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# Tolerance to Stress in Wheat

**Ana María Castro[1,2*] • Daniel Gimenez[1,2] • Erica Tocho[1,2] • María Tacaliti[1] • Mariana Barragán[1] • Rubén Bottini[3] • John Snape[4]**

[1] Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, CC31, 1900-La Plata, Argentina
[2] Instituto de Fisiología Vegetal, CCT- La Plata, Consejo Nacional de Investigaciones Científicas y Tecnológicas, CC31, 1900-La Plata Argentina
[3] Facultad de Agronomía, Universidad Nacional de Cuyo, Almirante Brown 500, M5528AHB-Chacras de Coria, Argentina
[4] John Innes Centre, Colney, Norwich NR4 7UH, UK

*Corresponding author*: * **amcastro@isis.unlp.edu.ar**

## ABSTRACT

Achieving tolerance to stress is one of the main objectives of wheat breeding, and genes or chromosomal regions with positive effects on tolerance to biotic and abiotic stresses need to be identified. The interaction between defence signaling pathways mediated by several phythormones is an important mechanism for regulating defence responses against various types of pathogens and herbivories. The response of bread wheat, *Triticum aestivum* (2n=6x=42) to greenbug attack or to exogenous application of the stress-induced hormones ethylene (E), jasmonic acid (JA), salicylic acid (SA) or ABA was analysed. In recent years, several components regulating the cross-talk between SA, JA and ET pathways have been identified. Treatment of plants with these hormones results in enhanced resistance to biotic challenge. However, the underlying physiological mechanisms are not well understood. Some of the main wheat physiological pathways affected by the cross-talk between biotic stress and stress-induced hormones are described below.

**Keywords:** ABA, aerial and root carbohydrates, aphids, ethylene, jasmonic acid, protein content, salicylic acid

## CONTENTS



## INTRODUCTION

Great progress has been achieved in enhancing the yield of hexaploid bread wheat (*Triticum aestivum*, 2n= *6x*= 42), as well as in improving end use quality and adaptation, obtaining varieties adjusted to diverse environments that show tolerance to pests, diseases, drought and salinity. The genetic control of many of these characters has been reviewed by Worland and Snape (2001).

Annual wheat production worldwide varies due to environmental problems, diseases and pests. Many stress-related genes and responsive elements have been cloned (Boyko *et al.* 2002; Cattivelli *et al.* 2002; Baek *et al.* 2006). The expression of some stress-related genes was reported to be linked to stress-tolerance QTLs, suggesting that these genes may represent the molecular basis of stress-tolerance. From the breeding point of view, stress tolerance can be described as the ability to maintain a constantly high performance (yield), regardless of any biotic or abiotic stresses imposed. The identification of the genetic components of stress tolerance is, therefore, a requirement to ensure further breeding progress (Cattivelli *et al.* 2002). Advances in the genetic and molecular understanding of stress responses have led to the identification of a number of single loci, QTLs, and genes related to stress tolerance in wheat (Laurie *et al.* 1994; Quarrie *et al.* 1994; Snape *et al.* 1996, 1997; Castro *et al.* 2008).

The genetic basis of the adaptation of wheat to environments has been reviewed by Cattivelli *et al.* (2002). Genes for tolerance to biotic stresses have been located on several chromosomes. Chromosome 3A of *T. dicoccoides* has a QTL providing resistance against one of the most important wheat disease, *Fussarium* head blight, two resistance genes analogs (RGA) map at 15 cM of the QTL peak (Otto *et al.* 2002). Several other QTLs have been located on chromosomes 2A, 3B and 6B. Chromosome 7D has been reported to carry a resistance gene to *Stagnospora nodorum* and a resistance gene against *Mycosphaerella graminis* (Arraiano *et al.* 2001), several genes conferring resistance against *Diuraphis noxia* (Castro *et al.* 1999; Liu 2001, 2002), resistance genes against greenbug (Castro *et al.* 1999). Most RGA and defense-related loci have been found on the high-density map of the *Aegilops tauschii* genome (Boyko *et al.* 2002).

Resistance against the main aphid pests in wheat (greenbug and Russian wheat aphid) have been located on three chromosomes: greenbug resistance genes *Gb2* (Tyller *et al.* 1987) and *Gb6* were identified as being on the wheat/rye translocation chromosome, 1RS/1AL (Porter *et al.* 1994); the gene *Gb3* was mapped on chromosome 7D (Hollenhorst and Joppa 1983) and recently located on 7DL linked to *Xgwm111* and *Xgwm428* (Weng and Lazar 2002). RWA resistance genes (*Dn1*, *Dn2*, and *Dn6*) are tightly linked to each other on 7DS, and other genes, *Dn5* (Heyns

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Invited Review

*et al.* 2006) and *Dn8,* on 7DL (Liu *et al.* 2001, 2002). Two other genes, *Dn4* and *Dn9*, were identified on chromosome 1D (located on 1DS and 1DL, respectively), (Liu *et al.* 2001, 2002). Antibiosis against greenbug and RWA was explained by two or three QTLs located either on 7DS or 7DL (Castro *et al.* 2004). Antixenosis to biotype I of RWA was associated with marker loci *Xpsr687* (7DS) and *Xgwm437* (7DL, Castro *et al.* 2004), and to biotype II with *Xgwm1293* and *Xgwm1150* on 6AL (Castro *et al.* 2005a). Antixenosis to greenbug was associated with *Xgwm1009* and *Xgwm1185* on the 6A centromere region (Castro *et al.* 2005a).

The application of cloning techniques to the analysis of cereal stress responses has led to the isolation of a large number of genes whose expression is affected by stresses. The analysis of stress-related gene sequences from a number of evolutionary distant plants reveals that the molecular response to stress is conserved (Cattivelli *et al.* 2002). Nonetheless, the precise function of many stress-related genes still remains unclear, although their expression patterns suggest a connection between their activity and stress tolerance. In *T. tauschii*, most of resistant genes and defence-response genes are organized into clusters found in the distal/telomeric regions of 1D, including genes for resistance against leaf rust, stem rust and powdery mildew. 2D that had the most of the RGA–41- and defence-response genes, 3D, carries 27 defence- related loci, 4D has16 loci mapped, 5D with the fewest number (e.g. 14 loci), 6D with 20 loci, and 7D with 18 loci (Boyko *et al.* 2002). Nonetheless, most of these RGA loci in the genetic map do not correspond to any known resistance genes. These authors suggested that these sites might possibly be the location of still unidentified or unmapped resistance genes (Boyko *et al.* 2002). The rate of evolution found for RGA is very high, since they are located in regions know to recombine highly, which suggests a rapid co-evolutionary race against rapidly evolving pathogen populations (Boyko *et al.* 2002; Bari and Jones 2008).

