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Inheritance of Resistance to Sorghum Downy Mildew Disease in Maize

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Abstract

Sorghum downy mildew (SDM) of maize caused by *Peronosclerospora sorghi* is a disease of great destructive potential because systemically infected plants seldom produce an ear. Breeding resistant varieties is one of the most effective and cheap methods to control this disease. The genetics of resistance is needed to be studied in any resistance breeding programme. In the present investigation, the inheritance study revealed that resistance was governed by two recessive genes in complementary (9:7) pattern in F_2 population of a cross, UMI 79 x UMI 936(w). The resistance behaved as a recessive character to susceptibility. Four SRR primers namely bnlg1035, bnlg420, Phi073 and bnlg1154 were found to be polymorphic between resistant and susceptible parents and they can be further effectively utilized in molecular mapping for SDM resistance.

Key words: *Maize, Sorghum Downy Mildew, Resistance, Genetics, Molecular markers*

Introduction

Maize breeding programmes are generally designed for the improvement of grain yield. However, several pests and diseases are responsible for major economic losses in maize. Among these, Sorghum downy mildew [*Peronosclerospora sorghi* (Weston and Uppal) C.G. Shaw] is one of the most serious diseases in maize which subsist in maize producing areas throughout the world. Although effective chemical measures (Anahosur and Patil, 1980, Odvody and Frederiksen, 1984a and 1984b, Anaso *et al*., 1989 and Sharma and Lal, 1998) are available for controlling Sorghum downy mildew (SDM) disease, breeding

resistant varieties and their cultivation has been a widely accepted phenomena in most of the crop improvement programmes (Shivanna and Anahosure, 1990). Effective breeding methods for producing sorghum downy mildew resistant inbreds and hybrids would depend primarily on the mode of inheritance of resistance to SDM disease. Genetic information relating to host plant resistance would provide more relevant basis for making breeding decisions. Considerable data have been reported concerning sources of resistance to SDM disease in maize (Craig *et al*., 1977, Schmitt *et al*., 1977, Lima *et al*., 1982, De Leon *et al*., 1993, Setty *et al*., 2001, Ajala *et al*., 2003 and Yen *et al*., 2004). Information on the mode of inheritance of resistance to sorghum downy mildew disease, however, is limited in maize. Hence, it is necessary to investigate the inheritance of resistance to SDM in maize.

Materials and Methods

The research work was carried out to study the inheritance of SDM in maize at the Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. The materials selected as parents for the present study was based on previous study and consisted of a highly susceptible maize inbred line, UMI 79 and highly resistant inbred line, UMI 936 (w). They were crossed by keeping UMI 79 as female parent and UMI 936 (w) as male parent following tassel bag method (Jugenheimer, 1976). F_1 was self pollinated to produce F_2 population.

In this present study, the study material consists of UMI 79, UMI 936 (w), F_1 and F_2 population (224 plants). A highly susceptible entry, CM 500 was included as check. They were screened against Sorghum Downy Mildew infection by conidial spray inoculation method under green house condition as described by Craig (1976). Conidial suspension was prepared daily as described by Cardwell *et al*. 1994. The disease reaction was assessed at 21 days after plant emergence of test entries in seedling spray inoculation method under green house condition. They were scored in per cent disease incidence after susceptible check, CM 500 showed 100 per cent infection by counting number of infected plants to total number of plants in each entry. Per cent downy mildew incidence was calculated as per standard procedure (Lal and Singh, 1984). The proportion of resistant and susceptible plant was calculated for F_2 population.

$$\text{Per cent downy mildew incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

The rating scale was followed as below.

Per cent downy mildew incidence (%)	Reaction
0 – 10	Resistance (R)
>10 – 30	Moderately resistance (MR)
>30 – 50	Moderately susceptible (MS)
>50	Susceptible (S)

Chi-square test

A Chi-square test was applied to test the fitness of the observed segregation ratio with standard expected monogenic (3:1) and digenic (15:1, 9:7 and 13:3) mendelian ratios to explain the inheritance of resistance to SDM in F_2 population obtained from a cross, UMI 79 x UMI936(w). The significance of the difference between observed and expected ratios was studied using the method given by Fisher, 1936. The calculated chi-square values were tested against table chi-square value with n-1 degrees of freedom (where, n is the number of classes). The null hypothesis (There is no deviation of observed data from expected ratio) was accepted wherever the calculated chi-square value was less than the table chi-square value and the presumed ratio was considered to be a fit and vice-versa.

