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in

Royo C. (ed.), Nachit M. (ed.), Di Fonzo N. (ed.), Araus J.L. (ed.).
Durum wheat improvement in the Mediterranean region: New challenges

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 40

2000
pages 505-509

QTL-mapping of genomic regions controlling gluten strength in durum (*Triticum turgidum* L. var. *durum*)

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SUMMARY – Gluten strength is the main determinant of the end-use quality in durum. Wheat gluten strength is affected by genetic variation at genes coding for gliadins and glutenins, and by environmental factors. We grew 110 recombinant inbred lines of the cross Jennah Khetifa x Cham1 in 13 environments (sites and years), and analyzed gluten strength in the resulting grain samples. We used Simple Interval Mapping (SIM) and simplified form of Composite Interval Mapping (sCIM) to estimate the positions of QTL and QTL x environment interactions on a 319-marker linkage map. Five QTL were detected. Two of these are on chromosome 6B, one (near *Xgwms644*) with a main effect across environments and one at *XPstIaagMseIcga9* with a QTL-by-environment interaction but no significant main effect. Two QTL exhibited both main effects and QTL-by-environment interactions: one near *XPstIaagMseIctg8* on chromosome 1A and one at the *Gli-B3* locus on chromosome 1B. A QTL near *Xcdo949* on chromosome 4B exhibited a QTL-by-environment interaction but no significant main effect. Together, these five QTL explained 35% of the total phenotypic variation for gluten strength. Our results show a clear combined effect of genes, precipitation, nitrogen fertilization, drought, and heat on gluten strength.

Key words: Durum wheat, gluten strength, QTL analysis, QTL x environment.

RESUME – *"Cartographie des QTL des régions du génome contrôlant la force du gluten chez le blé dur (*Triticum turgidum *L. var.* durum*)". La force du gluten est le principal déterminant de qualité pour l'utilisation finale du blé dur. La force du gluten de blé est affectée par la variation génétique de gènes codant pour les gliadines et les gluténines, et par des facteurs environnementaux. Nous avons cultivé 110 lignées endogames de recombinaison du croisement Jennah Khetifa x Cham1 dans 13 environnements (sites et années), et analysé la force du gluten dans les échantillons de grains résultants. Nous avons utilisé la cartographie d'intervalle simple (Simple Interval Mapping - SIM) et la forme simplifiée de cartographie d'intervalle composite (simplified form of Composite Interval Mapping - sCIM) pour estimer les positions des QTL et les interactions QTL x environnement sur une carte de linkage à 319 marqueurs. Cinq QTL ont été détectés. Deux d'entre eux se trouvent sur le chromosome 6B, un (près de* Xgwms644*) avec un effet principal à travers les environnements et un à* XPstIaagMseIcga9 *avec une interaction QTL-par-environnement mais pas d'effet principal significatif. Deux QTL ont montré tous deux des effets principaux et des interactions QTL-par-environnement : un près de* XPstIaagMseIctg8 *sur le chromosome 1A et un au locus* Gli-B3 *sur le chromosome 1B. Un QTL près de* Xcdo949 *sur le chromosome 4B a montré une interaction QTL-par-environnement mais pas d'effet principal significatif. Ensemble, ces cinq QTL ont expliqué 35% de la variation phénotypique totale pour la force du gluten. Nos résultats montrent clairement un effet combiné des gènes, des précipitations, de la fertilisation azotée, de la sécheresse, et de la chaleur sur la force du gluten.*

Mots-clés : *Blé dur, force du gluten, analyse QTL, QTL x environnement.*

Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) is a tetraploid with A and B genomes (AABB) and is the major source of semolina for the production of pasta, couscous and burghul. The gluten composition of durum cultivars is the main factor that determines the quality of the end products. Strong gluten cultivars produce pasta with greater after-cooking firmness and increased tolerance to overcooking (Payne, 1987; Pogna *et al.,* 1990). The application of electrophoresis to aneuploid stocks has confirmed that *Triticum* chromosomes 1A, 1B, and 1D carry genes for the majority of the glutenin subunits (Payne *et al.,* 1984). Chromosome 1B of durum wheat has been shown to have a special role in improving gluten strength (Pogna *et al.,* 1990). This was associated first with the presence of certain γ-gliadins bands (γ-45/γ-42), encoded at the *Gli-B1* locus (Joppa *et al.,* 1983), but further studies showed that the low molecular weight glutenin subunits (LMW2/LMW1), mapped to the *Glu-B3* on the short arm of chromosome 1B, are rather the causal factors of gluten strength than the gliadins (Payne *et al.,* 1984;

Nachit *et al.,* 1995a,b). Gluten strength was reported to be more influenced by genotypes than by environment or genotype-by-environment (GE) interaction; the genotype effect was around 60%, whereas environment and GE-interaction accounted only for 28% and 12%, respectively (Nachit *et al.,* 1995a).

