QUANTITATIVE TRAIT LOCI ANALYSIS FOR GRAIN PROTEIN PERCENTAGE IN DURUM WHEAT (Triticum turgidum L. var. durum)

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Abstract: Two crosses of high protein-black glumed x low protein-white glumed durum wheat (Triticum turgidum L. var. durum) genotypes were used for quantitative trait loci (QTL) analysis for grain protein percentage (GPP). The black glume colour was used as a genetic marker (M) in an individual marker locus model which allows for the estimation of gene effects and recombination frequencies by comparing marker classes means in the segregating generations (F₂, BC₁ and BC₂). The black glume character was monogenically controlled with codominance between the two alleles of the Bg gene. Black-glumed parental landraces showed greater GPP means (18.17) than the white glumed local cultivar (14.38), while the F₁ of the two crosses was intermediate (15.23). Meantime, the black-glumed (BgBg) F₂ and BC₁ plants of the two crosses showed higher GPP means than the two other types (Bgbg and bgbg) of glume colour. The white glumed (bgbg) F₂ and BC₂ plants had the lowest GPP means. The QTL analysis for GPP revealed that the recombination frequencies between the QTL for high GPP and the M marker for black glumes were 0.11 and 0.15 in the two tested crosses indicating a close linkage between the genes controlling the two characters. The additive (d) gene effects estimated from the QTL analysis were much greater in magnitude (1.67 and 2.28) than the dominance (h) effects (0.22 and 0.50) in the two crosses, respectively. Evidently, marker-assisted selection (MAS) for increasing grain protein percentage could be very effective utilizing the black glume gene as a marker in the segregating populations. The six generations means showed the adequacy of the additive-dominance model and the absence of non-allelic gene interaction. The additive (D) component of variance was greater in magnitude than the dominant (H) with moderate values of narrow-sense heritability being 0.54 and 0.63 in the two crosses.

Key words: Black glumes, QTL, MAS, grain protein percentage, durum wheat.

Introduction

Grain protein content of durum wheat is an important trait for the nutritional value of grain and the technological properties of flour (Blanco et al., 2006). Improvement of grain protein quantity and quality in cultivated wheats was achieved via transferring genes from the high protein wild type tetraploid wheat, T. turgidum var. dicoccoids (Avivi,
Moreover, several morphological and biochemical markers were found to be associated with high grain protein percentage (GPP) in wild tetraploid wheat. A strong linkage between the black glume gene ($B_g$) and the gene(s) coding for high GPP was reported by Levy and Feldman (1989b). A single gene for black glumes, $B_g$, has been reported in diploid, tetraploid and hexaploid wheat by Sikka et al. (1961), and was located on chromosome 1AS (Fletcher and McIntosh, 1974; Law and Chapman, 1974; Worland et al., 1987; Levy and Feldman, 1989a; Blanco et al., 1998).

The association between GPP and black glumes have allowed for QTL (quantitative trait loci) analysis and the location of genes for high GPP. Evidently, selection for characters with easily detectable phenotypes can simplify the recovery of genes of interest linked to them but difficult to score (Arus and Moreno-Gonzalez, 1993). Such associations is easily maintained by a strong linkage between alleles coding for these traits which found to be located on chromosome 1AS (Levy and Feldman, 1989c). Black glume colour might be used as a marker for genetic analysis in wheat, as well as for mapping of qualitative and quantitative genes. The method of QTL mapping provides the possibility for determining the inheritance of individual QTLs.

Arus and Moreno-Gonzalez (1993) described two genetic models for estimating the contribution of the marker-linked loci to the quantitative trait under study: (i) Individual marker locus, and (ii) Flanking marker loci. The models allow estimation of gene effects and recombinant frequencies by the method of comparison of marker class means. The experimental designs for estimating QTLs effects and map positions are extensions of the standard methods for mapping single genes, and are based on linkage disequilibrium between alleles at a marker locus and alleles at the linked QTL (Falconer and Mackay, 1996). There are two general sorts of methods for identifying and mapping QTLs: those based on crosses between lines that differ for the trait of interest, and methods based on segregating populations. The most efficient experimental designs for locating QTLs use crosses between lines that are fixed for alternate alleles at both the QTL and the marker loci. Most QTL analyses in plants involve populations derived from pure lines and several approaches have been developed to associate QTL with molecular markers in such populations. In autogamous species, QTL mapping studies commonly make use of $F_2$ or backcross progenies because they are the
This study was carried out to: (a) ascertain the mode of inheritance of the black glume colour; (b) determine the magnitude of linkage between the black glume gene and the gene(s) controlling grain protein percentage in durum wheat using QTL analysis.

