Assiut Journal of Agricultural Science, 37 (4) (25-35)

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

M.K. Omara; M.Y. Hussein; M.M. El-Defrawy and M.I.M. Hassan

Genetics department, Faculty of Agriculture, Assiut University, Assiut, Egypt.

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 (F_2, BC_1 and BC_2). The black glume character was monogenically controlled with co-dominance 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 F_1 of the two crosses was intermediate (15.23). Meantime, the black-glumed (*BgBg*) F_2 and BC_1 plants of the two crosses showed higher GPP means than the two other types (*Bgbg and bgbg*) of glume colour. The white glumed (*bgbg*) F_2 and BC_2 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,

1979, Avivi *et al.*, 1983 and Levy and Feldman, 1987). 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 (*Bg*) and the gene(s) coding for high GPP was reported by Levy and Feldman (1989b). A single gene for black glumes, *Bg*, 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

easiest and earliest to obtain (Asins, 2002).

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.

Parent No.	Name	Characteristics	
		Glume colour	Grain protein percentage
1	Sohag-3	White	Low
2	WK-12-2	Black	High
3	WK-12-4	Black	High

In the 2003-2004 winter season, two crosses were made among the parents to produce F_1 hybrid seeds, using Sohag-3 as common parent (Table 2).

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

Cross No.	Cross	Description
1	P_1 x P_2	white glume X black glume
2	P_1 x P_3	white glume X black glume

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).

2- Grain protein percentage (GPP), determined by the standard kjeldahl method according to **A.O.A.C. (1984)** on a milled grains sample.

Biometrical analyses:

I- The chi square test (χ^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.

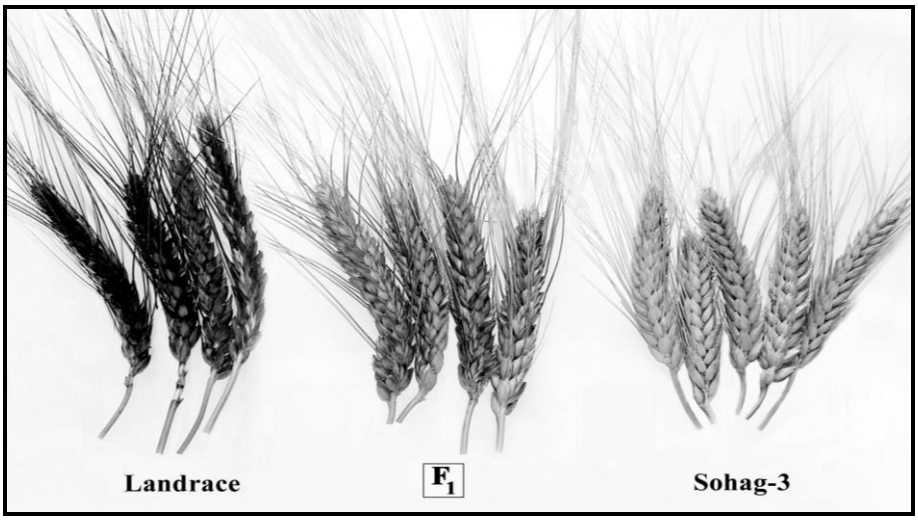

Fig.(3): Colour of the glumes for black-glumed parental landrace, white-glumed local parental cultivar (Sohag-3) and their F_1 hybrid.

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 co-dominance 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.

Cross	Generation	Number of plants for glume colour				Expected ratio	χ^2
		Black	Intermediate	White	Total		
1	BC_1	24	36	--	60	1:1	2.40
	BC_2	--	23	37	60	1:1	3.2667
	F_2	20	49	21	90	1:2:1	0.7333
2	BC_1	29	31	--	60	1:1	0.0667
	BC_2	--	27	33	60	1:1	0.60
	F_2	17	45	28	90	1:2:1	2.6889

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.

Cross / Generation	1	2
	Mean ± s.e.	Mean ± s.e.
P_1	18.06 ± 0.143	18.28 ± 0.135
P_2 (Sohag-3)	14.38 ± 0.146	14.38 ± 0.146
F_1	15.10 ± 0.162	15.36 ± 0.172
F_2	15.46 ± 0.176	15.53 ± 0.182
BC_1	16.44 ± 0.194	16.49 ± 0.197
BC_2	14.63 ± 0.174	14.68 ± 0.171

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).