Materials and methods

Population, field design, locations and genetic map

The map used was constructed using 110 recombinant inbred lines developed at CIMMYT/ICARDA durum breeding program for the Mediterranean dryland from a cross between two durum cultivars "Jennah Khetifa (Tamgurt)" and "Cham1" (Nachit *et al.,* 1995a). The population was grown in different locations and years (Table 1). The trial consisted of 6 blocks; within each block 5 checks and 19 lines were used using the statistical augmented field design. The linkage map is constructed with 319 probes of RFLPs, SSRs, AFLPs, and known genes (Nachit *et al.,* in press).

Table 1. Abiotic stress, nitrogen fertilization, and water regime of the locations x years where the Jennah Khetifa x Cham1 recombinant inbred lines were grown

Code	Location	Abiotic stress	Nitrogen (kg unit/ha)	Precipitation (mm)
SDS98Br	Breda-1997-1998, Syria	Severe drought and heat stress	40	229
SDS99Br	Breda-1998-1999, Syria	Drought and terminal heat	40	193
SDS98Rf	Tel Hadya-1997-1998, Syria	Moderate drought and heat	60	334
SDS99Rf	Tel Hadya-1998-1999, Syria	Moderate drought and heat	60	291
SDS98Lp	Tel Hadya-Late planting	Severe terminal stress (drought + heat)	60	270 + 30 (suppl. irrig.)
SDS99Lp	Tel Hadya-Late planting	Severe terminal stress (drought + heat)	60	290 + 30 (suppl. irrig.)
SDS99In	Tel Hadya-1998-1999, Syria	Favorable conditions	80	290 + 50 (suppl. irrig.)
SDS99Kf	Kfardan-1998-1999, Lebanon	Early cold and late heat	80	365
SDS98Tr	Terbol-1998-1999, Lebanon	Moderate cold and drought	100	450
SDS99Tr	Terbol-1998-1999, Lebanon	Moderate cold and drought	100	340
SDS98Ep	Tel Hadya-Early planting	Severe early cold stress	60	330 + 50 (suppl. irrig.)
SDS98Si	Sids -1997-1998, Egypt	No moisture stress, fertile soil, but heat stress	120	500 (full irrig.)
SDS98Nv	Nile-valley-1997-1998, Egypt	No moisture stress, but sandy soil and heat stress	120	500 (full irrig.)

Sodium dodecyl sulfate sedimentation test

The gluten strength was measured in 7 environments in 1997-1998 and 6 environments in 1998-1999 (Table 1). Grains from each line were tempered at 16% moisture and milled using Udy Cyclone mill equipped with a 100-mesh sieve. Three grams of the wheat meal sample were then used for SDS-test developed for durum wheat (Dick and Quick, 1983). The durum flour was suspended with bromophenol blue solution (1%). The protein hydration was facilitated by the addition of sodium dodecyl sulfate (SDS), which is a mild detergent, and lactic acid. Results are expressed in height (ml) of the interface line between solid (ground sample) and liquid (solution) into a measuring cylinder. The higher the sedimentation volume, the greater the gluten strength.

QTL analysis

The QTLs analysis was performed with software package MQTL (Tinker and Mather, 1995). We performed both Simple Interval Mapping (SIM) and simplified Composite Interval Mapping (sCIM), each with a test for main effect and a test for QTLxE interaction. For SIM, significance thresholds for main effects and QTLxE interactions were estimated using 5000 permutations and a genome-wide significance level of 0.05.

Results and discussion

Effect of environmental conditions on gluten strength

The results showed that the abiotic stressed environments of Breda (Br), Late planting (Lp), and Kfardan (Kf) induced a positive effect on gluten strength (Table 2). In contrast, in the moderate favorable environments (Tel Hadya-99In) and in the high input environments where high nitrogen fertilization is combined with irrigation such as in Sids (Si) and Nile Valley (Nv) of Egypt, medium values for gluten strength were registered. Moreover, the rain-fed environment (98Rf) which was relatively wet in 1998 season during the grain filling period and combined with low nitrogen fertilizer (60 kg/ha) application had negative effect on gluten strength (Table 2). Similar effects were also revealed in the early planting environment of 1997-1998 where in addition to high precipitation, supplementary irrigation was applied (Table 1). These results clearly demonstrate the importance of the effect of environmental conditions (precipitation, nitrogen fertilization, and drought) on gluten strength.

Table 2. Phenotypic mean and standard deviation by environment

Environment	Mean	SD†
SDS98Br	32.16	7.52
SDS99Br	29.38	4.98
SDS98Rf	16.39	5.57
SDS99Rf	30.81	6.5
SDS98Lp	31.6	7.15
SDS99Lp	36.57	8.16
SDS99In	31.05	7.77
SDS99Kf	25.15	6.92
SDS98Tr	21.04	5.21
SDS99Tr	24.3	7.64
SDS98Ep	12.32	4.04
SDS98Si	23.26	5.68
SDS98Nv	21.18	5.33

†Phenotypic standard deviation.

Quantitative trait loci

Five QTLs were detected by SIM: QTL1 on chromosome 1A, QTL2 on chromosome 1B, QTL 3 on chromosome 4B and QTL 5 and QTL 6 on chromosome 6B. In all of these regions of the genome, the sCIM test statistic scan was quite similar to the scan for SIM and did not reveal any additional QTL. QTL1, located 3.2 cM from *XPstlaag&Mselctg8*, showed both a main and an interaction with environments (Table 3).