**Materials and Methods**

The present study was carried out during the three growing seasons 2003-2004, 2004-2005 and 2005-2006 at the Experimental Farm of the Faculty of Agriculture, Assiut University, Assiut, Egypt. Two landraces of durum wheat (*Triticum durum* L.) with black glumes and a local cultivar with white glumes (Sohag-3) constituted the basic material of this study (Table 1). The landraces were originally collected from farmers' fields near Dandara Temple at Qena Governorate in 1993 (Omara, 1994) and were grown since then every year in order to ascertain the stability of the black glume character. Cytological analyses were also made which confirmed the tetraploid nature of the chromosome complement (2n= 4x= 28) and the regularity of meiosis.

**Table(1):** Descriptions of the three durum wheat parents used in the present study.

<table>
<thead>
<tr>
<th>Parent No.</th>
<th>Name</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Glume colour</td>
</tr>
<tr>
<td>1</td>
<td>Sohag-3</td>
<td>White</td>
</tr>
<tr>
<td>2</td>
<td>WK-12-2</td>
<td>Black</td>
</tr>
<tr>
<td>3</td>
<td>WK-12-4</td>
<td>Black</td>
</tr>
</tbody>
</table>

In the 2003-2004 winter season, using Sohag-3 as common parent (Table 2).

**Table(2):** Crosses established between the three parents.

<table>
<thead>
<tr>
<th>Cross No.</th>
<th>Cross</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P₁ x P₂</td>
<td>white glume X black glume</td>
</tr>
<tr>
<td>2</td>
<td>P₁ x P₃</td>
<td>white glume X black glume</td>
</tr>
</tbody>
</table>
In the 2004-2005 winter season, seeds of the two $F_1$ crosses and their respective three parents were sown in the field. Each of the $F_1$'s was backcrossed to its pertinent two parents to produce the first ($F_1XP_1$) and second ($F_1XP_2$) backcrosses ($BC_1$ and $BC_2$). In the meantime, crossing was made among the parents in order to produce $F_1$ seeds while $F_1$ seeds were planted to produce the $F_2$ seeds. In 2005-2006 winter season, the six basic generations ($P_1, P_2, F_1, BC_1, BC_2$ and $F_2$) of each of the two crosses were field evaluated in a randomized complete block design with three replications. Field observations and measurements for the six generations of each cross were recorded for the following characters on individual plant basis:

1- Glume colour (black, intermediate and white).


Biometrical analyses:

I- The chi square test ($\chi^2$):

The chi square test was used to test the expected numbers of the phenotypic classes according to the theoretical 1:2:1 ratio for glume colour (Black : intermediate : white glume colour, respectively) in the $F_2$'s, the 1:1 ratio (intermediate : black glume colour) in the $BC_1$'s and the 1:1 ratio (intermediate : white glume colour) in the $BC_2$'s against the observed numbers.

II- QTL analysis:

The QTL analysis was basically carried out according to the individual marker locus model (Arus and Moreno-Gonzalez, 1993).

III- Scaling tests:

The six generations model was employed for grain protein percentage as outlined by Mather and Jinks (1982) to establish the adequacy of the additive-dominance model and Jinks and Jones (1958) to determine types of gene effects involved.

Results and discussion

I- Inheritance of glume colour:

Glumes Colour was black $BgBg$ in each of the two parental landraces, white $bgbg$ in the local cultivar (Sohag-3), and intermediate $Bgbg$ in the $F_1$'s, (Fig. 1). For backcrosses to the black-glumed landraces, plants were divided into two groups: intermediate $Bgbg$ and black $BgBg$, while the backcrosses to the local white-glumed parent segregated into intermediate $Bgbg$ and white $bgbg$. 
**Fig. (3):** Colour of the glumes for black-glumed parental landrace, white-glumed local parental cultivar (Sohag-3) and their F$_1$ hybrid.