Molecular marker analysis

DNA extraction: Plant materials for DNA extraction consist of UMI 79 (orange kernel color), highly susceptible parent and UMI 936(w) (white kernel color) resistant parent to carry out SSR marker analysis. Leaf samples were collected from individual plants of two parents at 10th day and stored in -80°C for DNA extraction. DNA was extracted from the leaf samples of the two parents following CTAB method developed by Saghai-Maroof *et al*. (1984) with suitable modifications by Hoisington *et al*. (1994). DNA samples were stored at -20°C for parental polymorphic analysis.

SSR protocol: Ten SSR primers which were already reported to be linked with SDM resistance by many workers (George *et al.*, 2003, Nair *et al*., 2005 and Sabry *et al*., 2006) were selected. The sequence information for these ten SSR primers was downloaded from the maize genome data base http://www.maizegdb.org/ssr/php and synthesized by Sigma genosys, Bangalore. The SSR sequence information for each primer pair is listed in Table 1. Around 40 ng of template DNA was used for PCR with total reaction mixture volume of 20µl. PCR amplification was performed in a 96 well Gene Amp® PCR system 9700. The

temperatures and timings used for PCR cyclic profile were as follows: One cycle of an initial denaturation at 94°C for 3 minutes followed by 30 cycles of denaturation at 94°C for 1 minutes, primer annealing at 56 °C for 2 minutes and primer extension at 72°C for 2 minutes with one cycle of final extension at 72°C for 5 minutes. A four per cent agarose gel was used for resolution of the SSR amplified products. Electrophoresis post staining was carried out using ethidium bromide. The molecular weight marker of 100bp was used for comparison.

Results and Discussion

Inheritance of SDM disease resistance in maize

Ten species of fungi are known to cause downy mildew diseases in maize, of which, SDM is frequent in the humid areas of South India. Host resistance is described as the most efficient, effective and economic means for controlling the downy mildew diseases (Frederiksen and Renfro, 1977). The mode of inheritance of resistance in maize to SDM is essential in choosing breeding schemes and in determining the appropriate size of segregating populations to evolve SDM resistant maize lines / varieties. Therefore, in the present investigation mode of inheritance to SDM infection in maize was studied.

Parental lines (UMI 79 and UMI 936 (w)), F_1 and F_2 population (224 segregating lines) were tested for reaction to SDM infection under glasshouse condition and the results are presented in Table 2. The abaxial side of the leaves then were observed for sporulation of the pathogen 21 days after planting, which indicates susceptibility reaction to SDM infection. The percentage downy mildew infection of F_1 (66.67 per cent) differed largely from resistant parent, UMI 936(w) (2.63 per cent). Moreover, F_1 and F_2 progeny recorded susceptible reaction of 66.67 per cent and 60.71 per cent respectively. The percentage infection in the F_1 cross between resistant and susceptible parents was greater than the intermediate value of the parents and the disease reaction of the F_1 shifted to the susceptible parent, UMI 79 (100 per cent).

The percentage downy mildew infection of F_1 cross between UMI 79 and UMI 936 (w) was greater than the mid-parental value and the susceptibility of F_1 cross resembled that of the susceptible parent (UMI 79). The difference in percentage downy mildew infection between the F_1 progenies (66.67 per cent) and resistant parent (2.63 per cent) was sufficiently large as well as the disease reaction of both F_1 and F_2 progeny was inclining towards susceptibility. This indicated that dominant genes controlled susceptibility to SDM in maize.