## APHIDS AND STRESS-INDUCED HORMONES

Greenbug is one of the most important pests of wheat and barley, the aphid damages the leaf surface (Al-Mousawi *et al.* 1983) inducing lower photosynthesis rates in susceptible wheat plants after 5 days of infestation (Ryan *et al.* 1987). Nonetheless, aphid systemic effects have been found after 3 h of infestation, when the phosphate transport from root to the aerial parts decreases significantly in infested susceptible barley seedlings (Giménez *et al.* 1997). The mitotic index of apical and root meristems from tolerant and susceptible wheats were evaluated in greenbug infested and control plants (Tacaliti *et al.* 2002). The infested plants showed mitotic indexes higher than the controls for the tolerant cultivar.

A number of hormonal signals are enhanced in response to different biotic and abiotic stresses, therefore modulating expression of some genes and influencing different physiological plant responses. Ethylene (E) is a volatile plant hormone derived from methionine involved in numerous physiological processes such as seed germination, root and shoot growth, flower development, senescence and abscission of flowers and leaves, and ripening of fruit (Kende 1993). Ethylene also participates in the modulation of plant responses to a range of biotic and abiotic stresses and it is also involved in systemic acquired resistance (SAR) (Sticher *et al*. 1997; Vallad and Goodman 2004; Bari and Jones 2008; Kuppusamy *et al.* 2009). It has been reported that greenbug infestation induced a significant increase in plant E production (Castro *et al.* 1996b) either in susceptible or tolerant wheat, barley and oat cultivars. Although infestation induced similar E emissions in susceptible and tolerant cultivars, only tolerant plants maintained a higher growth rate when subjected to infestation and to increased doses of exogenous E (Castro *et al.* 1996a). Several wheat substitution lines showed tolerance to exogenous application of ABA (absisic acid), E, jasmonic acid (JA) and salicylic acid (SA) at the coleoptile stage (Castro *et al.* 2003).

Several lines of evidence indicate that JA is a primary regulator of vegetative storage proteins in soybean that may have some functions in plant defense (Mason and Mullet 1990; Staswick 1990; Anderson 1991). JA is the most potent plant growth regulator, acting as signal for various developmentally and environmentally induced changes in gene expression (Creelman and Mullet 1997; Baldwin *et al.* 1998). The role of JA in response to biotic stress from insects and pathogens, have been well documented (Cipollini and Bergelson 2001; Bari and Jones 2008; Vallad and Goodman 2004). JA leads to the synthesis of proteinase inhibitors, one of the main wound-induced phytochemicals, an expression of induced resistance (IR) (Vallad and Goodman 2004). Other authors described a dual role for JA in plant development and defense, inducing proteinase inhibitors and attracting insects' parasitoids (Creelman and Mullet 1997).

Evidence has been reported to show that SA plays a key role in triggering plant defense gene expression. Exogenous SA significantly affects resistance to pathogens and drives accumulation of pathogenesis-related proteins (White 1979; Enyedi *et al.* 1992). These observations have subsequently been confirmed in other systems and extended to various fungal and bacterial pathogens (Schneider *et al.* 1996; Cipollini 2002; Vallad and Goodman 2004).

ABA is an essential constituent of higher plants. Concentrations of ABA increase in response to stress, particularly drought stress. Many changes in physiology, morphology and development result from high ABA concentrations (Hall and McWha 1981; Quarrie 1982). ABA enhanced activation of an osmotin gene in tobacco (Raghothama *et al.* 1997) and induced gene expression in other plant species like rice (Mundy and Chua 1988).

There are few reports of the relationship between these stress-induced hormonal signals and main plant metabolites in terms of plant performance (Castro *et al.* 1996a; Giménez *et al.* 1997; Baldwing *et al.* 1998; Cipollini 2002; Castro *et al.* 2004). The genetic control of the synthesis of reduced, non-reduced and total non-structural carbohydrates and soluble proteins in aerial and rooting structures of wheat has not been analyzed under the effects of stress-induced hormones in terms of the concentrations of these essential metabolites in wheat, neither have aphid effects been related to stress-induced hormones (Castro *et al.* 2005b). The experimental application of defense hormones enables the phenotypic manipulation of defense in plants with the same genetic background, without the confounding effect of physical damage produced by aphids (Redman *et al.* 2001). In order to analyze the relationship between biotic stress and hormones, we identified the chromosome location of genes controlling plant defense promoted by stress-induced hormone treatments and greenbug infestation in terms of plant growth. The relationship between constitutive or induced plant defense genes and the contents of proteins and carbohydrates is discussed. The relationship of these hormone treatments with aphid resistance in wheat substitution lines between a greenbug and RWA susceptible cultivar ('Chinese Spring' (CS)) and a resistance donor ('Synthetic' (Syn)) is described in the current research.

## VARIATION IN PLANT GROWTH

Growth rate measured as foliar area showed significant differences between control and treated plants (**Table 1**). CS plants were significantly stunted by aphid infestation and hormone treatments, with the lowest rates under aphid infestation, E and SA. The leaf area in the 5B line was significantly inhibited by all treatments, while the aphid resistant parent Syn grew significantly less only when subjected to SA and ABA treatments. The 7D substitution line, under all treatments, showed an enhanced growth rate as compared to controls (**Table 1**). Similarly, the 6A and 4D substitution lines showed an over-expression of growth when infested, and after E and JA treatments. The rest of the treat-

**Table 1** Foliar area rate of 16 wheat substitution lines and both parents (CS and Syn)[a] subjected to 72 hrs of aphid infestation, hormone treatments and in control plants. (**Appendix 1**, unpublished data).

