Breeding sunflower for improved drought tolerance in Australia.

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Abstract

A germplasm enhancement program has been initiated for the development of sunflower hybrids with improved drought tolerance for Australian production environments. Based on data from computer simulations and research in other crops we have focused our efforts on selection for improved transpiration efficiency (TE - dry matter production per unit water transpired) in sunflower. Significant genetic variation for TE, as measured by the surrogate trait carbon-isotope-discrimination (delta), has been found. Genetic crosses segregating for delta have been established to construct a genetic linkage map of sunflower to identify molecular markers linked delta. To date three independent RFLP markers linked to delta have been identified. Using an alloplasmic set of sunflower lines a cytoplasmic effect on delta has been shown. The yield advantage of hybrids selected for improved TE (by indirect selection for delta) was tested by top-crossing low and high segregates from our mapping population to a common female. These experimental hybrids were tested at three locations and the high delta pool out-yielded the low delta pool by 35% in the droughted environments (2 of 3 environments). High TE material being developed by this project is currently being introgressed by the private seed companies.

Introduction

Exposure to moisture stress is one of the major limitations to productivity of sunflower in Australia. We have initiated a germplasm enhancement program to underpin development of sunflower hybrids with improved drought tolerance for Australian production environments. We are using crop modeling (see Wang et al these proceedings), plant breeding and molecular genetics to achieve this objective.

Using an enhanced sunflower crop simulation model developed in this project, six major types of moisture stress patterns were identified across nine locations from northern NSW.
to central Qld using 100 years of weather records. The most common stress pattern was
the terminal stress pattern where little or no rainfall was received after sowing.
Simulation runs over the same locations and 100 year weather record indicated that a
10\% increase in TE would lead to a yield improvement of 10-15\% in most years
(Chapman et al. 1999). These simulations suggest that improving TE is a worthwhile
objective for sunflower germplasm enhancement, consequently, selection for improved
TE has been the focus of our breeding program.

Selection for TE in wheat conducted by our colleagues at CSIRO Plant Industry Canberra
has successfully produced varieties with improved drought tolerance. For a group of 30
BC\textsubscript{2}F\textsubscript{4} high TE Hartog lines grown across eight environments (1995-8) there was up to an
11\% advantage over 30 BC\textsubscript{2}F\textsubscript{4} low TE Hartog lines (Rebetzke et al 2001).

**Carbon-isotope discrimination**

Significant genetic variation for TE, as measured by the surrogate trait carbon-isotope
discrimination (delta), has been found in sunflower (Lambrides et al 1999). A glasshouse
study has confirmed the association between TE and delta (r = 0.60) and also between TE
and specific leaf weight (r = 0.65) (Chapman et al 2000). Based on selection for delta,
material has been identified with potentially greater TE than exists in private sector
germplasm. This material represented a diverse range of genetic backgrounds including
several accessions selected from wild x cultivated crosses (Seiler 1991).

**Molecular markers for TE**

A cross between the high TE parent, HAR4, and the low TE parent, SA52, has been used
to develop a population for the construction of a framework genetic linkage map to
identify markers linked to low delta (high TE). Molecular markers aim to assist in plant
breeding programs by making selection more efficient and cheaper.
Based on phenotypic data collected from the F\textsubscript{3} generation grown in a single replicate field experiment, three RFLP markers accounting for 8.9, 12.8 and 12.0 % of the variation for delta, respectively, have been identified (Table 1). The population HAR4xSA52, currently at the F4 stage, is being inbred to develop a recombinant inbred population that will allow more accurate phenotyping and more precise molecular marker linkages to be obtained.

<table>
<thead>
<tr>
<th>marker</th>
<th>AA homozygous maternal</th>
<th>AB heterozygous</th>
<th>BB Homozygous paternal</th>
<th>AB or BB</th>
<th>Pr&gt;F R\textsuperscript{2} (% of total variation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mar 41</td>
<td>20.63 (12) ≈</td>
<td></td>
<td>20.86 (62)</td>
<td></td>
<td>0.0096*** 8.9</td>
</tr>
<tr>
<td>mar 46</td>
<td>20.65 (17)</td>
<td>20.83 (39)</td>
<td>20.95 (20)</td>
<td></td>
<td>0.0048*** 12.8</td>
</tr>
<tr>
<td>mar 63</td>
<td>20.67 (14)</td>
<td>20.82 (50)</td>
<td>21.01 (18)</td>
<td></td>
<td>0.0059*** 12.0</td>
</tr>
</tbody>
</table>

≈ number of observations for each genotypic class

**Cytoplasmic effects**

Using a set of alloplasmic sunflower lines (lines have the same nucleus but different cytoplasms) we determined that cytoplasmic effects also influence TE (Lambrides et al 2000). One cytoplasm, MAX1, reduced TE significantly in the presence of several different nuclear genomes. However, our screening of five additional cytoplasms was unable to detect any superior TE over cytoplasms currently used in commercial hybrid production. The opportunity still exists to find variation though, given that the genus *Helianthus* consists of almost 50 wild species and is a potentially rich source of cytoplasmic diversity and variation for delta.
Yield testing of CSIRO experimental hybrids selected for improved TE.

