Journey of Net Blotch: from Pathotype Diversity to useful Resistance in Barley

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Abstract

Studies on variation, occurrence and distribution of virulence in *Pyrenophora teres* f. *teres* are essential to identify effective sources of resistance for net type net blotch. Disease surveys suggested two different stains are prevalent in Western Australia and 13 in all around Australia. Sixty nine barley lines from different breeding groups in Australia and elsewhere were tested against most prevalent pathotypes. Majority of lines have partial to complete resistance while some have elite resistances to net type net blotch. Four lines out of 69 were chosen for further studies. These four lines: WA 4794 (103 IBON 91), Pompadour, CI 9214, and WPG 8412-9-2-1 were highly diverse and resistant to most of the isolates, and were crossed with Stirling-a highly adaptive but susceptible cultivar. Doubled haploids, F2s, and resistant x resistant crosses were studied against five prevalent isolates. Four genes from WA 4794 (all dominant), three (two dominant and one recessive) from Pompadour, five (two dominant and three recessive) from CI 9214, and two (one dominant and one recessive) from WPG 8412-9-2-1 were identified. In total, 11 different genes were operative against *P. teres* f. *teres* isolates. Molecular work is initiated to develop markers which would aid screening of the breeding populations for these resistances.

Introduction

Net type of net blotch of barley caused by *Pyrenophora teres* f. *teres* is a prominent leaf disease and occurs widely throughout the barley breeding areas of Australia. It reduces grain yield by up to 33% (Khan 1987) mainly through reduced grain size. Development of stable forms of resistance depends upon identification of resistances effective against the most prevalent isolates around Australia. In this paper we report the pathogen variability, sources of resistance and identification of genes resistance for net type net blotch in the selected barley lines which can be deployed in the breeding programs around Australia.

Material and methods

Two distinct net type net blotch isolates were found to be stable from Western Australia (Gupta and Loughman 2001) and 13 in total were identified around Australia (Greg *et al* 2000). In total five different isolates were used to identify useful net type net blotch resistances (Table 1).

Table 1: Net type net blotch isolates used for genetic studies.

Isolates	**Origin**	**Virulence Spectrum**
97NB1	Western Australia	Dampier, Prior, Stirling
95NB100	Western Australia	Beecher, Dampier, Prior, Stirling

NB50	Queensland	Rika, Franklin, Grimmett, Skiff, Corvette, Gilbert, Golf, Patty, Kaputar, Herta, Cameo, Betzes, Stirling
NB81	Queensland	Corvette, Prior, Cape, Prior, Clipper, Dampier, Betzes, Cameo, Stirling
NB52B	South Australia	Clipper, Skiff, Tallon, Patty, Herta, Golf, Kaputar, Cameo, Stirling

Sixty nine barley lines from different barley breeding groups around Australia and elsewhere to identify sources of resistance for breeding. Most of these lines have some resistance to net blotch and some represent elite resistances (Gupta *et al* 1999). Table 2 shows barley lines crossed with Stirling-a highly adaptive but susceptible cultivar of Western Australia. Doubled haploids, F2s from these four crosses, and resistant x resistant crosses were studied against 97NB1 (WA), 95NB100 (WA), NB50 (Qld), NB81 (Qld) and NB52B (SA) isolates. Inoculations were undertaken at the two leaf stage with a suspension of ~2 x 10^4 spores/ml. Plants were incubated at 19-20°C with complete leaf wetness for the first 24 hr and symptom severity was assessed on the ninth day using a scale by Tekauz (1985). The observed segregation ratios of F2s and doubled haploid populations were compared with expected ratios by Chi square tests. Joint segregation analysis was used to investigate the relationship of net type net blotch resistance genes in these resistant barley lines and between each of the four pathotypes by scoring same DH line from a particular cross, as described by Lupton and Macer (1962).

Table 2. Response of selected barley lines against different Australian pathotypes.