| Lines | Controls | Infested | Ethylene | Jasmonic acid | Salicylic acid | Aba |
|---|---|---|---|---|---|---|
| 3D | 115.0ab* | 95.7de | 96.4d | 113.0ab | 114.8ab | 114.4ab |
| 7B | 114.2ab | 105.8bc | 105.2bc | 107.6bc | 112.0ab | 111.3b |
| Syn | 112.1ab | 105.7bc | 106.3bc | 104.4bc | 92.4efgh | 100.6cd |
| 5D | 107.0bc | 97.9d | 92.3efgh | 102.6c | 96.4d | 96.8d |
| 1B | 106.9bc | 90.7efghi | 89.8fghi | 101.1cd | 87.5hijk | 83.7jkl |
| CS | 105.8bc | 72.7mnopq | 70.4nopq | 94.0ef | 79.3klmno | 90.4fghi |
| 1A | 101.7cd | 89.2fghij | 90.3efghi | 96.0d | 84.2ijkl | 85.8ijk |
| 7D | 93.6efg | 117.9a | 117.0a | 115.2ab | 116.0a | 118.6a |
| 5B | 80.7jklmn | 51.2wxyz | 56.8uvwx | 62.3rst | 57.1uvwx | 56.4stuvwx |
| 3B | 75.0lmnop | 73.0mnopq | 70.0nopq | 75.7lmnop | 74.6lmnopq | 74.1lmnopq |
| 6A | 68.7opqrs | 85.8ijk | 87.5hijk | 84.3ijkl | 66.2pqrst | 66.7pqrst |
| 1D | 66.1pqrs | 57.1tuvwx | 56.0uvwx | 69.5opqr | 70.8nopq | 70.9nopq |
| 4B | 61.3rstuv | 71.2mnopq | 70.4nopq | 66.6lpqrs | 67.0pqrst | 67.1pqrst |
| 2D | 55.5 uvwx | 45.7yzA | 46.0yzA | 66.5pqrst | 47.9xyzA | 48.6xyz |
| 3A | 48.0xyz | 47.3xyz | 48.1xyz | 51.0wxyz | 51.4wxyz | 51.1wxyz |
| 5A | 44.0yzA | 42.0zA | 42.3zA | 37.5AB | 41.6zA | 41.4zA |
| 4D | 41.8zA | 52.9vwxy | 51.6wxy | 51.3wxy | 28.1B | 27.6B |
| 6D | 41.4zA | 47.9xyz | 46.4yzA | 42.5zA | 39.8[a] | 45.0yzA |

[a]CS = Chinese Spring
Syn = Synthetic
* Values that share the same letters are not significant different ($P \geq 0.05$).

ments did not affect the 6A line growth rate, although these significantly inhibited the 4D line leaf area growth. The 4B substitution line showed a higher growth rate subjected to infestation and E treatments, like the 2D substitution line under the JA treatment; the other treatments did not affect leaf area growth in the 4B and 2D lines, but infestation and E significantly reduced the 2D line growth (**Table 1**).

Five substitution lines (3A, 5A, 3B, 7B and 6D) subjected to every treatment showed no significant differences with the control plants. Conversely, growth was significantly reduced, except under JA treatment, in another three substitution lines (1A, 1B and 5D). Finally, infestation and E significantly reduced leaf area in the 1D and 3D substitution lines.

There were no significant differences in aerial, root and total dry weights and in the ratio ADW: RDW between control and hormone treated plants of the parental varieties and the substitution lines. Similar results were previously reported (Clua *et al.* 2002).

There were nine substitution lines that showed tolerance to greenbug, and to one or more stress-induced hormones, (3A, 5A, 6A, 3B, 4B, 7B, 4D, 6D, 7D), in terms of plant growth, showing similar or higher aerial biomass than their controls. These lines and also the 2D line had a significant tolerance to JA, which might regulate the wound-inducible expression of defense genes (McConns *et al.* 1997). Comparing the current results with those previously reported (Castro *et al.* 2003), different genetic systems appeared to be involved in tolerance to stress-induced hormones at the coleoptile and at the 3rd leaf stages, due to the different responses of the same substitution lines subjected to similar treatments at two life stages. These results are in agreement with those found in barley, where two QTLs sets were found to control salt tolerance at germination (chromosomes 1H, 4H, 5H and 6H) and at the seedling stage (chromosomes 1H, 2H, 5H and 6H). These QTLs do not overlap, suggesting different genetic mechanisms of salt tolerance at different plant growth stages (Mano and Takeda 1997). Only substitution lines 6A and 7B showed tolerance to one or two hormones at the coleoptile stage, and to most of the treatments at an advance growth stage; nonetheless the 7B line was not tolerant to aphid feeding at the coleptile stage (Castro *et al.* 2003).

Growth rate showed no differences between control plants, JA treated and greenbug infested plants for the 7B substitution lines. The 1A and 1B substitution lines showed a normal growth rate after JA treatment, but 5B did not maintain a noticeable growth rate without treatment. Moreover, the 6A substitution line had an enhanced growth after greenbug infestation, ethylene and JA treatments. In agreement with current results, recently it has been found that the 6A chromosome carries genes for response to JA, E and ABA (Castro *et al.* 2008). *Arabidopsis* transgenic plants, carrying the SAR8.2 gene that is induced locally or systemically by biotic and abiotic stresses, grew faster with an enhanced rate and showed an over-expression of resistance against several pathogens, drought and salinity (Lee and Hwang 2006). These authors suggest that the SAR8.2 gene may function in the hormone-related signaling pathways. The over-expression of this gene leads to a greater amount of PR proteins that could act as positive regulators of plant defenses and activate the downstream defense related genes (Lee *et al.* 2004).

## VARIATION IN PROTEIN CONTENTS

Protein contents showed significant differences between control parental lines (**Table 2**), with the highest values for Syn. The protein contents were not significantly affected by hormone treatments in either parent, except in the resistant parent Syn that was significantly reduced by the SA treatment. The highest protein values for control plants significantly different from CS were for the 6A and 1D substitution lines.

Infestation induced a significant increase in protein contents in the 1B, 7B and 7D substitution lines compared to their controls (**Table 2**). Aphid feeding provoked a significant protein decrease in Syn and in four substitution lines (3A, 6A, 1D, 3D), compared to their controls.