The suggested mode of inheritance of glume colour as based on their segregation pattern in BC$_1$, BC$_2$ and F$_2$, fitted well the expected Mendelian distribution for one gene model, according to the Chi-square test. A close to 1:2:1 segregation ratio, was observed in the F$_2$'s and a 1:1 segregation ratio was obtained in BC$_1$ and BC$_2$, the results of the Chi-square analysis (Table 3) showed non-significant differences in BC$_1$, BC$_2$ and F$_2$ of the four crosses, indicating monogenic model with codominance for the inheritance of glume colour in this material. These results are in agreement with those reported on black glume colour in wheat as summarized by Sikka *et al.*, (1961); Fletcher and McIntosh (1974); Law and Chapman (1974); Worland *et al.*, (1987); Levy and Feldman (1989a) and Blanco *et al.*, (1998).

**Table (3):** Segregation of BC$_1$, BC$_2$ and F$_2$ plants for glume colour in the two crosses.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Generation</th>
<th>Number of plants for glume colour</th>
<th>Expected ratio</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Black</td>
<td>Intermediate</td>
<td>White</td>
</tr>
<tr>
<td>1</td>
<td>BC$_1$</td>
<td>24</td>
<td>36</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>BC$_2$</td>
<td>--</td>
<td>23</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>F$_2$</td>
<td>20</td>
<td>49</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>BC$_1$</td>
<td>29</td>
<td>31</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>BC$_2$</td>
<td>--</td>
<td>27</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>F$_2$</td>
<td>17</td>
<td>45</td>
<td>28</td>
</tr>
</tbody>
</table>
II- QTL analysis:

The landraces with black glumes consistently displayed greater means for grain protein percentage GPP (18.06% and 18.28% in the two crosses, respectively) with an average of 18.17% whereas the local cultivar with white glumes displayed 14.35% GPP. The F$_1$ means of the two crosses were intermediate being 15.10% and 15.36% with an average of 15.23% (Table 4). These results indicated an association between black glume and high grain protein percentage in accordance with the results of Levy and Feldman on _T. L. var_ dicoccoides (1989b and 1989c).

**Table(4):** Means of grain protein percentage of P$_1$, P$_2$, F$_1$, F$_2$, BC$_1$ and BC$_2$ generations for the two crosses.

<table>
<thead>
<tr>
<th>Generation</th>
<th>Cross</th>
<th>Mean ± s.e.</th>
<th>Mean ± s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>P$_1$</td>
<td>18.06 ± 0.143</td>
<td>18.28 ± 0.135</td>
<td></td>
</tr>
<tr>
<td>P$_2$ (Sohag-3)</td>
<td>14.38 ± 0.146</td>
<td>14.38 ± 0.146</td>
<td></td>
</tr>
<tr>
<td>F$_1$</td>
<td>15.10 ± 0.162</td>
<td>15.36 ± 0.172</td>
<td></td>
</tr>
<tr>
<td>F$_2$</td>
<td>15.46 ± 0.176</td>
<td>15.53 ± 0.182</td>
<td></td>
</tr>
<tr>
<td>BC$_1$</td>
<td>16.44 ± 0.194</td>
<td>16.49 ± 0.197</td>
<td></td>
</tr>
<tr>
<td>BC$_2$</td>
<td>14.63 ± 0.174</td>
<td>14.68 ± 0.171</td>
<td></td>
</tr>
</tbody>
</table>

The means of grain protein percentage of the BC$_1$, BC$_2$ and F$_2$ plants of each of the two crosses within the three marker classes MM, Mm and mm are presented in Table 5. The results showed that plants with black glumes uniformly displayed greater means of grain protein percentage which exceeded those of the two other types of glume colour.

**Table(5):** Means of grain protein percentage of F$_2$, BC$_1$ and BC$_2$ plants in the two crosses within three marker classes MM (black glume), Mm (intermediate) and mm (white glume).

