Appl Genet 91: 353–360
Singh SD (1995) Downy mildew of pearl millet. Plant Dis 79: 545–550
Singh SD, Talukdar BS (1998) Inheritance of complete resistance to pearl millet downy mildew. Plant Dis 82: 791–793
Sreedhara HS, Nandini BA, Shetty SA, Shetty HS (1995) Peroxidase activities in the pathogenesis of *Sclerospora graminicola* in pearl millet seedlings. Int Jr Trop Plant Dis 13: 19–32
Van Loon LC, Van Strien EA (1999) The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. Physiol Mol Plant Pathol 55: 85–97
Vezvaei A, Hancock TW, Giles LC, Clarke GR, Jackson JF (1995) Inheritance and linkage of isozyme loci in almond. Theor Appl Genet 91: 432–438
Warnke SE, Douches DS, Branham BE (1998) Isozyme analysis supports allotetraploid inheritance in tetraploid creeping bentgrass (*Agrostis palustris* Huds.). Crop Sci 38:801–805
Wyatt SE, Pan SQ, Kuc J (1991) β-1,3-glucanase, chitinase, and peroxidase activities in tobacco tissues resistant and susceptible to blue mold as related to flowering, age and sucker development. Physiol Mol Plant Pathol 39: 433–440