Different substitution lines responded differentially to the hormone treatment. Ethylene treatment did not affect the protein contents except in the 1A and 7D substitution line that showed a significantly lower value compared to their controls (**Table 2**). JA treatment induced a significant increase in protein contents in the 1A, 5B, and 7B substitution lines, and a significant decrease in the 1D line, compared to their controls (**Table 2**). The SA treatment produced a significant accumulation of proteins in the 3B, 2D and 6D lines. Conversely, contents were reduced by SA in the 1A substitution line (**Table 2**). Finally, ABA treated plants of the 1A, 5A, 1B and 7D substitution lines showed significantly higher protein contents.

Several infested and treated substitution lines showed similar protein contents to their controls (4B, 2D, 4D and 5D). Protein contents of the substitution lines that showed greenbug tolerance (3A, 5A, 6A, 3B, 4B, 7B, 4D, 6D, 7D) were reduced by aphid feeding in the 3A and 6A lines, and increased in the 7B and 7D lines. The protein contents were

**Table 2** Protein contents of aerial biomass of 16 wheat substitution lines and both parents (CS and Syn)[a], subjected to 72 hrs of aphid infestation or hormone treatments and in control plants. (**Appendix 1**, unpublished data).

| Lines | Controls | Infested | Ethylene | Jasmonic acid | Salicylic acid | ABA |
|---|---|---|---|---|---|---|
| 1A | 24.69defghijk* | 29.66abcdef | 13.26nop | 35.25ab | 10.36op | 35.94a |
| 3A | 22.18fghijkl | 9.83pq | 24.35defghijk | 23.03efgijkl | 23.67efghijkl | 24.11defghijk |
| 5A | 23.76efghijkl | 22.55fghijkl | 21.73ghijklm | 28.65bcdefg | 15.64lmnop | 31.7abcd |
| 6A | 29.36abcdef | 16.05lmnop | 18.15ijklmno | 31.47abcd | 23.34efghijkl | 20.49hijklmn |
| 1B | 15.73 lmnop | 27.91bcdefg | 12.32nop | 23.55efghijkl | 10.33op | 34.91abc |
| 3B | 16.85 lmnop | 17.08lmnop | 12.15nop | 18.96jklmno | 27.33cdefgh | 15.68lmnop |
| 4B | 18.92ijklmno | 15.39lmnop | 10.54opq | 22.66fghijkl | 12.45nop | 14.05mnop |
| 5B | 16.23lmnop | 20.35hijklmn | 10.41opq | 26.03cdefghi | 11.63opq | 23.73efghijkl |
| 7B | 18.75ijklmno | 35.31ab | 13.54nopq | 36.84a | 11.45opq | 17.36klmnop |
| 1D | 30.08abcde | 22.21fghijkl | 25.44defghij | 21.81fghijklm | 28.36bcdefg | 35.99a |
| 2D | 14.85mnop | 14.53mnop | 23.15efghijkl | 20.53hijklmn | 22.52fghijkl | 15.17lmnop |
| 3D | 13.88nop | 5.42q | 10.26opq | 8.06pq | 9.43pq | 8.32pqq |
| 4D | 20.92hijklmn | 17.41lmnop | 21.78ghijklm | 15.48lmnop | 23.45efghijkl | 16.84klmnop |
| 5D | 19.46hijklmno | 12.34nop | 26.16cdefghi | 12.33nop | 25.33defghij | 15.65lmnop |
| 6D | 15.54lmnop | 14.73mnop | 19.46hijklmno | 11.39opq | 28.35bcdefg | 14.73lmnop |
| 7D | 20.85hijklmn | 31.49abcd | 11.54opq | 20.21hijklmn | 12.16nop | 29.06abcdef |
| CS | 19.87hijklmno | 19.78hijklmno | 17.61klmnop | 17.54 klmnop | 15.51lmnop | 19.11ijklmno |
| Syn | 29.21abcdef | 17.16lmnop | 21.81fghijklm | 23.40efghijkl | 18.6jklmno | 22.48fghijkl |

[a]CS = Chinese Spring. Syn = Synthetic

* Different letters within a column indicate significant differences according to Duncan's multiple range test ($P < 0.05$ )

not affected in the remaining aphid tolerant lines. Ethylene applied as ethephon showed its activity by differential induction of acquired resistance and pathogenesis related protein gene expression in tobacco (Brederode *et al.* 1991). Moreover, previous reports had demonstrated an E-responsive LEA (late embryo-abundant) like protein in response to drought in tomato (Zegzouti *et al.* 1997), and pathogenesis related protein genes that have been induced by E (Xu *et al.* 1994). In this work the exogenous application of E significantly increased protein content only in the 2D substitution line. These results would indicate that this line has an activity to protect the plants against environmental stresses such as drought, and against disease or pest attacks. The higher protein content could explain the lack of differences in the foliar area growth rate between greenbug infested and ethylene treated plants of the 2D substitution line.

Jasmonates play an important role in plant insect and disease resistance, since JA activates genes encoding protease inhibitors that could protect plants from insect damage (Dunaevsky *et al.* 2005), or JA promotes the expression of genes encoding antifungal proteins such as thionin (Becker and Apel 1992), osmotina (Xu *et al.* 1994), PDF (Penninckx *et al.* 1996), the ribosome-inactivating protein RIP60 (Chaudhry *et al.* 1994). Jasmonates also modulate the synthesis of infection barriers such as PRP cell wall proteins (Creelman *et al.* 1992). In this context, the 1A, 5A, 6A, 1B, 5B, and 7B substitution lines showed the highest values due to a significant change in protein contents, or did not show differences with their controls after JA treatment. The 1B, 7B and 7D substitution lines showed a significant increase in protein content after greenbug infestation, and these substitutions have also been reported to reduce greenbug and RWA fertilities (Castro *et al*. 2001, 2003).

Salicilic acid and SA-glucosides are immediately synthesized after pathogens infection, and these metabolites have been reported to induce PRs and *Tobacco mosaic virus* resistance in tobacco. This is consistent with the central role that SA occupies in the induction of systemic acquired resistance genes (Malamy *et al.* 1996; Vallad and Goodman 2004). Eleven substitution lines (3A, 5A, 6A, 3B, 4B, 7B, 1D, 2D, 3D, 6D, 7D) showed tolerance to SA treatment by means of growth rate, and simultaneously were tolerant to greenbug, except for the 1D and 3D lines. The 3B, 2D and 6D substitution lines showed higher protein values after SA treatment, and these lines did not show differences in protein contents after greenbug infestation. These substitution lines could have a set of genes encoding pathogenesis-related (PR) proteins associated with a large number of viral, fungal and bacterial pathogens that are responsive to SA signals. No report has previously related SA tolerance and insect resistance, but our current results show that substitution lines 3B and 6D, with higher protein contents after SA treatment, simultaneously showed a similar growth rate as control plants, and after greenbug infestation. SA treatment did not affect the protein contents in the other eight substitution lines compared with their controls.

