Introgression of Russian Wheat Aphid Resistance from Tetraploid Wheat Germplasm

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Introduction

The Russian wheat aphid (RWA), *Diuraphis noxia* (Mordvilko), is a serious pest in arid and semi-arid wheat (*Triticum aestivum*) production areas of the Great Plains (Stoetzel, 1987). Direct and indirect economic impact from RWA was estimated at over $800 million dollars between 1987 and 1993 (Morrison and Peairs, 1998), leading to extensive research efforts to identify resistance for incorporation into adapted cultivars. Several cultivars with resistance conferred by the *Dn4* resistance gene have been released and widely grown in Colorado, beginning with the release of 'Halt' in 1994 (Quick et al., 1996).

Biotypic variation in RWA populations has been reported in several areas of the world (Puterka et al., 1992). In North America, a single RWA biotype was thought to exist until the identification of an isolate in 2003 that was capable of causing damage on genotypes carrying the *Dn4* resistance gene (Haley et al., 2004). This biotype has since been designated as RWA biotype 2 (Porter et al., 2005).

Most of the resistance sources identified in North America since the introduction of RWA in 1987 have been localized to the D genome of common wheat. While many new sources of resistance to RWA biotype 2 have been identified in common wheat, broadening of the genetic base of RWA resistance beyond the D genome of wheat would be highly desirable. Several examples exist in the literature of the use of tetraploid wheat (i.e., *Triticum dicoccoides*) as a source of genes for applied wheat breeding. As accessions of *T. dicoccoides* resistant to RWA biotype 2 have been identified (unpublished data), these may be useful as sources for transfer to common wheat.

Figure 1: The Russian Wheat Aphid.

Objectives

1) Effect transfer of RWA resistance from three tetraploid wheat accessions to two different hexaploid wheat genotypes.

2) Determine inheritance of RWA resistance from the tetraploid wheat accessions.

3) Confirm the level of expression of RWA resistance from the tetraploid wheat accessions in a hexaploid wheat background.

Materials and Methods

Plant Materials

The Iranian tetraploid accessions PI 624903, PI 624904, and PI 624908 were identified as carrying resistance to RWA biotype 2 in standard greenhouse seedling screening tests. These accessions were crossed as the male parent to the hexaploid spring wheat varieties 'Len' (CItr 17790) and 'Coteau' (CItr 17749). F_1 plants from these crosses were advanced to the F_2 by self-pollination and were used as females in backcrosses to the susceptible hexaploid wheat parnets. The BC_2F_1 was derived similarly following screening the BC_1F_1 generation for RWA biotype 2 resistance.

Ploidy Confirmation

To confirm the ploidy level of the three resistant parents, chromosome counts were done using root tip squashes. Root tips were collected, placed in ice cold water for 24 hours, fixed with 3:1 95% ethanol: glacial acetic acid, and stained in 0.7% acetocarmine solution. The D-genome specific markers *Xcfd57* and *Xcfd161* were used to amplify DNA extracted from parental tissue of the tetraploid wheat parents.

RWA Screening Procedures

Greenhouse grown seedlings were infested with RWA by placing a piece of leaf tissue with 4-7 aphids at the base of each plant. Leaf rolling scores were assigned on a basis of 1-3, with 1 indicating unfolded leaves, 2 folded leaves, and 3 tightly rolled leaves. Chlorosis scores were assigned on a scale of 1-9 with 1 =no chlorosis and 9 = death of the plant.

Data Analysis

Resistance scores for the F_1 were compared using Proc GLM in SAS (SAS Institute, Cary, NC). Chi-square analysis was used to determine goodness of fit in segregating generations.

Results and Discussion

Table 1. Russian Wheat Aphid Biotype 2 Resistance Scores of the Parents and the F_1 Pentaploid Progeny.

