Analysis of the genetics underlying ozone and drought tolerance in wheat

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The estimated yield loss due to different stresses in various crops

- Ozone
- Salt
- Drought

* maize, rice, wheat

Wheat yield is ‘sensitive’ to O$_3$/soil water deficit/salinity

Considerable intra- and interspecific variation in susceptibility

Lower O$_3$, but higher drought, tolerance exhibited by modern varieties compared with early accessions
Unlike drought, there has so far been little effort to improve crop responses to O$_3$ through breeding or biotechnology. A combination of approaches, including QTL mapping and other genetic and breeding tools, will likely be needed to significantly improve the abiotic stress tolerance of crops in the field.

O$_3$/drought tolerance are quantitative traits, governed by the additive effects of several genes.

The latest stage in the on-going programme at Newcastle has been the production of near isogenic lines (NILs) which focus on 12 regions of the bread wheat genome where major QTL clusters for a number of traits have been located using a much-studied Chinese Spring (CS) x SQ1 mapped population.
enables us to find out which chromosome house targeted genes

use of marked lines has the major advantage that we can find out exactly what individual genes do ..... Functional genomics in its purest sense: employing a bottom-up strategy (i.e. physiological approaches) rather than a top-down approach (gene tagging, transformation, targeted mutations) to ascribe likely gene functions
Genetic map of Chinese Spring x SQ1 from 96 doubled haploids

Loci in **BLUE**: RFLP markers
Loci in **RED**: PCR-based markers
Loci in **GREEN**: morphological, physiological and isozyme markers
Loci in **PURPLE**: SSR markers mapped in the SUSTAIN project

Other loci are AFLPs

Distances given in cM (Kosambi function)

Approximate position of centromeres shown in black.

Genetic map from a population of 96 doubled haploid lines from the cross Chinese Spring x SQ1 population of 95 doubled haploid lines. Tick marks show the position of mapped loci in the mapping population and dashed lines join homoeologous loci, shown in bold, mapped with the same marker. Different types of marker are shown in different colours. Underlined markers were located to chromosomes using nulli-tetras.
Thickness of bars proportional to level of significance: from $P<0.05$ to $P<0.0001$.

Bars to the left of chromosome: increasing allele from SQ1
Bars to the right of chromosomes: increasing allele from CS

Figure 3.11. The wheat chromosome map showing localisation of QTLs for the investigated traits. Blue circles show the regions where the ‘ozone-specific’ clusters are localised.
Chromosomes and markers for selecting QTL+/- NILs for the indicated traits.
The backcrossing programme into SQ1:

SQ1 BB (4DL) × CSDH12 AA (4DL) = 50% SQ1

SQ1 BB (4DL) × BC1 AB = 75% SQ1

BC2 BB+AB = 87.5% SQ1

(BC2 seeds sown Sep 2006 and back-crossed Dec 2006)

BC2 BB+AB (BC2 seeds sown Mar 2007 and backcrosses made without screening)

BC3 B+BB+AB = 93.75% SQ1

(BC3 seeds harvested Aug 2007)

BC3 B+BB+AB (BC3 seeds sown March 2008, backcrossed Jun 2008 and marker screened after backcrossing)

6.9% SQ1

(BC4 seeds harvested August 2008)

6.9% SQ1 (BC4 seeds sown Mar 2009, back-crossed Jun 2009)

(BC5 seeds harvested August 2009 and sown Sep 2009 without genotyping. Marker screened in 2010, and 2011)

SQ1 (Ears of all plants self-pollinated Dec 2009, harvested Feb 2010, and marker screened in 2011)

(C5S sown May 2010 to bulk up seeds. Leaves sampled for DNA to identify AA and BB homozygotes.)

(Bulk up in the glasshouse in spring 2011, with some phenotyping, and collect seeds to sow in autumn 2011 for further phenotyping.)
Ozone trial: open-top chambers
- control (NFA) vs **ozone treatment**
- 10 weeks (booting stage-full maturity)
- NFA plus 50 ppb 8 h d⁻¹

**Drought trial**: growth chambers
- Control (fully irrigated) vs **drought treatment**
- 5 weeks (flag leaf emerging-milk stage of grain filling)
- about 50% less water than in control

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The Feekes scale of wheat development

<table>
<thead>
<tr>
<th>Tillering</th>
<th>Stem Extension</th>
<th>Heading</th>
<th>Ripening</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>one shoot</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>tillering begins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>tillers formed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4 leaf sheaths lengthen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5 leaf sheaths strongly</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6 first node of stem visible</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7 second node visible</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>8 last leaf just visible</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>9 ligule of last leaf just visible</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10 in “boot” visible</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.1</td>
<td>10.1 head visible</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.5</td>
<td>10.5 flowering (wheat)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