Cross / Marker class	1	2
MM (F_2)	16.98	16.38
Mm (F_2)	15.55	15.77
mm (F_2)	13.86	14.69
MM (BC_1)	17.11	17.14
Mm (BC_1)	15.99	15.90
Mm (BC_2)	15.57	15.73
mm (BC_2)	14.10	13.81

The means of grain protein percentage of the black-glumed F_2 plants were 16.98 and 16.38% for the two cross, respectively. Meanwhile, the black-glumed plants of the BC_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_2, BC_1 and BC_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_2 and BC_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. The recombination frequency between QTL (quantitative trait locus for grain protein percentage) and M (black glume gene) loci were 0.11 and 0.15 in the two crosses, respectively. These results indicate a tight linkage between the gene for black glumes and the gene(s) for high grain protein percentage in agreement with Levy and Feldman (1989b and 1989c).The magnitude of the additive (d) gene effect in the two crosses (1.67 and 2.28 in the two crosses, respectively) was greater than those of the dominant (h) gene effects (0.22 and 0.50). Evidently, the genetic system governing grain protein percentage is largely additive (the major component) while dominance was partial with a dominance ratio of 0.13 and 0.22 for the two crosses.

Table(6): Estimates of the genotypic effects on grain protein percentage, the recombination frequency, and dominance ratio in the two crosses.

Cross / Value	1	2
r	0.11	0.15
d	1.67	2.28
h	0.22	0.50
h/d	0.13	0.22

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

Cross / Value	1	2
	Scale ± s.e.	Scale ± s.e.
A	-0.29 ± 0.443	-0.65 ± 0.451
B	-0.22 ± 0.411	-0.38 ± 0.409
C	-0.80 ± 0.802	-1.25 ± 0.830

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.

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

** = 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

A.O.A.C. 1984. Official method of analysis. 14th Ed. Association of Official Analytical Chemists, Washington, D.C., U.S.A.

Arus, P. and J. Moreno-Gonzalez 1993. Marker-assisted selection. In: M.D. Hayward; N.O. Bosemark; I. Romagosa and M. Cerezo eds., Plant breeding. Chapman and Hall, pp. 314-331.

Asins, M.J. 2002. Review: Present and future of quantitative trait locus analysis in plant breeding. Plant Breeding, 121: 281-291.

Avivi, L. 1979. Utilization of *Triticum dicoccoides* for the improvement of grain protein quantity and quality in cultivated wheats. Monografia of Genetica Agraria, 4: 27-38.

Avivi, L.; A.A. Levy and M. Feldman 1983. Studies of high protein *durum* wheat derived from crosses with the wild tetraploid wheat *Triticum turgidum* var. *dicoccoides*. Proc. 6th Int. Wheat Genet. Symp., Kyoto, Japan, pp. 199-204.

Blanco, A.; M.P. Bellomo; A. Cenci; C.De Giovanni; R. D'Ovidio; E. Iacono; B. Laddomada; M.A. Pagnotta; E. Porceddu; A. Sciancalepore; R. Simeone and O.A. Tanzarella 1998. A genetic linkage map of durum wheat. Theor. Appl. Genet., 97: 721-728.

Blanco, A.; R. Simeone and A. Gadaleta 2006. Detection of QTLs for grain protein content in durum wheat. Theor. Appl. Genet., 112: 1195-1204.

Falconer, D.S. and T.F.C. Mackay 1996. Introduction to quantitative genetics. 4th eds. Longman Group Ltd, pp. 356-378.

Fletcher, R.J. and R.A. McIntosh 1974. Isolation and identification of the chromosome arm bearing *Rg* determining brown glume colour in Federation wheat. EWAS Newsletter, 4: 65-66. (C.F. Zeven, A.C. (1983). The character brown ear of bread wheat: a review. Euphytica, 32: 299-310.).

Jinks, J.L. and R.M. Jones (1958). Estimation of the components of heterosis. Genetics. Princeton, 43: 223-224.

Law, C.N. and V. Chapman 1974. An inhibitor of glume colour. EWAS Newsletter, 4: 8-9. (C.F. Zeven, A.C. (1983). The character brown ear of bread wheat: a review. Euphytica, 32: 299-310.).

Levy, A.A. and M. Feldman 1987. Increase of grain protein percentage in high-yielding common wheat breeding lines by genes from wild tetraploid. Euphytica, 36: 353-359.

Levy, A.A. and M. Feldman 1989a. Genetics of morphological traits in wild, *Triticum turgidum* var. *dicoccoides*. Euphytica, 40: 275-281.