<table>
<thead>
<tr>
<th>Marker class</th>
<th>Cross</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM (F$_2$)</td>
<td>16.98</td>
<td>16.38</td>
<td></td>
</tr>
<tr>
<td>Mm (F$_2$)</td>
<td>15.55</td>
<td>15.77</td>
<td></td>
</tr>
<tr>
<td>mm (F$_2$)</td>
<td>13.86</td>
<td>14.69</td>
<td></td>
</tr>
<tr>
<td>MM (BC$_1$)</td>
<td>17.11</td>
<td>17.14</td>
<td></td>
</tr>
<tr>
<td>Mm (BC$_1$)</td>
<td>15.99</td>
<td>15.90</td>
<td></td>
</tr>
<tr>
<td>Mm (BC$_2$)</td>
<td>15.57</td>
<td>15.73</td>
<td></td>
</tr>
<tr>
<td>mm (BC$_2$)</td>
<td>14.10</td>
<td>13.81</td>
<td></td>
</tr>
</tbody>
</table>
The means of grain protein percentage of the black-glumed F\textsubscript{2} plants were 16.98 and 16.38\% for the two cross, respectively. Meanwhile, the black-glumed plants of the BC\textsubscript{1} of the two crosses showed higher means of 17.11\% and 17.14\%. The plants with intermediate glume colour (Mm) showed moderate values of 15.55, 15.99 and 15.57\% for cross 1, and 15.77, 15.90 and 15.73\% for cross 2 in the F\textsubscript{2}, BC\textsubscript{1} and BC\textsubscript{2}, while the white glumed plants have the lowest values (13.86 and 14.10\% for cross 1, and 14.69 and 13.81\% for cross 2 in the F\textsubscript{2} and BC\textsubscript{2}).

Estimates of the QTL effects on grain protein percentage, and the recombination frequencies and dominance ratio for each of the two crosses are presented in Table 6.

<table>
<thead>
<tr>
<th>Cross Value</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>0.11</td>
<td>0.15</td>
</tr>
<tr>
<td>d</td>
<td>1.67</td>
<td>2.28</td>
</tr>
<tr>
<td>h</td>
<td>0.22</td>
<td>0.50</td>
</tr>
<tr>
<td>h/d</td>
<td>0.13</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Where: r is recombination frequency between Q and M, d and h are the additive and dominance effects respectively, and h/d is the dominance ratio.

**III- Scaling tests:**

The A, B, and C values for grain protein percentage were not significantly deviating from zero in the two crosses, indicating the adequacy of the additive-dominance model for the inheritance of grain protein percentage (Table 7).
Table(7): The A, B, and C values of the scaling tests in the two crosses

<table>
<thead>
<tr>
<th>Cross</th>
<th>Value</th>
<th>1 Scale ± s.e.</th>
<th>2 Scale ± s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-0.29 ± 0.443</td>
<td>-0.65 ± 0.451</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>-0.22 ± 0.411</td>
<td>-0.38 ± 0.409</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>-0.80 ± 0.802</td>
<td>-1.25 ± 0.830</td>
<td></td>
</tr>
</tbody>
</table>

Types of gene effects for grain protein percentage:

The additive gene effect [d] was highly significant in each of the two crosses (Table 8). The additive component of variance (D) was greater in magnitude than the dominance (H) in the two crosses, indicating the major role of the additive gene effects as compared to the dominance effects in the control of variation in grain protein percentage. The narrow-sense heritability values were of moderate magnitude being 0.54 and 0.63 in the two crosses, respectively.

Table(8): Estimates of the additive and dominance parameters, the components of variance and the narrow-sense heritability for the two crosses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cross</th>
<th>1 Effect ± s.e.</th>
<th>2 Effect ± s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>m</td>
<td>15.93** ± 0.882</td>
<td>16.11** ± 0.901</td>
<td></td>
</tr>
<tr>
<td>[d]</td>
<td>1.84** ± 0.102</td>
<td>1.95** ± 0.100</td>
<td></td>
</tr>
<tr>
<td>[h]</td>
<td>-1.05 ± 2.132</td>
<td>-1.56 ± 2.164</td>
<td></td>
</tr>
<tr>
<td>Variance Components</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>3.0404</td>
<td>3.7801</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>2.3652</td>
<td>1.5911</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>0.6806</td>
<td>0.6938</td>
<td></td>
</tr>
<tr>
<td>$h^2_{ns}$</td>
<td>0.54</td>
<td>0.63</td>
<td></td>
</tr>
</tbody>
</table>

