GENETIC RESISTANCE TO GREENBUG IS EXPRESSED WITH HIGHER CONTENTS OF PROTEINS AND NON-STRUCTURAL CARBOHYDRATES IN WHEAT SUBSTITUTION LINES

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Abstract: This paper studied the endogenous levels of reduced, non-reduced, total non-structural carbohydrates, soluble proteins and biomass in aerial and rooting structures of bread wheat, *Triticum aestivum* (2n = 6x = 42), in response to aphids, as a first step for understanding the cascade of transductional events that may account for antixenosis, antibiosis and tolerance to greenbug. Up to now, few studies have been made on the relationship between aphid resistance and these traits. A set of wheat intervarietal chromosome substitution lines, with "Chinese Spring" (CS, a greenbug susceptible line) as a recipient and a synthetic wheat (*Triticum dicoccum* x *T tauschii*, = [Syn]) as the donor, and both parents were used. Plants were cultivated in hydroponic solutions to the fully expanded 3rd leaf stage. Half of the plants of every genotype were infested 72 h with greenbugs, and the remaining uninfested plants were used as controls. Carbohydrate and protein contents and dry matter mass were determined for aerial and root tissues Lines 5A and 6A had lower aerial, root and, consequently, total dry weights in both control and infested plants. These lines have been previously reported to be antixenotic against greenbug and Russian Wheat Aphid (RWA), implying these lines carry genes for constitutive defences. Four substitution lines (1A, 1B, 7B and 7D) showed significant increases in protein content when infested, compared to their controls and to the CS susceptible parent. Considering that these substitution lines have been previously reported to reduce greenbug and RWA fertilities and longevities, the antibiotic

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resistance to greenbugs may be related to gene expression for enhanced protein levels. Most of the D genome substitution lines showed an increase of total root carbohydrates with the greatest increase in total root and aerial carbohydrates under infestation in the 1D and 6D substitution lines. Since these lines have been reported as being tolerant to greenbug, their highest carbohydrate contents probably protect them against biotic stress by enhancing growth. Greenbug resistance genes have been mapped only on the 1A, 6A, 7A and 7D chromosomes. Nonetheless, it was possible to identify other substitution lines that showed effects in the photosynthesis, the C and N metabolisms in the cascade of transductional signals that account for antixenosis, antibiosis and tolerance to greenbug in wheat

Keywords: greenbug, host plant resistance, non-structural carbohydrates – proteins, *Schizaphis graminum*, substitution lines, wheat

INTRODUCTION

Greenbug (*Schizaphis graminum* Rond) is one of the most economically important insect pests of wheat, It causes decreases in yield and even seedling death, depending on the intensity and the moment of infestation, In susceptible cultivars greenbug infestation diminishes total root biomass, and volume, thereby reducing the capacity for water and mineral uptake (Castro et al. 1988). After 4 days of infestation it leads to shorter leaves decreasing leaf area (Burton 1986, Castro et al. 1994) and reducing photosynthesis (Ryan et al. 1987). In susceptible genotypes of barley, oat and sorghum, greenbug inhibits differentiation of new leaf primordia and nodal roots after 48 h of infestation (see Castro et al. 2001). It has been claimed that these alterations in aerial and root growth were consequence of disturbances in nutrient uptake and transport, (Giménez et al. 1990), suggesting that greenbug induces systemic damage (Giménez et al. 1997). Accordingly, tolerant cultivars of barley and wheat showed no decrease in growth or production under aphid infestation (Castro et al. 1988, 2001). These previous results are consistent with those found in studies of plant responses to insect attack in the *Nicotiana attenuata-Manduca sexta* system (Hermsmeier et al. 2001). Changes in the metabolism of infested plants involved constitutive and induced defences, and these responses are based on very complex transcriptional regulation that include genes coding for both primary and secondary metabolites, wound- and jasmonate-elicited responses induced or repressed photosynthesis, carbon and nitrogen metabolisms (Kessler and Baldwin 2001, 2002).

The genetic control of the synthesis of reducing, non-reducing and total non-structural carbohydrates and soluble proteins in aerial and root tissues of wheat seedlings has been reported in a set of substitution lines (Clúa et al. 2002). The same set was used to study the chromosomal effects on the different mechanisms of greenbug resistance (Castro et al. 2001). These authors reported the Chinese Spring/Synthetic substitution lines that contributed the highest levels of antixenosis (5A and 6A), antibiosis (1A, 1B and homoeologous group 7 chromosome) and

tolerance (1D and 6D) to greenbug. However, to date aphid R genes in wheat have been located only on the 1A, 1D, 7D (McIntosh et al. 2003), 7A (Boyko et al. 2004) and 6A chromosomes (Castro et al. 2005). Since, the relationship between wheat chromosomes involved in the control of total biomass, contents of carbohydrates and proteins, and the plant responses to aphids has not been studied, the aim of this paper was to evaluate these plant traits as a first step towards the knowledge of the relationship between aphid resistance and these three metabolisms in the cascade of events that account for antixenosis, antibiosis and tolerance to greenbug. By restricting the variability to a single chromosome, it should be possible to isolate the effects of chromosomes carrying genes involved with photosynthesis, and with C and N metabolisms in the infested plants.