Barley Line	**97NB 1**	**95NB 100**	**NB 50 NB 81 (Scale 1-10)**		**NB 52B**
WA 4794 (103 IBON 91) (Pedigree: Arupo 'S'*2/3/PI 2325/Maf 102//Cossack)	1	2	2	1.5	1
Pompadour (Pedigree: FDO192/Patty)	1	2	3	3	2
CI 9214 (Pedigree: Collected from South Korea)	1	1.5	1.5	2	2
WPG8412-9-2-1 (Pedigree: Bowman//Ellice/TR451)	2	2	3.5	1	2
Stirling (Pedigree: Dampier//Prior/Ymer/3 /Piroline)	7	7	7	8.5	7.5

Results and Discussion

The F1s were resistant from WA 4794, Pompadour and CI 9214 but was intermediate in response from WPG8412-9-2-1 when these lines were crossed with Stirling. Genetic ratios from F2s and doubled haploid populations are shown in Tables 3a-d. In WA 4794 x Stirling all the observed ratios were not significantly different from the expected ratios at $P<0.05$ significance. These ratios were confirmed with F2 populations which also provided the information on the nature of resistance genes. All the genes operative against different isolates are dominant and are independent in nature.

For Pompadour x Stirling, all the observed ratios were not significantly different from the expected ratios at P<0.05 significance except DH lines tested against NB81 isolate. But we found a good fit to a one gene ratio from the F2 population against this isolate. The higher Chi Square value indicated that there might be some genetic distortion among the DH population or possible misclassification from the disease ratings. All the genes identified are dominant except one recessive gene operative against NB50 isolate. Genes are independent in action.

Table 3: Genetics studies in doubled haploid and F2 populations against net type net blotch isolates

Table 3a: WA 4794 x Stirling F2 and DH Population

Isolate	Generation	Res.	Sus.	Total	Exp. Ratio	Chi Square	Genes (Number & Nature)
97NB 1 (WA)	F2	280	25	305	15 : 1	1.98	Two Dominant Independent
	DH Lines	237	62	299	3 : 1	2.90	
95NB 100 (WA)	F2	167	16	183	15 : 1	1.94	Two Dominant Independent
	DH Lines	182	76	258	3 : 1	2.73	
NB 50 (Qld)	F2	175	61	236	3 : 1	0.09	One Dominant
	DH Lines	117	90	207	1 : 1	3.52	
NB 81 (Qld)	F2	203	11	214	15 : 1	0.45	Two Dominant Independent
	DH Lines	154	65	219	3 : 1	3	
NB 52B (SA)	F2	-	-	-	-	-	Two Genes
	DH Lines	106	26	132	3 : 1	1.98	

Table 3b: Pompadour x Stirling F2 and DH Population

Isolate	Generation	Res.	Sus.	Total	Exp. Ratio	Chi Square	Genes (Number & Nature)
97 NB 1 (WA)	F2	214	90	304	3 : 1	3.44	One Dominant
	DH Lines	143	155	299	1 : 1	0.48	
95 NB 100 (WA)	F2	155	48	203	3 : 1	0.19	One Dominant
	DH Lines	134	156	290	1 : 1	1.66	
NB 50 (Qld)	F2	183	50	233	13 : 3	1.12	One Dominant One Recessive Independent
	DH Lines	211	75	286	3 : 1	0.23	
NB 81 (Qld)	F2	179	60	239	3 : 1	0.00	One Dominant Gene
	DH Lines	122	174	296	1 : 1	9.13*	
NB 52B (SA)	F2	-	-	-	-	-	Two Genes
	DH Lines	209	63	272	3 :1	0.49	

* Significant at 5% level of significance

The third cross, CI 9214 x Stirling, was more complex. This is mainly because more than one dominant and recessive genes against the different isolates were identified. All the ratios were not significantly different from the indicated expected ratios at P<0.05 significance and the doubled haploid ratios were confirmed by F2s.

In the last cross, WPG8412-9-2-1 x Stirling, all the ratios from doubled haploid and F2 populations were not significantly different from the expected ratios at P<0.05 significance except DH lines tested against NB 81 and NB 52B isolates. There was a single gene action against three isolates and a recessive gene along with dominant gene operable against NB 50 and NB 52B.