58 NILs
OTC ozone data (20th June – 29th August)

Drought trial data - water status in the pots* (30th April – 1st June)
*averaged across NILs
### Agronomic traits measured in ozone and drought trial (QTL++ and QTL-- analysis will be presented for those highlighted in red)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Ozone trial</th>
<th>O/C</th>
<th>Drought trial</th>
<th>D/C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ozone</td>
<td>Control</td>
<td>O/C</td>
<td>Drought</td>
</tr>
<tr>
<td>Days to heading</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>53.3</td>
</tr>
<tr>
<td>Productive tillers</td>
<td>2.5</td>
<td>2.6</td>
<td>0.96</td>
<td>2.1</td>
</tr>
<tr>
<td>Sterile tillers</td>
<td>0.2*</td>
<td>0.1</td>
<td>2.00*</td>
<td>0.8**</td>
</tr>
<tr>
<td>Total tillers</td>
<td>2.7</td>
<td>2.7</td>
<td>1.00</td>
<td>2.9</td>
</tr>
<tr>
<td>Stem height (cm)</td>
<td>57.6</td>
<td>58.8</td>
<td>0.98</td>
<td>42.9</td>
</tr>
<tr>
<td>Spike length (cm)</td>
<td>6.9</td>
<td>6.9</td>
<td>1.00</td>
<td>8.6</td>
</tr>
<tr>
<td>Fertile spikelets/spike</td>
<td>17.5</td>
<td>18.1</td>
<td>0.97</td>
<td>14.4</td>
</tr>
<tr>
<td>Sterile spikelets/spike</td>
<td>1.5</td>
<td>1.0</td>
<td>1.50**</td>
<td>3.1**</td>
</tr>
<tr>
<td>Total spikelets/spike</td>
<td>19.0</td>
<td>19.1</td>
<td>0.99</td>
<td>17.5</td>
</tr>
<tr>
<td>Yield/plant (g)</td>
<td>1.28</td>
<td>1.71</td>
<td>0.75**</td>
<td>1.10</td>
</tr>
<tr>
<td>Kernels/plant</td>
<td>42.5</td>
<td>49.0</td>
<td>0.87**</td>
<td>36.4</td>
</tr>
<tr>
<td>Mass of 1000 grains (g)</td>
<td>29.9</td>
<td>34.8</td>
<td>0.86**</td>
<td>30.5</td>
</tr>
<tr>
<td>Biomass/plant (g)</td>
<td>2.88</td>
<td>3.62</td>
<td>0.80**</td>
<td>3.67</td>
</tr>
<tr>
<td>Harvest index</td>
<td>0.45</td>
<td>0.47</td>
<td>0.96**</td>
<td>0.30</td>
</tr>
</tbody>
</table>

* and ** indicate differences between stress and non-stress means at $P < 0.05$ and $0.01$, respectively
Target region: 3AS (Gwm2)

NILs++ (with CS alleles in an SQ1 background), NILs-- (with SQ1 alleles in an SQ1 background)

*, ** and *** indicate differences between NILs++ and NILs-- means at $P < 0.05$, $0.01$ and $0.001$. respectively
**Target region: 4BL (Dupw43)**

**NILs++** (with CS alleles in an SQ1 background), **NILs--** (with SQ1 alleles in an SQ1 background)

*, ** and *** indicate differences between **NILs++** and **NILs--** means at $P < 0.05$, 0.01 and 0.001, respectively
NILs++ (with CS alleles in an SQ1 background), NILs-- (with SQ1 alleles in an SQ1 background)

*, ** and *** indicate differences between NILs++ and NILs-- means at $P < 0.05$, 0.01 and 0.001, respectively
To sum up

Several loci related with productive traits enhance yield in elevated ozone and/or drought stress. Several loci shown to be drought-specific, but only one appeared to be ozone specific.

Preliminary results suggest that there is no evidence of genetic cross-tolerance linkages between the two stresses when applied separately in tested NILs.

Next steps
• to extend testing of our NILs under O$_3$ and drought/heat/salinity stress
• dissect the genes involved in governing the traits of interest
• Extend trials to the field with a view to crossing into commercial breeding material
NILs++ (with CS alleles in an SQ1 background), NILs-- (with SQ1 alleles in an SQ1 background)

*, ** and *** indicate differences between NILs++ and NILs-- means at $P < 0.05$, 0.01 and 0.001, respectively
Spearman's correlation coefficients ($r$) and scatter graphs showing the relationship between productive traits in ozone and drought treatment.

* $r$ – Correlation coefficient significant at the 0.05 probability level

**Spearman's correlation coefficients ($r$) and scatter graphs showing the relationship between productive traits in ozone and drought treatment.**