MATERIALS AND METHODS

Plant Material

A set of intervarietal chromosome substitution lines of wheat, *Triticum aestivum* L, (Law and Worland 1996) was used in the current research The lines 2A, 4A, 7A, 2B and 6B were not included because the molecular characterizations of these chromosomes were not correct "Chinese Spring" (CS) was the recipient variety into which chromosomes from a synthetic wheat (*T dicoccum* x *T tauschii* = Syn) were introduced. Two hundred seeds of each of the 16 substitution lines and of the parental lines were sown singly in 20 ml plastic vials perforated at the base, on a substrate of vermiculite. The vials were then placed in trays under natural conditions of light and temperature in a glasshouse, in La Plata, Argentina (34° 55′ SL, 57° 57′ WL). The trays were filled with nutrient solution (Hoagland and Arnon 1959) to enable a free supply of water and minerals and to maintain the volume constant along the experiments.

Source of Aphids

Greenbugs were collected from wheat plants in the vicinity of Tres Arroyos, Argentina (38° 20′ SL, 60° 15′ WL), and reared under controlled conditions. The clone aisolated from this population used in the trail was characterized as biotype C (Castro et al. 2004).

Assay Procedures

The responses of wheat plants to aphid infestation were evaluated when plants had reached the fully expanded 3rd leaf stage. Half of the plants of every genotype were transferred to another tray and infested during 72 h with 10 adult aphids per plant. The remaining uninfested plants were used as controls. Afterwards, aphids were removed, plants were harvested and divided into aerial and root tissues and the fresh weight of each portion was determined Four plants of every genotype

in each treatment were sampled together, representing one replicate. At least 20 replicates per genotype and treatment were analysed for protein (Bradford 1976) and carbohydrate contents (Cronin and Smith 1979). The remaining aerial and root biomass of the 18 genotypes was oven dried at 60°C until constant weight, and root (RDW) and aerial dry weights (ADW) were determined ANOVA was applied for all the parameters studied, and Duncan's test was used to determine significant differences between means (SAS 1998).

RESULTS AND DISCUSSION

There were few significant differences in RDW between control or infested plants of the parental varieties and the corresponding substitution lines. The infestation caused an increase in the RDW of both parental varieties and in most of the substitution lines. The 7B substitution line showed a significantly higher RDW than parental varieties either with or without infestation (Table 1) and the 5A and 6A substitution lines showed a significantly lower RDW compared to the parental controls and infested plants. The aerial dry weight (ADW) and the total dry weight (TDW) were reduced by greenbug feeding in most of the substitution lines, nonetheless, these differences were not significantly different, except for 5A and 6A substitution lines that showed significantly lower ADW and TDW

Table 1. Root (RDW), aerial (ADW), and total dry weights (TDW) of 16 wheat substitution lines and both parents (CS and Syn), subjected to aphid infestation and in uninfested control plants

Lines	RDW		ADW		TDW	
	Control	Infested	Control	Infested	Control	Infested
1A	508efgh	524defg	1254abcdefg	1314abcd	1762abcdefghi	1838abcde
3A	558bcdef	580bcde	1214bcdefgh	1224bcdefgh	1772abcdefgh	1804abcdefg
5A	**402ijk**	**434hij**	**960o**	**962°**	**1362mopq**	**1416mnop**
6A	**412ijk**	**458ghij**	**818op**	**824op**	**1230pqr**	**1272opqr**
1B	454ghij	486fghi	996 klmn	1040ijklmn	1450lmno	1526jklmn
3B	552bcdef	558bcdef	1218 bcdefgh	1134 fghijkl	1770abcdefgh	1692defghijk
4B	522defg	568bcdef	1282abcdef	1326abcd	1804abcdefgh	1894abc
5B	516defgh	536defg	1130fghijkl	1042 ijkllmn	1646efghijkl	1578hijklm
7B	**624ab**	**636a**	1200 cdefg	1220 bcdefgh	1824abcde	1904ab
1D	596abcd	624abc	960mno	994klmn	1556ijklmn	1618fghijklm
2D	500efgh	508efg	1010jklmn	960mno	1510jklmn	1487klmn
3D	592abcd	606abc	1106ghijmn	1006jklm	1698cdefghijk	1612fghijklm
4D	580bcde	634a	1226 bcdefgh	1194cdefghi	1806abcdefg	1848abcde
5D	525defg	562bcdef	1223 bcdefgh	1251 abcdefg	1748bcdefghi	1813abcdef
6D	572bcde	574bcde	1178defghi	1082hijklmn	1750abcdefghi	1656efghijkl
7D	550bcdef	616abc	1232 bcdefgh	1214 bcdefgh	1782abcdefgh	1830abcde
CS	512defgh	540cdef	1137fghijkl	1114ghijklm	1681deghijk	1665efghijkl
Syn	526defg	578bcde	1346abc	1336abcd	1872abcd	1914ab