The proportions of resistant and susceptible plants in F_2 population allowed the study of SDM reaction in the form of a qualitative trait. Chi-square analysis was carried out to find the goodness of fit of the observed phenotypic ratio of susceptible and resistant plants with in F_2 segregating population to those expected ratios based on monogenic (3:1) and digenic (15:1, 9:7 and 13:3) ratios (Table 3). In F_2 population the actual phenotypic frequency differed significantly from the hypothesized 3:1 monogenic ratio and 15:1 and 13:3 digenic ratios. However, chi-square values based on mendelian ratios showed that the actual phenotypic frequency of F_2 population did not differed significantly from expected ratio of 9:7 (9 susceptible : 7 resistant) (Table 2). The F_2 population segregated into 9:7 ratio for susceptiblility and resistant to SDM. This indicated that, two loci were involved and segregating in a 9:7 complementary gene action for resistance to SDM. The presence of either or both genes together in homozygous recessive state conferred resistance while presence of both genes together in dominant state resulted in susceptibility. Hence, SDM resistance was controlled genetically by two pairs of genes with complementary type of interaction involved in its genetics.

Most of the genetic studies of downy mildew resistance in maize have used generation mean analysis. Craig (1982b) studied the inheritance of resistance to SDM in the F_1, F_2, F_3 and back cross progenies from a cross of the resistant corn inbred Tx601 and the susceptible, N28 and indicated that susceptibility was partially dominant by two linked genes. Lima *et al.* (1982) observed intermediate resistance level in the variety cross and inferred that resistance to SDM was largely due to additive gene action. Bockholt and Frederiksen (1972) and Frederiksen *et al.* (1973) investigated the inheritance of resistance to SDM and they inferred that susceptibility was dominant and they concluded that two or three genes control the reaction to SDM. In Thailand, using open pollinated maize varieties, Jinahyon (1973) reported that resistance to SDM was controlled by many genes.

Parental screening with SSR primers

Since phenotypic selection for SDM resistance is cumbersome and could be hampered by the occurrence of disease escapes due to non conducive environmental conditions, the integration of marker aided selection (MAS) at specific stages in breeding for SDM resistance could be highly effective. Further, if tightly linked molecular markers to SDM resistant genes are identified, they can be effetely utilized to screen large number of germplasm / genotypes under laboratory condition without artificial inoculation or natural infection under field condition. In the

present study, molecular marker analysis was attempted to identify polymorphic SSR primers between resistant (UMI 79) and susceptible (UMI 936(w)) parents.

Two maize inbred lines with extreme reaction to SDM disease were selected for molecular marker analysis in order to identify polymorphic primers between resistant [UMI936(w)] and susceptible [UMI79] parents. Ten SSR primers, which, were reported to be linked with SDM resistant gene by earlier workers (Nair *et al*., 2001, George *et al*., 2003, Nair *et al*., 2005 and Sabry *et al*., 2006) were used for parental screening. The SSR allelic profiles of two extreme phenotypes are given in Plate 1. Out of ten SSR primers analyzed, four SSR primers *viz*., bnlg1035, bnlg420, Phi073 and bnlg1154 showed polymorphism between resistant and susceptible lines. The other six primers were found to be monomorphic. Identification of more polymorphic SSR primers and bulked segregant analysis with a large F_2 population could help to identify SSR markers which are tightly linked to SDM resistance in maize and they can be further used in Molecular Assisted Selection (MAS).

Conclusion

Sorghum downy mildew of maize remains important constraints to establish sustainable crop production worldwide as it causes severe yield losses in warm, moist areas of the tropical and sub-tropical world. Although effective chemical measures are available for SDM disease management, breeding resistant varieties and their cultivation have been widely accepted phenomena in most of the crop improvement programmes. Genetic information relating to host plant resistance would provide more relevant basis for making breeding decisions. The inheritance study revealed that SDM resistance was governed by two recessive genes in complementary (9:7) pattern in F_2 population of a cross UMI 79 x UMI 936 (w). The resistance behaved as a recessive character to susceptibility. Four SRR primers, bnlg1035, bnlg420, Phi073 and bnlg1154 were found to be polymorphic between resistant and susceptible parents and they can be effectively utilized in molecular marker mapping for SDM resistance.

References

Ajala, S.O., J.G. Kling, S.K. Kim and A.O. Obajimi. 2003. Improvement of maize populations for resistance to downy mildew. *Plant Breed*., **122:** 328-333.