** = Highly significant (P < 0.01)
Conclusion

With most traits of economic importance being quantitative and controlled by a fairly large number of genes, marker-assisted selection (MAS) could be used in targeting genes with larger effects (QTL) which are large enough to be detected and mapped on the genome. Evidently, marker-assisted selection (MAS) for increasing grain protein percentage could be very effective utilizing the black glume marker in the segregating populations. In the present study, the association between grain protein percentage and black glume colour provide a method to improve grain protein percentage in durum wheat using black glume character in marker-assisted selection program. The implication for marker-assisted selection is that in the QTL analysis aiming to determine which regions are to be transferred using markers; it is of utmost importance to examine whether those regions contain QTLs for other traits that will affect the total performance of the genotypes. The present results indicate that a breeding strategy for improvement of grain protein percentage utilizing black glume colour as a genetic marker should be successful since the black glume gene is associated with high GPP and can be easily selected which might improve the response to selection since the marker traits are not affected by the environment.

References


تحليل مواقع الصفات الكمية لصفة النسبة المئوية لبروتين الحبوب في قمح المكرونة

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تم استخدام مجموعتين من مجموعتين أربية سوداء المنتشرة في النسبة المئوية لبروتين الحبوب وصنع محلل من مزرع أبيض القالب منخفض في نسبة البروتين في حول موقع الصفات الكمية لصفة النسبة المئوية لبروتين الحبوب. استخدم لدي تسجيل الوراثة الأصول وراثي في نموذج الوراثة المفردة والذي يسمح بتقدير الأثير الجيني وتكرار الإحصادات الجديدة بطريقة مقارنة متوسطات فئات الوراثات في الأجيال الإنجذابية (الجيل الثاني) وكل من التهجين الرجعي للجريب الأول والتهيجين الرجعي للجريب الثاني). وجد أن صفة لون القالب السوداء تقع تحت تحكم جين مفرد (Bg) ذو علاقة سيدة مشتركة بين الالي. أظهرت السلالات الأردنية ذات القالب السوداء الأولى نسبة لبروتين الحبوب (18.17) بالمقارنة بالصنف المحلي ذو القالب البيضاء (14.18) بينما أظهرت النباتات الجيل الأول قيمة وسطية في كلا الجينين (15.13)، وفي نفس الوقت أظهرت النباتات ذات القالب السوداء في كل من الجيل الثاني وجيل التهجين الرجعي للأب الأول في كلا الجينين متوسطات لبروتين أعلى من نباتات الطرازات الأخريين من لون القالب. وكانت النباتات بيضاء القالب في كل من الجيل الثاني وجيل التهجين الرجعي للأب الثاني منخفضة في النسبة المئوية لبروتين الحبوب. أظهر تحليل مواقع الصفات الكمية لصفة النسبة المئوية لبروتين الحبوب أن تكرار الإحصادات الجديدة بين موقع المتحكم في صفة النسبة المئوية لبروتين الحبوب والجريب الوراثة السينوز عن لون القالب الأسود 0.11 و 0.15 في كلا الجينين المحترمين مما يشير إلى ارتباط وثيق بين الموقعين المتحكمين في كلا الصفات. وكانت قيم الأثير الجيني المضيف (1.67 و 2.28) والتي تم حسابها من تحليل مواقع الصفات الكمية، أعلى من البار الجينية السينوزية (0.22 و 0.50) في كلا الجينين على التوالي. وهذا يوضح بشكل جلي فائدة استخدام لون القالب الأسود كمصدر في الاتصال لزيادة النسبة المئوية لبروتين الحبوب في الأجيال الإنجذابية. أظهر تحليل متوسطات النسب الأساسية لتحديد النظام الوراثي وmetry الجين السينوز للفئة المنوية لبروتين الحبوب انطلاق نموذج إضافية - سيدة وغياب التفاعل الجيني غير الأولي في الوراثة، وتمكنت الإضافي للتبين أكبر في المدار من المكون السينوز. وكانت قيمة المكاني الموراثي بالمعنى الضيق متوسط الارتفاع (0.54 و 0.63) في الوراثة على التوالي.