ABA plays a key role in stress-tolerance promoted by temperatures (heat and cool), drought and salinity, and this role is based on the synthesis of metabolites that are osmotically active, such as proteins or carbohydrates, for avoiding water loss. Under various stress conditions, including osmotic or salinity stress, several mRNAs and proteins are accumulated. These that can be divided into a first set of mRNAa/proteins that are inducible by exposure to both stress and ABA. A second set are inducible only by exposure to stress, and finally a set that are only inducible by ABA but not by the stress imposed (Luo *et al.* 1992). The *Em* gene encodes a hydrophilic protein of the LEA (late-embryo abundant) class that is one of the most abundant proteins in embryos of cereal grains such as wheat. Levels of *Em* transcripts normally increase during maturation of wheat and maize embryos, but it is also expressed in osmotically stressed vegetative tissue (Bostock and Quatrano 1992). Current results showed that substitution line 7D was tolerant to ABA treatment with an enhanced growth. Another 10 substitution lines did not show differences between ABA treated plants and controls (3A, 5A, 6A, 3B, 4B, 7B, 1D, 2D, 3D, 6D). ABA treatment induced higher protein contents in four lines (1A, 5A, 1B, 7D). These results could indicate that the activity of any of these genes encode stress-induced proteins in wheat. The current results are in agreement with those previously reported, where a major QTL affecting drought-induced ABA accumulation was mapped on the long arm of chromosome 5A, close to the locus controlling frost resistance and tightly linked to the dehidryn locus, suggesting a genetic linkage between ABA accumulation and stress-tolerance (Quarrie *et al.* 1994). Moreover, a gene conferring tolerance to environmental stresses, located on the long arm of the homoeologus group 2 chromosomes, has been sequenced (Baek *et al.* 2006), and having an ABA responsive element in the promoter. Chromosome 7D has also been reported to promote positive effects on water use efficiency (Gorny 1999). Likewise, 7D has been reported to carry a resistance gene to *Stagnospora nodorum* and a resistance gene against *Mycosphaerella graminis* (Arraiano *et al.* 2001), several genes of resistance to Russian wheat aphid (Liu *et al.* 2001, 2002; Castro *et al.* 2004), *Gb3* (Weng and Lazar 2002), and two QTLs conferring resistance to greenbug (Castro *et al.* 2004).

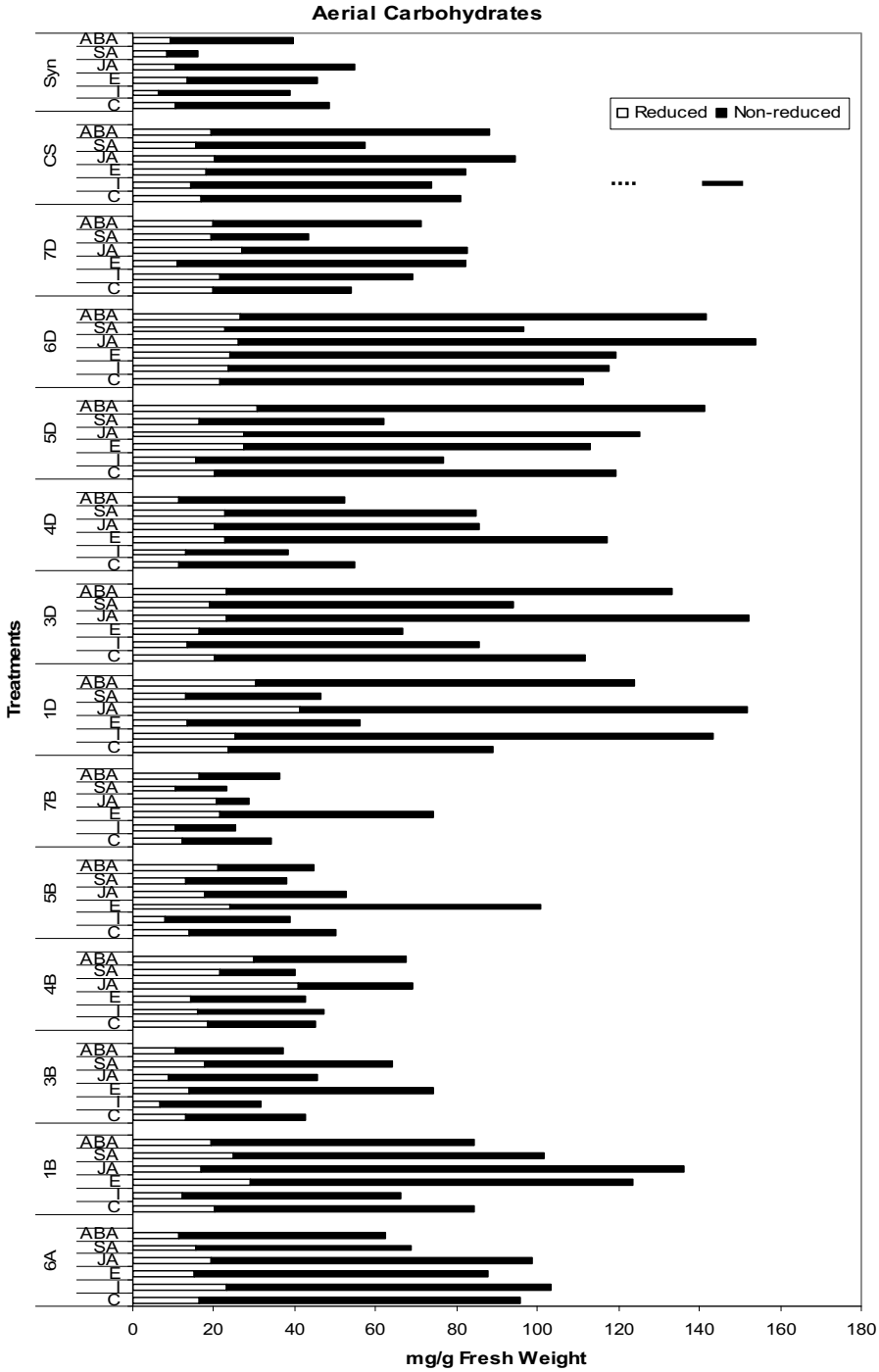


**Fig. 1 Reduced, non reduced and total carbohydrates in the aerial biomass of 12 wheat substitution lines and both parents (CS and Syn), controls (C), plants subjected to aphid infestation (I), or exogenously treated with ethylene (E), jasmonic acid (JA), salicylic acid (SA) and ABA.** The horizontal bars represent the Standard Errors for reduced (□) and non reduced (■) carbohydrates (**Appendix 1** unpublished data).

