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Phenotypic and molecular genetic analysis of partially resistant bread wheat cultivars against root lesion nematode (*Pratylenchus thornei*)

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INTRODUCTION

At least eight species of the Root Lesion Nematode (RLN) (genus Pratylenchus) are known to attack small grain cereals (1). On wheat, *P. thornei* is the most studied species being found in Syria, Yugoslavia, Mexico, Australia, Canada, Israel, Morocco, Turkey, Pakistan, India, Algeria and Italy and with clear documented economic yield losses ranging from 38-85% in Australia, 12-37% in Mexico and 70% in Israel (2).

The primary limitation to developing wheat germplasm with resistance to RLN is the limited information available about the genetic basis of resistance. In this study, using both phenotypic screening and molecular genetic characterisation of a F2 half diallel of three partially resistant sources (Australia GS50A, Iraqi land race AUS4930 7.2[1], and CIMMYT synthetic derivative CROC_1/AE.SQUARROSA (224)//OPATA[2] abbreviated to Croc) and two susceptible wheat varieties, Pastor from CIMMYT and Janz from Australia, the genetics and nature of inheritance of nematode resistance in these partially resistant sources was investigated. CIMMYT germplasm identifiers for the two partial resistant sources include; [1]AUS4930 7.2 (CID 390521; SID 20; SH: BW30063-7AUS(JN)-2GHB-0Y), and [2]CROC_1/AE.SQUARROSA (224)//OPATA (CID 72726; SID 531; SH: CMBW91Y00935S-80Y-11KBY-1KBY-010M-1Y-2M-0Y-0SY).

MATERIALS AND METHODS

The three partially resistant x two susceptible wheat populations were studied (Table 1). To assay resistance for the AUS4930 7.2 x Pastor (n=93), each F9 line and the parents of the population were grown with 7 replicates using the improved screening method (3). For all other F2 populations single plants were grown in a pot without replicates. Seven replicates of each of the parents were grown.

Plants were grown according to a randomized complete block design in small tubes (15x100mm) with a 70:29:1 sand : field soil : organic matter mix. The tubes were placed in large trays containing perlite. Sterilized seed were pre-germinated and planted one seedling per tube with approximately 3 equidistant seminal roots about 1cm long. All seedlings were then inoculated one week after planting with 400 *P. thornei*/plant in a 1ml water medium obtained from single nematode carrot cultures from Adana in the south east of Turkey. Plants were watered as necessary to maintain soil moisture and were harvested after 13 weeks. All plants were kept in a controlled environment room with 16 hour light supplied by fluorescent lights followed by 8 hours dark and maintained at temperatures between 20-25 °C. At harvest time plant shoots were removed and the soil and roots extracted using a modified Baermann funnel and Mister extraction (Toktay, unpublished).

Numbers of *P. thornei* per 1 ml of extracted sample were counted in a counting dish under a compound low powered microscope and final results were expressed as number of nematodes per plant. Progeny and parents from each population were phenotyped at the Plant Protection Research Institute, Adana, Turkey. DNA was extracted from leaf tissue and seeds from all progeny from the populations.

Genetic Analysis of data was achieved by log transformation (ln x+1) and analysis with ANOVA. Combining ability effects for the F2 populations were calculated according to Griffing's (1) experimental model 1 (fixed effects) and method 4 (no parents or reciprocals) using diallel analysis.

Molecular genetic characterization was undertaken on individuals of the AUS4930 x Pastor and Croc x Pastor populations only using simple sequence repeat (SSR) markers previously shown to be associated with RLN resistance (3,4,6). Polymorphism screening was initially undertaken using agarose gels, and was completed using polyacrylamide gels, as described in (5).

RESULTS AND DISCUSSION

In this study, *P. thornei* resistance was shown to be polygenic and additive in gene action.

The General and Specific (GCA & SCA) of each of the Partial resistance sources with each of the Susceptible Checks , Pastor and Janz. Numbers in **bold** indicate negative values which infer a contribution from the parent(s) towards greater resistance in the progeny.

Table 1. The nine F2 half diallel populations developed.

	Janz	GS50A (T34.8 REP 2)	AUS4930 7.2	Croc
Pastor		1* (133)**	2 (93)***	3 (106)
Janz		4 (245)	5 (229)	6 (207)
GS50A(T34.8 REP 2)			7 (170)	8 (205)
AUS4930 7.2				9 (135)

*1-9:Populations. ( )Number of individuals in the population. *** F9 population

Table 2. The General and Specific (GCA & SCA) of each of the Partial resistance sources with the Susceptible Check, Pastor .