To test the value of delta as a trait to enhance drought tolerance we selected 6 low delta F3 segregates and 6 high delta segregates from the mapping population HAR4 x SA52 and top-crossed these to a common genetic male sterile tester (supplied by Pacific seeds). These hybrids were then evaluated at three locations during autumn 2000.

One trial was sown in the field under a 30 m x 10 m rainout shelter located at Gatton Qld. The trial was watered up with 50 mm of irrigation and given an additional 50 mm during the early vegetative phase. No additional moisture was provided to the plot. Location two, planted at Gatton at the same time and adjacent to the rainout shelter, was rain-fed and given supplementary irrigation as needed. Location three was planted in the Central Highlands Qld at Capella under dry-land conditions. The Capella location was severely water stressed from shortly after planting until flowering. The Gatton locations included three replicates of each hybrid sown in single-row 3 m plots. The Capella trial included two replicates of two-row 6m plots.

Delta signatures were determined from all hybrids sown at Gatton. Grain yield, oil content and days to first flower were obtained for all hybrids at each location. Statistical contrasts were then used to detect differences between the low delta vs. high delta pools.

At both Gatton locations the low vs. high delta pools of experimental top-cross hybrids were significantly different for delta by an average of 0.35 per mil, indicating that the phenotyping of the F3 individuals used as male parents was accurate (Table 2). This mean difference indicated that we were successful in developing pools of hybrids differing for delta. While not measured, we expect that the difference for delta between the pools would also have been observed at Capella given the low genotype by environment interaction observed for this trait.
Table 2 Mean delta values (per mil) for low vs high delta pools of experimental top-cross hybrids made from F3 segregates of the population HAR4 x SA52 and evaluated at two Gatton locations in autumn 2000.

<table>
<thead>
<tr>
<th></th>
<th>Gatton irrigated</th>
<th>N (no of obs)</th>
<th>Gatton dryland</th>
<th>N (no of obs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High delta pool</td>
<td>20.66</td>
<td>18</td>
<td>21.97</td>
<td>18</td>
</tr>
<tr>
<td>Low delta pool</td>
<td>20.32</td>
<td>18</td>
<td>21.61</td>
<td>18</td>
</tr>
<tr>
<td>Pr &gt; F</td>
<td>0.0022***</td>
<td></td>
<td>&lt;0.0001***</td>
<td></td>
</tr>
</tbody>
</table>

*** P < 0.005

The low delta pool significantly out-yielded the high delta pool in the two droughted locations by 35% (Table 3). There was no significant difference in grain yield between the pools at the well-watered location in Gatton (Table 3). There was also no significant difference between the pools for oil content and maturity (data not shown) indicating that the delta trait does not appear to be associated with these key agronomic traits. Yield evaluations of experimental hybrids from low and high delta parents are being conducted this season (2000-1) and will be repeated next season (2001-2).

Table 3 Grain yield (t/ha) for low vs high delta pools of experimental top-cross hybrids made from F3 segregates of the population HAR4 x SA52 and evaluated at three locations in autumn 2000.

<table>
<thead>
<tr>
<th></th>
<th>Gatton irrigated</th>
<th>N (no of obs)</th>
<th>Gatton dryland</th>
<th>N (no of obs)</th>
<th>Capella dryland</th>
<th>N (no of obs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High delta pool</td>
<td>0.82 (100) ≈</td>
<td>18</td>
<td>0.48 (100)</td>
<td>18</td>
<td>1.01 (100)</td>
<td>12</td>
</tr>
<tr>
<td>Low delta pool</td>
<td>0.89 (108)</td>
<td>18</td>
<td>0.65 (135)</td>
<td>18</td>
<td>1.37 (135)</td>
<td>12</td>
</tr>
<tr>
<td>Pr &gt; F</td>
<td>0.52 ns</td>
<td>&lt;0.0246*</td>
<td>0.0098 **</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* percentages given in parentheses are relative to the high delta group
*P < 0.05  **P < 0.01
It should be noted that these preliminary experiments were carried out with unadapted material, consequently, the 35% yield difference observed between the two pools may not be the yield advantage expected in adapted commercial material with the high TE trait added. The yield advantage expected for commercial material might be closer to the 15% as indicated by our computer simulation studies.

The current results suggest three important features of hybrids selected for low delta (high TE): firstly, there appears to be a clear benefit of the delta trait under moisture stress conditions; secondly, the trait does not carry a yield penalty under more favorable moisture conditions; and thirdly, the delta trait is not negatively associated with the important agronomic traits oil content and maturity.

**Introgression of the TE trait into private sector breeding programs.**

We have aligned our breeding project closely with those in each of the three private sector sunflower breeding programs (Agseeds, Pacific and Pioneer) operating in Australia. Material identified in this project as having higher TE has been crossed with elite parental lines from the private sector to introgress this trait into those programs. In addition, we are now crossing our best TE lines with elite private sector female lines to make experimental hybrids that will be yield tested under dryland conditions in many of the sunflower production areas of Australia. All yield testing of hybrids developed from CSIRO material will be carried out by the three private programs.

**Conclusion**

Considerable progress has been made in developing sunflower germplasm with enhanced drought tolerance for Australian production environments. The benefits of this research should reach the market place in the next five years.
Acknowledgments

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References