Anahosur, K.H. and S.H. Patil. 1980. Chemical control of sorghum downy mildew in India. *Plant Dis.*, **64(11):**1004-1006.

Anaso, A.B., P.D. Tyagi, A.M. Emechebe and S.K. Manzo. 1989. Control of sorghum downy mildew (*Peronosclerospora sorghi*) of maize by seed treatment in Nigeria. *Crop Prot.*, **8(2):**82-85.

Bockhold, A.J. and R.A. Frederiksen. 1972. Breeding corn for resistance to sorghum downy mildew. *Agron. J.*, **64:** 3.

Cardwell, K.F., Bock, C., Akinnioye, O.F., Onukwa D., Adenle V. and Adetoro, A.O. (1994). Improving screening methods for resistance to downy mildew of maize in Nigeria. Plant Health Management Research Monogrphs, 22: 22-25.

Craig, J. (1976). An inoculation technique for identifying resistance to sorghum downy mildew. *Plant Dis. Reptr.*, 60: 350-352.

Craig, J. 1982b. Inheritance of resistance to sorghum downy mildew in corn. *Phytopathology*, **72(7):**943.

Craig, J., A.J. Bockholt, R.A. Frederiksen and M.S. Zuber. 1977. Reaction of important corn inbred lines to *Sclerospora sorghi*. *Plant Dis. Reptr.*, **61(7):** 563-564.

De Leon, C., G. Granados, R.N. Wedderburn and S. Pandey. 1993. Simultaneous improvement of downy mildew resistance and agronomic traits in tropical maize. *Crop Sci.*, **33:**100-102.

Frederiksen, R.A. and B.L. Renfro. 1977. Global status of maize downy mildew. *Annu. Rev. Phytopathol.*, **15:**249-275.

Frederiksen, R.A., A.J. Bockholt, L.E. Clark, J.W. Cosper, J. Craig, J.W. Johnson, B.L. Jones, P. Matocha, F.R. Miller, L. Reyes, D.T. Rosenow, D. Tuleen and H.J. Walker. 1973. Sorghum downy mildew: A disease of maize and sorghum. Research monograph**. 2:1-32.** The Texas Agricultural Experiment Station, Texas A & M University, College Station, Texas, U.S.A.

George, M.L.C., B.M. Prasanna, R.S. Rathore, T.A.S. Setty, F. Kasim, M. Azrai, S. Vasal, O. Balla, D. Hautea, A. Canama, E. Regalado, M. Vargas, M. Khairallah, D. Jeffers and D. Hoisington. 2003. Identification of QTLs conferring resistance to downy mildew of maize in Asia. *Theor. Appl. Genet.*, **107:**544-551.

Hoisington, D., M. Khairallah and D. Gonzalez-de-Leon. 1994. Laboratory protocols: CIMMYT applied molecular genetics laboratory. CIMMYT, Mexico.

Jeger, M.J., E. Gilijamse, C.H. Bock and H.D. Frinking. 1998. The epidemiology, variability and control of the downy mildews of pearl millet and sorghum, with particular reference to Africa. *Plant Pathol.,* **47(5):**544-569.

Jugenheimer, R.W. (1976). Corn: Improvement, seed production and uses. A Wiley Inter science Publications, USA. Pp: 124-129.

Jinahyon, S. 1973. The genetics of resistance and its implications to breeding for resistance in corn. Proc. 9th Inter-Asian Corn Improv. Workshop, Malayia, pp: 30-39.

Lal, S. and Singh, I.S. (1984). Breeding for resistance to downy mildews and stalk rots in maize. *Theor. Appl. Genet.*, 69:111-119.

Lima, M., N. Gimenes-Fernandes, J.B. Miranda Filho and J.C.V.A. Pereira. 1982. Introduction of maize germplasms as sources for downy mildew resistance. *Maydica*, **27:** 159-168.

Nair, S.K., B.M. Prasanna, A. Garg, R.S. Rathore, T.A.S. Setty and N.N. Singh. 2005. Identification and validation of QTLs conferring resistance to sorghum downy mildew (*Peronosclerospora sorghi*) and Rajasthan downy mildew (*Peronosclerospora heteropogoni*) in maize. *Theor. Appl. Genet.*, **110:**1384-1392.