Genotype†	Leaf Rolling †	Chlorosis †	Resistant Parent Difference ‡	Susceptible Parent Difference ‡
Len	3.0	7.5		
Coteau	3.0	6.5		
PI 624903	2.0	1.3		
PI 624904	2.0	2.0		
PI 624908	2.0	1.3		
Len/PI 624903	2.0	2.3	0.56	<0.01
Len/PI 624904	2.0	3.0	0.32	<0.01
Len/PI 624908	2.0	2.0	0.99	<0.01
Coteau/PI 624904	2.0	2.0	1.00	<0.01
Coteau/PI 624908	2.0	1.3	1.00	<0.01

† Leaf Rolling scores are based on a scale of 1-3 with 1 indicating unfolded leaves and 3 indicating tightly rolled leaves. Chlorosis scores are based on a scale of 1-9 with 1 =no chlorosis and 9 = death of the plant. The numbers are averages from 3-6 plants.
‡ Differences expressed as a p-values from LS Means separation of chlorosis scores of F_1 t respective resistant and susceptible parent.

Expression of RWA Resistance in a Hexaploid Background

Based upon data obtained from F_1 pentaploid progeny (Table 1), the level of expression of RWA resistance genes from tetraploid parents are not significantly different when transferred to a hexaploid background. The p-values for differences in expression from the respective resistant parent ranged from 0.32 – 1.00. However, when the progeny were compared to their respective susceptible parent they were all significantly different with p-values of <0.01.

Table 2. Russian Wheat Aphid Biotype 2 Resistance in F_2's.

Genotype†	No. of Plants		χ^2 Values for Expected Ratios		
	Resistant	Susceptible	3:1	13:3	15:1
Coteau/ PI 624904-1	15	6	NS	NS	***
Coteau/ PI 624904-3	11	4	NS	NS	***
Coteau/ PI 624904-4	4	8	*	*	***
Coteau/ PI 624904-5	5	13	***	***	***
Coteau/ PI 624908-2	13	8	NS	*	***
Coteau/ PI 624908 B1	5	5	*	*	***
Coteau/ PI 624908 B2	5	8	***	***	***
Len/ PI 624903-1	2	8	***	***	***
Len/ PI 624903-2	1	7	***	***	***
Len/ PI 624903-6	4	18	***	***	***
Len/ PI 624903-7	2	22	***	***	***
Len/ PI 624904-4	6	14	***	***	***
Len/ PI 624904 B1	4	4	NS	***	***
Len/ PI 624904 B2	3	4	NS	***	***
By Parent					
Coteau	58	52	*	*	***
Len	22	77	*	*	***
PI 624903	9	55	*	*	***
PI 624904	48	53	*	*	***
PI 624908	23	21	*	*	***

*Significant at the α=0.05 level.
*** Significant at the α=0.01 level.
† Dashed numbers indicate seeds taken from heads of a single plant. B numbers indicate bulked populations.

Inheritance of Resistance from Tetraploid Wheat Accessions

Five of the 14 different groups of F_2 plants (Table 2) met the statistical criteria for a 3:1 resistant: susceptible ratio indicative of single gene inheritance. When analyzed for a 13:3 segregation ration, two of the 14 F_2 lines met the statistical criteria. However, the data could be confounded due to reproductive barriers caused by uneven chromosome numbers at meiosis. Furthermore, when analyzed by the parents used in the cross, none of the segregation ratios are met. 'Len' and PI 624903 seemed to produce an excess of susceptible progeny where the other parents produced equal numbers of both resistant and susceptible progeny.

Table 3: Russian Wheat Aphid Biotype 2 Resistance in BC_1F_1.

Genotype‡	# Resistant	# Susceptible	P-value†
Coteau/ PI 624904 B1	7	7	1.00
Coteau/ PI 624908-1	1	0	0.32
Len/ PI 624903-1	1	2	0.48
Len/ PI 624903-2	2	3	0.56
Len/ PI 624903-3	3	11	0.03
Len/ PI 624903-4	1	4	0.20
Len/ PI 624904-1	3	3	1.00
Len/ PI 624904-2	4	8	0.25
By Parent			
Coteau	8	7	0.72
Len	14	31	0.01
PI 624903	7	20	0.01
PI 624904	14	18	0.48
PI 624908	1	0	0.32

† P-values are based upon a chi-square test for fit of a 1:1 resistant: susceptible segregation ratio indicating single gene inheritance in a backcross.
‡ Dashed numbers indicate seeds taken from heads of a single plant. B numbers indicate bulked populations.

Table 4: Russian Wheat Aphid Biotype 2 Resistance in BC_2F_1.