Table 3c: CI 9214 x Stirling F2 and DH Population

Isolate	**Generation**	**Res.**	**Sus.**	**Total**	**Exp. Ratio**	**Chi Square**	**Genes (Number & Nature)**
97 NB 1 (WA)	F2	108	35	143	13 : 3	3.07	One Dominant One Recessive Independent
	DH Lines	234	60	294	3 : 1	3.30	
95 NB 100 (WA)	F2	134	33	167	13 : 3	0.11	One Dominant One Recessive Independent
	DH Lines	221	71	292	3 : 1	0.07	
NB 50 (Qld)	F2	175	28	203	55 : 9	0.012	One Dominant Two Recessive Independent
	DH Lines	252	27	279	7 : 1	2.03	
NB 81 (Qld)	F2	198	15	213	15 : 1	0.29	Two Dominant Independent
	DH Lines	216	55	271	3 : 1	3.20	
NB 52B (SA)	DH Lines	240	39	279	3 : 1	0.55	Three Independent

Table 3d: WPG 8412-9-2-1 x Stirling F2 and DH Population

Isolate	**Generation**	**Res.**	**Sus.**	**Total**	**Exp. Ratio**	**Chi Square**	**Genes (Number & Nature)**
97 NB 1 (WA)	F2	140	50	190	3 : 1	0.17	One Dominant
	DH Lines	157	130	287	1 : 1	2.54	
95 NB 100 (WA)	F2	137	48	185	3 : 1	0.08	One Dominant
	DH Lines	162	130	292	1 : 1	3.50	
NB 50 (Qld)	F2	188	37	225	13 : 3	1.05	One Dominant One Recessive Independent
	DH Lines	212	69	281	3 : 1	0.03	
NB 81 (Qld)	F2	177	53	230	3 : 1	0.47	One Dominant
	DH Lines	168	123	291	1 : 1	6.95*	
NB 52B (Qld)	DH Lines	199	95	294	3 : 1	8.37*	Two Independent

*Significant at 5% level of significance

F2 populations from resistant x resistant crosses in all possible combinations from four resistant

parents were studied against the same set of isolates. From each cross roughly 200 to 500 seeds were tested as individual seedlings. The segregation/non-segregations were determined to establish the diversity of the resistance genes present in the candidate parental lines. The pattern of segregation/non-segregation was found to be as expected except in two cases – WA 4794 x Pompadour and WA 4794 x CI 9214 against 97NB1 and NB 50 respectively. We established this inaccuracy only after studying the interrelationship of the genes (Table 5).

The interrelationships of the genes were derived from the joint segregation analysis. Symbols for postulated genes are given alphabetically and capital letters indicate the dominant rather than recessive resistance. Table 5 indicates the action of the genes is differential in some cases and same in other cases with respect to the isolates. The dominant gene 'A' present in WA 4794 is also present in Pompadour. Recessive gene 'f' is common among parents Pompadour, CI 9214 and WPG 8412-9-2-1. All other genes are different among these parents. Results indicate the possibility of 11 different genes among these parents.

Table 5: Number and distribution of resistance genes in WA 4794, Pompadour, CI 9214 and WPG8412-9-2-1 against respective net type net blotch isolates

Population	**Putative number of genes from WA 4794, Pompadour, CI 9214 and WPG8412-9-2-1**					**Proposed Genes**
Isolates	97NB 1 (WA)	95NB 100 (WA)	NB 50 (Qld)	NB 81 (Qld)	NB 52B (SA)	
WA 4794 x Stirling	2 (AB)	2 (AC)	1 (B)	2 (AC)	2 (BD)	4 (ABCD)
Pompadour x Stirling	1 (A)	1 (A)	2 (Ef)	1 (A)	2 (Ef)	3 (AEf)
CI 9214 x Stirling	2 (Gh)	2 (Gh)	3 (Gif)	2(GJ)	3 (Gif)	5 (fGhiJ)
WPG 8412-9-2-1 x Stirling	1 (K)	1 (K)	2(Kf)	1(K)	2 (Kf)	2 (fK)
Different genes in total = 11						

These resistance genes are very useful for net-type net blotch resistance breeding around Australia. We propose to develop linkage maps by employing bulk segregant analysis followed by amplified fragment length polymorphism (AFLP) or microsatellite technique. This will lead to identification of molecular markers linked to these resistances for marker-assisted selection in the near future.

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