Levy, A.A. and M. Feldman 1989b. Location of genes for high grain protein percentage and other quantitative traits in wild wheat *Triticum turgidum* var. *dicoccoides*. Euphytica, 41: 113-122.

Levy, A.A. and M. Feldman 1989c. Intra- and inter-population variations in grain protein percentage in wild tetraploid wheat, *Triticum turgidum* var. *dicoccoides*. Euphytica, 42: 251–258.

Mather, K. and J.L. Jinks 1982. Biometrical genetics. 3rd (eds), Chapman and Hall, London.

Omara, M.K. 1994. Collection, maintenance, and gene banking of germplasm of wheat, barley, berseem clover, maize and sorghum from moisture deficient areas in Upper Egypt. Final Report of project No. A-5-4, NARP, Egypt.

Sikka, S.M.; K.B.L Jain and K.S. Parmer 1961. Inheritance of some morphological characters in intervarietal crosses of *Triticum aestivum* L. J. Indian Bot. Soc., 40: 217-233 (C.F. Plant Breed. Abstr., 32: 315, p.57).

Worland, A.J.; M.D. Gale and C.N. Law 1987. Wheat genetics. In: F.G.H. Lupton eds., Wheat breeding, its scientific basis. Chapman and Hall Ltd, pp. 129-171.

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

محمد قدري عمارة، محمد يونس حسين، محمد محمود حسيب الدفراوي، محمد إبراهيم محمد حسن

قسم الوراثة - كلية الزراعة - جامعة أسيوط – أسيوط- جمهورية مصر العربية.

تم استخدام هجينين في قمح المكرونة ما بين سلالتين أرضيتين سوداء القنابع مرتفعة في النسبة المئوية لبروتين الحبوب وصنف محلي منزرع أبيض القنابع منخفض في نسبة البروتين في تحليل مواقع الصفات الكمية لصفة النسبة المئوية لبروتين الحبوب. استخدم لون القنابع الأسود كواسم وراثي في نموذج الواسم المفرد والذي يسمح بتقدير الأثار الجينية وتكرار الإتحادات الجديدة بطريقة مقارنة متوسطات فئات الواسمات في الأجيال الإنعزالية (الجيل الثاني وكل من التهجين الرجعي للأب الأول والتهجين الرجعي للأب الثاني). وجد أن صفة لون القنابع السوداء تقع تحت تحكم جين مفرد (*Bg*) ذو علاقة سيادة مشتركة بين أليليه. أظهرت السلالات الأرضية ذات القنابع السوداء أعلى نسبة لبروتين الحبوب (18.17) بالمقارنة بالصنف المحلي ذو القنابع البيضاء (14.18) بينما أظهرت نباتات الجيل الأول قيماً وسطية في كلا الهجينين (15.13)، وفي نفس الوقت أظهرت النباتات ذات القنابع السوداء في كل من الجيل الثاني وجيل التهجين الرجعي للأب الأول في كلا الهجينين متوسطات للبروتين أعلى من نباتات الطرازين الأخرين من لون القنابع. وكانت النباتات بيضاء القنابع 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 الاتحادات الجديدة بين الموقع المتحكم في صفة النسبة المئوية لبروتين الحبوب والجين الواسم المسئول عن لون القنابع الأسود 0.11 و 0.15 في كلا الهجينين المختبرين مما يشير إلى إرتباط وثيق بين الموقعين المتحكمين في كلا الصفتين. وكانت قيم الأثار الجينية المضيفة (1.67و 2.28) ، والتي تم حسابها من تحليل مواقع الصفات الكمية، أعلى من الآثار الجينية السيادية (0.22 و 0.50) في كلا الهجينين على التوالي. وهذا يوضح بشكل جلي كفاءة استخدام لون القنابع الأسود كواسم في الانتخاب لزيادة النسبة المئوية لبروتين الحبوب في الأجيال الانعزالية. أظهر تحليل متوسطات الستة أجيال الأساسية لتحديد النظام الوراثي وطرز فعل الجين لصفات النسبة المئوية لبروتين الحبوب إنطباق نموذج إضافة - سيادة وغياب التفاعل الجيني غير الأليلي في الهجينين، وكان المكون الإضافي للتباين أكبر في المقدار من المكون السيادي. وكانت قيمة المكافئ الوراثي بالمعني الضيق متوسطة الإرتفاع (0.54 و 0.63) في الهجينين على التوالي.