Nair, S.K., T.A. Setty, R.S. Rathore, R. Kumar, N.N. Singh and B.M. Prasanna. 2001. Towards molecular marker mapping of genes conferring resistance to sorghum downy mildew (*Peronosclerospora sorghi*) in maize. *Maize Genetic Co-operation Newsletter*, **75**.

Odvody, G.N. and R.A. Frederiksen. 1984a. Use of systemic fungicides metalaxyl and fosetyl-Al for control of sorghum downy mildew in corn and sorghum in South Texas. I: seed treatment. *Plant Dis.,* **68(7):** 604-607.

Odvody, G.N. and R.A. Frederiksen. 1984b. Use of systemic fungicides metalaxyl and fosetyl-Al for control of sorghum downy mildew in corn and sorghum in South Texas. II: foliar application. *Plant Dis.,* **68(7):** 608-609.

Sabry, A., D. Jejjers, S.K. Vasal, R.A. Frederiksen and C. Magill. 2006. A region of maize chromosome 2 affects response to downy mildew pathogens. *Theor. Appl. Genet.*, **113:** 321-330.

Saghai-Moroof, M.A., K. Soliman, R.A. Jorgensen and R.W. Allard. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc. Natl. Acad. Sci., USA,* **81:** 8014-8018.

Schmitt, C.G., G.E. Scott and R.E. Freytag. 1977. Response of maize diallel cross to *Sclerospora sorghi*, cause of sorghum downy mildew. *Plant Dis. Reptr.*, **61(7):**607-608.

Setty, S., G.R. Ramaswamy, K.T. Pandurangae Gowda, T.B. Anilkumar, T.A. Puttaramanaik and Srinivasachary. 2001. Reaction of some maize genotypes against sorghum downy mildew. *Plant Dis. Reptr*., **16(1):** 127-129.

Sharma, R.C. and S. Lal. 1998. Maize diseases and their management. *Indian Farming*, **48(1):**92-96.

Shivanna, H. and K.H. Anahosur. 1990. Breeding downy mildew resistant sorghum varieties. *Indian Phytopathol*., **43(3):** 372-374.

Yen, T.T.O., B.M. Prasanna, T.A.S. Setty and R.S. Rathore. 2004. Genetic variability for resistance to sorghum downy mildew (*Peronosclerospora sorghi*) and Rajasthan downy mildew (*Peronosclerospora heteropogoni*) in the tropical / sub-tropical Asian maize germplasm. *Euphytica*, **138:** 23-31.

Table 1. Base sequence information for ten SSR primers

Sl. No.	SSR primer	Bin locations	Forward	Reverse
1.	bnlg1018	2.04	CGAGGTTAGCACCGACAAAT	CGAGTAAATGCTCTGTGCCA
2.	bnlg371	2.05	CAACGCGAAGCAGAGATAAAA	TCGTCGCATGACCATAGTAGC
3.	bnlg1893	2.09	AATCCTGTAGCGTGTGTCCC	TAACTGAGTTGTTGAAGGAAATTG
4.	umc1223	3.04	TTCAACAGATTCAGAGAAAGCACA	TTGATAATTAATCCGCAGCTCTCTC
5.	bnlg1035	3.05	TGCTTGCACTGTCAGGAATC	CAGCTCTGACACACCACACA
6.	bnlg420	3.05	CTTGCGCTCTCCTCCCCTT	GGCCAGCTCACTGCTCACT
7.	Phi073	3.05	GTGCGAGAGGCTTGACCAA	AAGGGTTGAGGGCGAGGAA
8.	bnlg1154	6.04	GGGTGATCACATGGGTTAGG	AAATCAATGCTCCAAATCGC
9.	bnlg1702	6.05	TTATCATCAAATGGAGGACACG	AAAGACACACGCTAATGGGC
10.	umc1859	6.06	ATATACATGTGAGCTGGTTGCCCT	GCATGCTATTACCAATCTCCAGGT