	Line	Disease Reaction[1]	Transformed mean[2]	Equivalent mean[3]	GCA	Significance
GCA	Gs50a	PR	3.06	36.73	0.04	**
	AUS 4930	PR	1.78	9.65	**-0.61**	**
	Croc	PR	0.33	5.48	**-0.05**	**
	Pastor	S	3.64	107.82	0.62	***
SCA	GS50a x AUS 4930	PR x PR	0.41	15.42	0.10	**
	GS50a x Croc	PR x PR	0.39	11.53	**-0.41**	***
	GS50a x Pastor	PR x S	0.45	28.07	-0.41	
	AUS 4930 x Pastor	PR x S	4.58	293.31	**-0.41**	***
	AUS 4930 x Croc	PR x PR	1.48	27.53	0.31	**
	Croc x Pastor	PR x S	2.9	60.92	0.10	***

*, **, *** Significant at P=0.05, P = 0.01 and P = 0.001, GCA general combining ability, SCA Specific combining ability.
1 Disease reaction. PR, partially resistant; R, Resistant; S, susceptible.
2.Data transformed by log (ln x+1)
3.Equivalent mean values expressed a *P. thornei* per plant (including roots and soil).

Table 3. The General and Specific (GCA & SCA) of each of the Partial resistance sources with the Susceptible Check, Janz

	Line	Disease Reaction[1]	Transformed mean[2]	Equivalent mean[3]	GCA	Significance
GCA	Gs50a	PR	3.06	36.73	-0.06	
	AUS 4930	PR	0.33	5.48	**-0.63**	***
	Croc	PR	3.64	107.82	**-0.33**	***
	Janz	S	2.36	41.28	1.02	***
SCA	GS50a x AUS 4930	PR x PR	1.17	27.55	-0.05	
	GS50a x Croc	PR x PR	0.39	11.53	**-0.29**	***
	GS50a x Janz	PR x S	0.45	28.07	-0.29	
	AUS 4930 x Janz	PR x S	4.58	293.31	**-0.29**	**
	AUS 4930 x Croc	PR x PR	1.71	25.52	0.34	***
	Croc x Janz	PR x S	7.05	1492.41	**-0.05**	***

*, **, *** Significant at P=0.05, P = 0.01 and P = 0.001, GCA general combining ability, SCA Specific combining ability.
1 Disease reaction. PR, partially resistant; R, Resistant; S, susceptible.
2.Data transformed using ln x+1
3.Equivalent mean values expressed a *P. thornei* per plant (including roots and soil).

The General and Specific combining ability analysis (GCA and SCA) with both *P. thornei* susceptible parents indicate that AUS9430, followed by Croc and then Gs50a in this order, provide the best general combining abilities. Considering specific crosses (SCA): GS50a and Croc combinations work extremely well for combining the resistance; both susceptible parents combine best with AUS4930 PR parent; the Croc x Janz population is also promising .

Previous studies have identified QTL for resistance to *P. thornei* on chromosomes 1B, 2B, 3B, 4D and 6D (3, 6) with the 6D locus present in all 5 resistance sources studied to date and the 2B locus in 4 of the 5 sources; the 1B, 3B and 4D loci appear to be more source-specific. Molecular genetic analysis of the AUS4930 and Croc sources of resistance has identified putative resistance loci on chromosomes 1B, 2B, 6D and 1B, 3B, respectively (5), supporting the identification of 'common' and 'less common' resistance loci. These latter loci offer opportunities for gene pyramiding; transgressive segregation was found when GS50a was combined with either Croc or AUS4930, however this effect was not as evident with Croc in combination with AUS4930. Further work is required to determine if the QTLs identified in AUS4930 and/or Croc are the same, or allelic, or linked but different resistance loci to those previously identified, and to determine if these two sources contain other novel resistance loci.

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REFERENCES

1 Griffing B 1956. A generalised treatment of the use of diallel crosses in quantitative inheritance. Heredity 10, 31-50.

2 Nicol, J., Rivoal. R., Taylor, S. and Zaharieva, M, 2004. Global Importance of Cyst (*Heterodera spp*.) and Lesion Nematodes (*Pratylenchus spp*.) on Cereals: yield loss, population dynamics, use of host resistance and integration of molecular tools. Nematology Monographs and Perspectives 2: 233-251.

3 Schmidt, A. L., McIntyre, C. L., Thompson J., Seymour N. P., Liu C. J., 2005. Quantitative trait loci for root lesion nematode (*Pratylenchus thornei*) resistance in Middle-Eastern landraces and their potential for introgression into Australian bread wheat. Australian Journal of Agricultural Research 56: 1059–1068.

4 Toktay, H., Şahin, E., Tülek, A., Nicol, J., Elekçioğlu, H. And Bolat, N. 2004. Optimising a Resistance Screening Assay for Cereals Against Cereal Cyst Nematode *Heterodera filipjevi* (Madzhidov) and Root Lesion Nematode *Pratylenchus thornei* (Sher et Allen) Under Greenhouse Conditions. 1st National Pathology Congress, Samsun, Turkey, 8-10th Sept. pp.111.

5 Toktay, H., McIntyre, L., Nicol, J.M., Ozkan, H. and Elekcioglu, H.I. 2006. Identification of common root lesion nematode (*Pratylenchus thornei*) Sher et Allen) loci in bread wheat. Genome 49: 1-5.

6 Zwart, R.S., Thompson J.P., Godwin I.D., 2005. Identification of quantitative trait loci for resistance to two species of root-lesion nematode (*Pratylenchus thornei* and *P. neglectus*) in wheat. Australian Journal of Agricultural Research, 56: 345 – 352.