## VARIATION IN NON-STRUCTURAL CARBOHYDRATES

Significant differences were found in the contents of non-reduced and total aerial carbohydrates between the parental lines, and these differences remained under the infestation and the hormone treatments (**Fig. 1**), with CS having the highest values. Conversely, the parental lines did not differ in the contents of reduced carbohydrates except when subjected to JA and ABA treatments.

Infestation induced a significantly higher content of reduced carbohydrates in the 6A line and in non-reduced and total carbohydrates of 1D plants, with significantly lower values in the 5A, 1B, 3D, 4D and 5D substitutions (**Fig. 1**).

Sugar metabolites were not significantly increased by hormone treatments in the A-genome substitution lines. Most of the substitution lines of the B genome subjected to the hormone treatments showed significantly higher contents in the three metabolities in aerial tissues, or did not show differences compared to their controls.

Ethylene significantly diminished the different sugar metabolites in the 3A, 5A, 1D and 3D lines. Conversely, E significantly increased the sugar contents in the 1B, 3B, 5B, 7B, 4D and 7D lines.

JA treatment induced a significant increase in the sugar contents in lines 1B, 4B, 7B and in every D-genome substitution line, except for 2D and 5D. Salicilic acid affected the sugar contents, reducing (in 6A, 1D and 5D) or increasing them (in 1B, 3B and 4D) (**Fig. 1**). Finally, ABA treatments induced significantly lower carbohydrates in aerial tissues in the 6A line and a significantly increase in the 4B, 1D, 3D, 5D and 6D lines.

There were no significant differences in the root re-

duced carbohydrates between control, JA-, SA-, and ABA-treated plants of CS and Syn (**Fig. 2**). Conversely, the parental lines showed significantly different reduced carbohydrates under infestation and the E treatment. Infestation significantly increased the non reduced and total carbohydrates in Syn. Non-reduced and total carbohydrates in the root of control, JA and ABA treated plants of the parental lines were significantly different, with CS having the highest contents (**Fig. 2**).

Infestation produced a significant increase of reduced carbohydrates in roots of the D genome substitution lines, except for 6D and 7D, when compared to their controls (**Fig. 2**). Ethylene treatment induced a significant increase of reduced carbohydrates in the root in Syn and in the 5A substitution line, and significant lower contents in five lines (4B, 1D, 4D, 6D, 7D), comparing to their controls and parental lines. JA treatment conditioned significantly higher reduced carbohydrates in the 4B, 1D, 2D and 5D substitution lines. The SA treatment significantly lowered root reduced carbohydrates in the 3B, 4B, 1D, 4D and 7D substitution lines. ABA treated plants showed significantly higher reduced carbohydrates in five lines (4B, 5B, 1D, 2D, 5D) (**Fig. 2**).

Non-reduced and total carbohydrates in the roots were significantly increased by infestation in the substitution lines of the B and D genomes, except for the 3D and 7D lines. Ethylene treatment produced significantly lower non-reduced and total carbohydrates in the roots of the 4B, 1D, 6D and 7D lines (**Fig. 2**). JA induced an increase in non reduced and total carbohydrates in the D genome lines (except in 3D). SA-treated plants of the 3B, 4B, 1D, 4D and 7D lines showed significantly lower non-reduced and total carbohydrates in the roots. Finally, the ABA treatment significantly increased non-reduced and total sugars in the 4B, 5B and D genome substitution lines (**Fig. 2**).

Ethephon was reported to have some indirect effects on carbohydrate translocation, moving newly fixed assimilates downward from the leaf source toward the roots. This partitioning pattern determined the inhibition of the apex growth in tomato seedlings (Woodrow *et al.* 1988). Conversely, current results showed that the partitioning pattern of total and non reduced carbohydrates maintained a high content in aerial tissues, with neither inhibition of growth, nor a significant reduction in roots in 3B, and 7B substitution lines that showed the highest contents. Nonetheless, the partitioning pattern was affected by ethylene treatment in the 4D and 7D lines, which showed an enhanced growth rate treated with ethylene and infested with greenbug.

Ethylene also promoted both the elongation of rice coleoptiles and the increase of dry weights with higher transport and accumulation of sucrose, fructose and glucose from the scutellum to the coleoptile in rice (Ishizawa and Esashi 1985). The increased transport was attributed to the activation of sucrose unloading into growth, conditioning the change of imported sucrose into glucose and fructose (Ishizawa and Esashi 1988). In our current results, the 7B, 4D and 7D substitution lines that showed a higher growth rate after E treatment, and simultaneously had an increased accumulation of sucrose, fructose and glucose in the aerial tissues.