Table 2. SDM reaction of materials used in this study

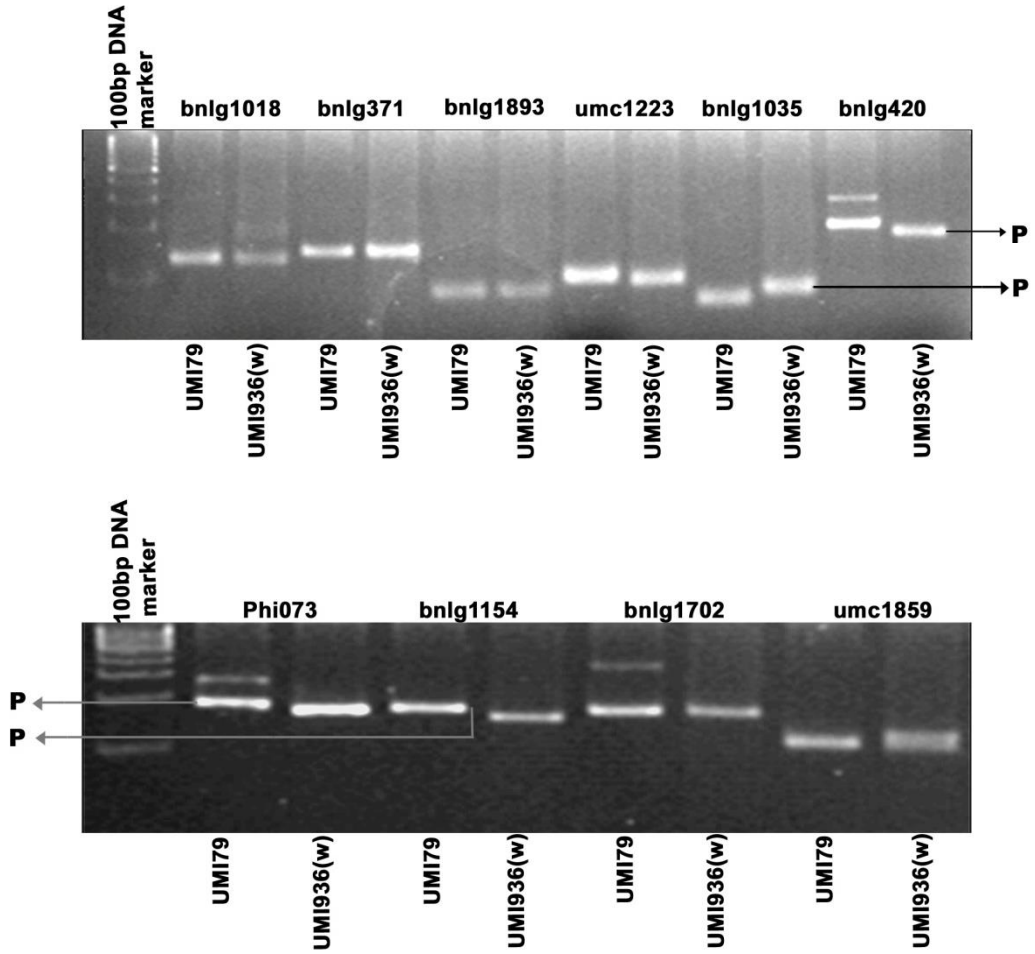
Sl. No.	Entries / Population	SDM infection (per cent)	Reaction
1	UMI 79	100.00	Susceptible

2	UMI 936 (w)	2.62	Resistant
3	F_1	66.67	Susceptible
4	F_2	60.71	Susceptible

Table 3. Chi square test for inheritance pattern of SDM resistance in UMI 79 (susceptible) x UMI 936 (w) (resistant) cross

Presumed Genetic Ratios and Gene action	Observed ratio (susceptible : resistant)	Expected ratio (susceptible : resistant)	$X^2_{calculated}$	X^2_{table}
3:1 (Complete Dominance)	136S : 88R	168S : 56R	24.38**	3.84
15:1 (Duplicate gene action)	136S : 88R	210S : 14R	417.22**	
9:7 (Complementary gene action)	136S : 88R	126S : 98R	1.81^{ns}	
13:3 (Inhibitory gene action)	136S : 88R	182S : 42R	62.01**	

Plate 1. Parental screening for polymorphism using SSR primers



Where, P – polymorphic markers