Values in bold are significantly different ($P \geq 0.05$). Values within RDW, ADW or TDW column with a similar letter are not significantly different.

than CS either with or without infestation (Table 1). The rest of the substitution lines did not show differences from parental varieties or uninfested controls. The substitution lines that showed significantly lower ADW, RDW and TDW (5A, and 6A) compared to CS both with and without greenbug infestation, have been reported to contribute to antixenosis to greenbug and RWA infestation (Castro et al. 2001) and a new gene for greenbug antixenotic resistance has been mapped on the 6A (Castro et al. 2005). These chromosomes may carry genes for constitutive defences that affect photosynthesis yielding a poor plant biomass, which made these genotypes unattractive for aphid feeding, or induce repellency. Since antixenosis to aphids has been associated with secondary compounds, the lower plant weights could be the result of the costs devoted by these genotypes to constitutive synthesis of secondary plant metabolites (Martin et al. 2003), distracting resources from plant growth and production regardless of the presence of insects (Cipollini 2002). On the other hand, the 1A, 1D, and 7D substitution lines that are reported to carry aphid R genes (McIntosh et al. 2003), showed no lowering in plant biomass under greenbug infestation.

Protein content showed highly significant differences between the control and infested plants of the parental lines (Table 2). Protein contents significantly increased as a result of greenbug infestation in the 1A, 1B, 7B and 7D substitution lines compared to both parents and to their controls. The 7B line showed the highest value and another seven substitution lines (3A, 6A, 4B, 1D, 3D, 4D and 5D) showed a significantly lower protein content than their controls and CS under infestation. The remaining substitution lines did not show significant differences between control and infested plants The 1A, 1B, 7B and 7D substitution lines were previously reported to reduce greenbug and/or RWA fertilities and longevities (Castro et al. 2001). Increases in proteins have also been found in microbe infections (Martin et al. 2003) and after *Manduca sexta* attack due to ethylene, jasmonic acid and salicylic acid elicited a significant number of genes involved with N metabolism under the stress of *Manduca sexta* attack (Hui et al. 2003). Therefore the increases in protein content registered on 1A, 1B, 7B and 7D substitution lines could be then consequence of an enhanced protein synthesis necessary to support high enzymatic requirements due to the over-expression of genes in the downstream chain of events of plant defence that implies a greater metabolic activity under stress. The R genes carried by chromosome 7D (Castro et al. 2004) may interact with aphid elicitors to induce a cascade of transductional events involved with the higher protein synthesis that affect greenbug and RWA life cycles and fertilities (Castro et al. 2001). The remaining substitution lines (1A, 1B, 7B) have genes involved with N metabolism (McIntosh et al. 2003) that in the absence of aphid-specific R genes (located on Synthetic 7D), could induce proteins related with indirect defences (ie: defence proteins, peroxidases) that also affected aphid performance. Moreover, 121 candidate R-genes have been physically located with most mapping on the 1A, 1B and 7B chromosomes (Dilbirligi and Gill 2003, Dilbirligi et al. 2004). Further characterization is necessary to find out the relationship between these R-gene candidates, N metabolism and the antibiosis to greenbug.