The pattern of translocation for reduced carbohydrates in the Syn and 5A substitution line showed greater values in roots after ethylene treatment. These results could be very important for assessing plants resistant to flooding or drought. Roots of plants under flooded conditions require a large amount of carbohydrate due to the inefficiency of anaerobic respiration compared to aerobic respiration. In addition, flooding induces a complete inhibition of most protein synthesis, and new stress-induced proteins are synthesized. These anaerobic induced proteins are mostly glycolytic proteins. Therefore, anaerobic respiration is strongly increased by the requirement for carbohydrates. As a result, root tissues rapidly become depleted in carbohydrates unless the plant has stored an excessive amount of carbohydrates before flooding occurred. Several authors have described this situation as "carbohydrate starvation" during flooding (Setter *et al.* 1987). Carbohydrate starvation is further enhanced in roots because translocation of carbohydrates from leaves to roots is inhibited during flooding conditions (Brandle 1991). One QTL affecting drought-induced ABA accumulation has been mapped on chromosome

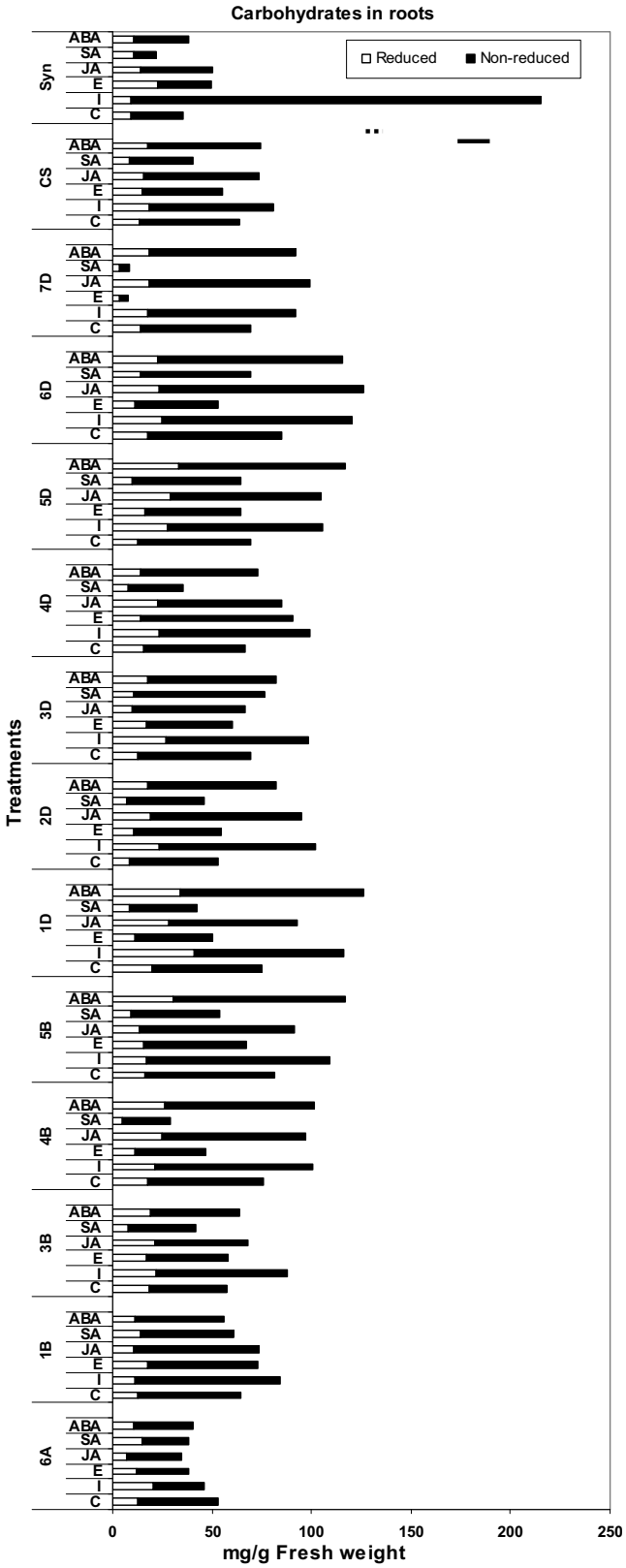


**Fig. 2 Reduced, non reduced and total carbohydrates in the root biomass of 12 wheat substitution lines and both parents (CS and Syn), controls (C), plants subjected to aphid infestation (I), or exogenously treated with ethylene (E), jasmonic acid (JA), salicylic acid (SA) and ABA.** The horizontal bars represent the Standard Errors for reduced (□) and non reduced (■) carbohydrates (**Appendix 1** unpublished data).

5A (Quarrie *et al.* 1994), close to loci controlling frost tolerance (Snape *et al.* 1997).

There are evidence that sugars and MeJA act synergistically and induce the accumulation of vegetative storage protein mRNAs in soybean leaves and cell cultures (Mason *et al.* 1992). During the wound induction of proteinase inhibitor I in tomato, a similar requirement for sucrose was observed (Doares *et al.* 1995), and for proteinase inhibitor IIK in potato. The wound induction of some plant genes may require both, metabolic and hormonal signals for optimal expression. Moreover, JA treatment has been related with tolerance to oxidative stress in plants (Kumari *et al.* 2006). The 1B, 4B and the D genome substitution lines showed higher contents of carbohydrates, in aerial tissues and roots, and these current results could represent JA action in defense mechanisms: if the JA is the signal molecule for gene transcription induction, the sugars could be the energy source that enhance this process, as was suggested by Mason *et al.* (1992). In a previous report, the 1D and 6D substitution lines showed the greatest increase in total root carbohydrates and high contents in aerial tissues when subjected to greenbug infestation (Castro *et al.* 2005b). These authors suggested that since these substitution lines were reported tolerant to aphid infestation (Castro *et al.* 2001), probably, higher sugar contents allowed a better protection against biotic stress. Plants accumulate large amounts of carbon and nitrogen in specific cells and tissues and then both are mobilized for their use in other parts of the plant; these events also occur during vegetative growth. In this sense, JA was suggested to play an important role in protein and sugar storage and mobilization since jasmonate levels are high in vegetative sinks (Creelman and Mullet 1995). In six-week-old soybean seedlings, JA levels are higher in young growing leaves (that are importing carbon and nitrogen) than in older fully expanded leaves (Creelman and Mullet 1995). Current results indicate that exogenous application of JA promoted a significant protein increase in the 1A, 5B and 7B lines, and a different partitioning pattern of total carbohydrates. The highest accumulation of sugar in the aerial tissues was found in 1B, 4B, 7B, 1D, 3D and 6D substitution lines. Similarly, the 4B, 1D, 2D, 4D, 5D, 6D and 7D lines showed significantly higher sugar accumulation in roots. It is noteworthy that JA did not alter the pattern of carbohydrates distribution in lines of the A genome.