Genotype‡	# Resistant	# Susceptible	P-value†
Coteau/ PI 624904¥	7	8	0.72
Coteau/ PI 624904 B1£	13	18	0.37
Coteau/ PI 624908-1-1	0	2	0.16
Len/ PI 624903-1-1	5	3	0.48
Len/ PI 624903-2 B1€	6	1	0.07
Len/ PI 624903-2-2	2	7	0.11
Len/ PI 624903-2-4	9	4	0.17
Len/ PI 624903-3 B1€	2	3	0.56
Len/ PI 624904-4-1	1	0	0.32
Len/ PI 624904-1 B1£	8	1	0.03
Len/ PI 624904-2 B2£	6	5	0.68
By Parent			
Coteau	20	28	0.25
Len	39	24	0.06
PI 624903	24	18	0.35
PI 624904	35	32	0.70
PI 624908	0	2	0.16

† P-values are based upon a chi-square test for fit of a 1:1 resistant: susceptible segregation ratio indicating single gene inheritance in a backcross.
‡ Dashed numbers indicate seeds taken from heads of a single plant. B numbers indicate bulked populations.
£ Bulked in the BC_2F_1 and the BC_1F_1.
€ Bulked in the BC_1F_1.
¥ Bulked in the BC_1F_1.

The BC_1F_1 (Table 3) and BC_2F_1 (Table 4) data show a strong indication of single gene inheritance. One group of plants of eight and one group of 11 in the BC_1F_1 and BC_2F_1, respectively, do not meet the statistical criteria for goodness of fit of a 1:1 resistant: susceptible segregation ratio indicating single gene inheritance. Furthermore, when lines were analyzed by parent, all but two in the BC_1F_1 meet the criteria for a goodness of fit for a 1:1 resistant: susceptible ratio. Therefore, RWA resistance in PI 624903, PI 624904, and PI 624908 is most likely inherited as a single major gene.

This is different from what is seen in the F_2 plants where only a limited number met any goodness of fit for the segregation ratios. This could be attributed to the ability of certain eggs in the F_1 pentaploids carrying the A, B, and D-genomes to produce viable seeds when pollinated with the hexaploid recurrent parents.

These findings however only really apply to PI 624903 and PI 624904. In the BC_1F_1 and BC_2F_1, PI 624908 only produced one resistant and two susceptible progeny, respectively. When these data are compared to the F_2 data, no sounds conclusions can be made on the inheritance of RWA Biotype 2 resistance in this accession.

The data for PI 624903 differ between the BC_1F_1 and the BC_2F_1. In the BC_1F_1, there are excess susceptible progeny for crosses with PI 624903. This could be due to a failed cross that lead to self-pollination. In the BC_2F_1, crosses with PI 624903 meet the expected 1:1 segregation ratio with a p-value 0.35.

Single gene inheritance is strongly indicated for PI 624904 in the F_2, BC_1F_1, andBC_2F_1. Four of the five groups of F_2's that meet the criteria for a 3:1 segregation ratio have PI 624904 as the resistant parent. Groups of plants in the BC_1F_1 with PI 624904 as the parent also meet a 1:1 segregation ratio indicating inheritance of a single dominant gene. In the BC_2F_1, there is a group of plants that do not meet the 1:1 segregation ratio. This group is also a bulked population and when the data is combined and analyzed overall, PI 624904 meets the criteria for single dominant gene inheritance with a p-value of 0.70.

Differences also occurred based on the susceptible parent used in the cross. None of the F_2 plants combined to meet any statistical ratio for either 'Len' or 'Coteau'. In the BC_1F_1 and BC_2F_1, crosses with 'Coteau' met a 1:1 segregation ratio with p-values of 0.72 and 0.25, respectively. Crosses with 'Len' produced an excess of susceptible progeny in the BC_1F_1, but met the criteria for a 1:1 ratio in the BC_2F_1 with a p-value of 0.06.

Figure 2: A susceptible parent (left) and resistant F_1 pentaploid (right).

Conclusions

1) Successful transfer of RWA Biotype 2 resistance gene(s) occurred from wild tetraploid wheat accessions to a hexaploid cultivar and it's expression is not significantly different from the resistant parent.

2) Resistance is most likely inherited as a single dominant gene in PI 624903 and PI 624904. However, no sound conclusions can be made about inheritance of RWA resistance in PI 624908.

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