Table 2. Protein content of aerial biomass, reduced and non reduced carbohydrates in the aerial and root biomass of 16 wheat substitution lines and both parents (CS and Syn), subjected to aphid infestation and in uninfested control plants

Lines	Protein		Aerial carbohydrates				Root Carbohydrates			
			Reduced		Non Reduced		Reduced		Non Reduced	
	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested
1A	2469f	**2966cd**	1275qrst	62BC	4636uv	3283z	987GH	1175CD	3942yz	3549B
3A	2218hi	983v	1241rstu	936xy	6471q	8666i	1329yz	1268zAB	4516w	5069t
5A	2376gh	2255hi	2561a	2243cd	9828d	6689pq	1367xy	772 K	4614uv	3199C
6A	2936cd	1605op	**1648kl**	**2326b**	7941kl	8029k	1296z	2045 i	3981y	2574D
1B	1573pq	**2791de**	2023gh	1217stu	6459q	5387t	1275zAB	1151CE	5207r	7253h
3B	1685no	1708no	1324opqr	675BC	2947B	2468D	1857k	2229g	3908z	6563lm
4B	1890lm	1539pqr	1863ij	1589lm	2652C	3145A	1778n	2084h	5822o	7989c
5B	1623op	2035jk	1402no	816zA	3627x	3074AB	1619opq	173 o	**6518m**	**9185b**
7B	1875l	**3531a**	1209tuv	104x	2208E	1495F	1079EF	614 L	2713C	2389E
1D	3008bc	2221hi	**2354ef**	**255a**	**6528q**	**11771b**	**1985 j**	**4114a**	5516q	7501ef
2D	1485qrs	1453rs	1118wx	731AB	3394z	3004B	879 J	2363e	4401x	7856cd
3D	1388st	542x	2089fg	1342nopq	9132f	7201no	1245B	2684c	5674p	7178i
4D	2092 jk	1741mn	1124vwx	1297pqrs	4322w	2518D	1559rs	2328f	5109st	7617e
5D	1946kl	1234tu	2001h	155m	9955c	6124r	**128**[a]	**2728b**	5636pq	7813d
6D	1554pqr	1473qr	**2142ef**	**2373b**	**8961g**	**9377e**	1782mn	2458d	**6747k**	**9607a**
7D	2085 jk	**3149b**	**1947hi**	**2132ef**	3483y	4782u	1388uw	1793 m	552q	7423fg
CS	1942kl	1978kl	1716jk	1417mn	6526q	5936rs	1403tu	1841 ll	5119rs	6212n
Syn	2921cd	1716no	1055x	653BC	3779x	3243z	934 I	918 I	2615CD	2058F

Values in bold are significantly different ($P \geq 0.05$). Values within a trait category with a similar letter are not significantly different.

The parental lines showed significant differences in the reducing carbohydrates (RC) of the aerial tissues, with a significantly lower value for Syn (Table 2). Only infested plants of the 6A, 1D, 6D and 7D substitution lines showed a significantly higher RC than their controls and also than CS. The contents of non reducing carbohydrates (NRC) showed significant differences between control and infested plants of both parental lines, CS showing the highest value (Table 2). The greatest values of NRC in the infested plants were recorded for the 6D and 1D substitution lines with the latter showing the highest significant increases, consequently these substitution lines also showed the highest total aerial carbohydrates. There are significant differences in reducing carbohydrates in roots between control and infested plants of both parents RC values were significantly increased in CS and in most of the substitution lines Infested plants of the 1D and 5D lines showed the highest contents compared to CS and to their controls. There were significantly greater differences in non-reducing carbohydrates of the roots in control and infested plants of both parental lines, and CS showed the highest values (Table 2). Infestation significantly increased the NRC in CS and in most of the lines, with the highest values for 5B and 6D The total carbohydrates resulted significantly higher in 1D and 6D. Greenbug infestation was reported to produce a reduction in carbohydrates either due to ingestion or to chloroplast membrane injury (Al-Mousawi et al. 1983). Carbohydrate contents play an important role in the osmoregulation and osmoprotection of winter cereals. The current results show that the contents of different categories of carbohydrates were significantly different in several substitution lines subjected to aphid infestation. Most of the B and D genome substitution lines showed a significant increase in root carbohydrates but only 1D and 6D showed the highest increases in both the root and aerial parts under infestation. These substitution lines have been previously reported as tolerant to greenbug and RWA infestation (Castro et al. 2001). Nonetheless, only chromosome 1D has been reported to carry aphid R genes (McIntosh et al. 2003) Possibly on chromosome 6D there are genes that cope with an increase in C metabolism. The metabolism of C is known to be one of the most reconfigurated under infestation (Ryan et al. 1987, Behle et al. 1994, Kessler and Baldwin 2002, Hui et al. 2003). The responses that could minimize the fitness consequences of insect attack represent the tolerance responses, a largely unstudied mechanism of defence (Cipollini 2002). In the current research, the 1D and 6D lines showed significant increases in total carbohydrates of aerial and root parts, which could be related to an improved performance for nutrient uptake under stress. Nevertheless further research should be performed in order to understand the complex regulation of the tolerance to greenbug.

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