Plants treated with SA showed significantly different total aerial carbohydrates only in the 1B, 3B and 4D substitution lines, meanwhile in roots, no substitution line showed higher values than untreated controls. Substitution lines 3B and 4D showed tolerance to greenbug by means of growth rate and proteins content. Nonetheless, after SA treatment, the 3B line maintained a normal growth rate but the 4D line grew significantly less. The 3B chromosome has been reported to carry QTLs conferring resistance to Fussarium head blight (Otto *et al.* 2002). Although, the relationships between endogenous contents of carbohydrate and the effects of exogenous SA remain unknown, current information could be of interest in breeding wheat resistant to diseases and pests. Recently, 12 genes for SA early responses have been identified in *Arabidopsis* and 20% of them are related to photosynthesis (Blanco *et al.* 2005).

ABA treatment increased tolerance to frost in bromegrass and also stimulated a three to four-fold increase of sucrose (Robertson *et al.* 1994). In agreement with this, ABA treated plants of the 4B, 1D, 3D, 5D, and 6D substitution lines showed the highest values for non reduced and total carbohydrates in aerial tissues. Similarly, the 4B, 5B, 1D, 2D, 5D, 6D lines showed the highest values in the roots, as a response to exogenous ABA treatment. These results could indicate there are genes activated by ABA that would enable an enhancement of drought and/or salinity tolerance in wheat. Considerable information is available concerning salinity-induced ABA accumulation in plants. As a result of osmotic (salt) stress, plants respond by producing metabolites such as proline and reducing sugars which are thought to counteract the loss of water due to the osmotic imbalance (Yamaguchi-Shinozaki and Shinozaki 2006).

## CONCLUDING REMARKS

Nine substitution lines showed tolerance to greenbug and to most of the stress-induced hormones in terms of plant growth, protein content and aerial and root soluble carbohydrates. Nonetheless, stress-induced hormone treatments were insufficient to closely mimic events associated with aphid feeding stress in plant growth since 1D, 2D and 3D substitution lines suffered a significant decreased in their leaf area under greenbug infestation and ethylene treatment, and showed an over expression of aerial growth when subjected to JA treatment (2D). Conversely, the 4D substitution line, that showed tolerance to aphid feeding, remained susceptible to SA and ABA treatments. Current results are in agreement with those previously reported for other host-pest interactions (Baldwin and Preston 1999; Felton and Korth 2000; Ozawa *et al.* 2000; Halitschke *et al.* 2001; Voelckel *et al.* 2001). The eight substitution lines that were tolerant to greenbug (3A, 5A, 6A, 3B, 4B, 7B, 4D, 6D and 7D) simultaneously were tolerant to the different hormones. Greenbug resistance genes have been previously mapped only on four chromosomes, but in this work it was possible to identify other chromosomes that showed positive effects with respect to growth responses and protein and sugar levels, suggesting a complexity in the cascade of transductional signals that account for the crosstalk between tolerance to aphids and to stress-induced hormones.

Previously, it has been reported that genes for salt tolerance in wheat were associated with the group 5 chromosomes (Koebner *et al.* 1996). Several lines have been reported to carry other genes that give adaptation to the environment. Vernalization responsive genes (*Vrn1*) have been located on the long arm of chromosomes 5A, 5B and 5D (Galiba *et al.* 1995; Snape *et al.* 1997; Sutka *et al.* 1999). Loci with major effects on frost tolerance are carried by chromosomes 5A and 5D (Sutka and Snape 1989; Snape *et al.* 1997). Homoeologus group 2 of chromosomes has also been identified as carrying ABA-responsive elements (Baek *et al.* 2006). Finally, the short arm of chromosome 6A has been reported to carry ABA-, JA-, E- and SA-responsive gene/genes (Castro *et al.* 2008).

Current results give an insight into the expression of several genes induced by exogenous treatment of stress-induced hormones. We found several chromosomes that have not been previously reported to carry genes of interest for adaptation to the environment. These lines probably carry genes for several distinct metabolic pathways, and could be useful for improving the level of tolerance, and are worth incorporating in new improved wheat cultivars.

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## APPENDIX 1

Wheat inter-varietal substitution lines with known chromosomal locations for genes for resistance against greenbug, Russian wheat aphid and *Septoria tritici* were used in the current research. "Chinese Spring" (CS) was the recipient variety into which individual chromosomes from a synthetic wheat (*T. diccocoides* x *Aegilops squarrosa*) were introduced by crossing and cytological selection. The precise genetic stocks were developed by Dr. A. Worland (John Innes Centre, Norwich, UK). Three hundred seeds of each of 16 substitution lines and of the parental lines were washed for 2 h and disinfected for 5 min in a solution of NaHClO ($5g.l^{-1}$) before being placed in a Petri dish to germinate for 24 h at 21°C in darkness. Afterwards, 240 germinating seeds were sown in 20 ml vials perforated at the base, one seed per vial on a substrate of vermiculite, and placed in trays in a glasshouse under natural conditions of light and temperature at La Plata (36° 36' S) in early spring. Plants with the 3rd leaf fully expanded were selected, and the trays were filled with 2 l of Hoagland (1959) solution to enable a free supply of water and minerals; that volume was maintained during the whole assay. Hormone solutions, prepared in distilled water and Tween 20® (0.01%, w/v), were injected into the soil as 50 mM Ethrel®, $10^{-5}$M JA, 50mM SA and $10^{-6}$M ABA (Ethrel, JA, SA and ABA were purchased from Sigma Chem Co, MO, USA). Control plants were treated with distilled water + 0.01% Tween 20. A set of plants was infested with 20 greenbug/plant. At the onset of the trial, leaf area was recorded, and this evaluation was repeated 72 h later. After 3 days, control and treated plants were harvested, the roots washed and the samples prepared for protein and sugar measurements. Twenty plants of every genotype in each treatment were separated into aerial and root tissues, these parts were pooled and their fresh weights determined. Ten samples of 1 g each for aerial and root tissue were used to determine proteins according to Bradford (1980). Another 10 samples of 0.5g were used for carbohydrate determination following the Cronin and Smith method (1978). Total, reducing and non-reducing carbohydrates were assessed for the aerial and root tissues. The rest of the aerial and root biomass of the 18 genotypes was oven dried at 60°C until constant weight to determine aerial, root and total dry weights. The ratio aerial dry weight: root dry weight was calculated. An ANOVA was applied for all the parameters measured, and Duncan's multiple range test was used to separate the means with significant differences among lines (SAS, 1998).