The position of QTL2 corresponds exactly to that of the *Glu-B3,* which controls LMW-GS. This QTL showed both a main effect and an interaction with environments, and had the greatest effect with up to +8.28 ml in the environment late planting99 (SDS99Lp) (Table 3).

QTL3 is located near *XCDO949*. It showed the largest QTLxE interaction (Table 3). Its effect ranged from -1.64 ml in Breda99 to 5.01 ml in Terbol99 (SDS99Tr). QTL4 and QTL5 are 82 cM apart, with QTL4 near *XPstlaag&Mselcga9* (8.4 cM from *Gli-B2*) and QTL5 at 2 cM from *Xgwm644*. QTL4 exhibited a QTLxE interaction, while QTL5 exhibited a main effect across environment but no interaction (Table 3).

Table 3. Estimated QTL main effects for all environments merged and for each environment

QTLs	QTL1	QTL2	QTL3	QTL4	QTL5
SDS98Br	5.36	6.89	0.04	1.64	2.64
SDS99Br	3.98	3.73	-1.64	1.34	1.97
SDS98Rf	2.61	2.44	1.2	2.27	1.31
SDS99Rf	4.3	5.42	1.89	-0.09	2.51
SDS98Lp	2.85	6.88	-1.46	0.76	2.59
SDS99Lp	4.92	8.28	-0.28	3.16	3.7
SDS99In	3.91	6.89	-0.66	2.97	2.83
SDS99Kf	2.86	3.99	3.79	2.75	3.22
SDS98Tr	2.08	3.99	3.04	0.24	1.89
SDS99Tr	1.38	4.6	5.01	1.82	5.14
SDS98Ep	0.88	0.5	-0.75	0.08	1.1
SDS98Si	-0.67	4.52	0.07	-0.3	3.39
SDS98Nv	1.75	3.45	-0.64	-0.5	2.67
Mean	2.78	4.74	0.74	1.26	2.69

The 5 QTLs explained 35% of the total gluten strength variability. Actually, the QTLs on the group 1 chromosomes explained 27% of the total variability (77% of the 5 QTLs). These results apparently confirm that the LMW glutenin subunits are the major genes controlling gluten strength (Payne, 1987; D'Ovidio, 1993; Nachit *et al.,* 1995a; Elouafi *et al.,* 1998), especially the *Glu-B3* gene coding for the B-LMW-type. In contrast, the *Glu-A1* and *Glu-B1* genes (coding the HMW glutenin subunits) on the long arm of chromosome 1A and 1B, respectively, seems to have no effect on the gluten strength. Actually, the association between HMW glutenin subunits and quality characteristics of durum wheat has not been shown to be as close as that in the bread wheat, although certain HMW alleles can influence the viscoelastic properties of durum wheat dough (Autran and Feillet, 1987). In our analysis this lack of effect could be explained by the fact that HMW7+8 and HMW20 (for Jennah Khetifa and Cham1, respectively) have a similar effect on gluten strength.

There have been no previous reports of genes on chromosome 4B affecting gluten strength. This chromosome is known to be very important for abiotic stress and carries the alpha-lipoxigenase gene. In our analysis, this QTL seems to interact positively with high nitrogen fertilization and highland conditions. The two QTLs detected on chromosome 6B are not similar to *Gli-B2* genes.

Conclusion

In the present work, we detected 5 QTLs related to gluten strength. Environments used were diverse in terms of precipitation, nitrogen fertilization, drought, cold and heat stresses, allowing the detection of QTLs that were expressed consistently across environments as well as those that interact with environment. The 5 QTLs explained 37% of all the variability. Pyramiding these QTLs could allow the durum breeding programs to improve further the grain quality.

References

Autran, J.C. and Feillet, P. (1987). Genetic and technological basis of protein quality for durum wheat in pasta. In: *Protein Evaluation in Cereals and Legumes,* Pattakou V. (ed.), pp. 59-71.

D'Ovidio, R. (1993). Single-seed PCR of LMW glutenin genes to distinguish between durum wheat cultivars with good and poor technological properties. *Plant Mol. Biol.,* 22: 1173-1176.

Elouafi, I., Martin, A., Martin, L.M. and Nachit, M.M. (1998). Association of introgressed *T. dicoccoides* seed storage protein subunits with gluten strength and protein content in durum (*T. turgidum* L. var. *durum*). In: *International Wheat genetics Conference. Proceedings of the International Wheat Genetics Symposium* 9, Saskatoon (Canada), 2-7 August 1998, Vol. 4, Skinkard, A.E. (ed.). University of Saskatoon, Saskatoon, pp.145-147.

Joppa, L.R., Khan, K. and Williams, N.D. (1983). Chromosomal location of genes for gliadin polypeptides in durum wheat *Triticum turgidum. Theor. Appl. Genet.,* 64: 289-293.

Liu, C.Y. (1995). Identification of a new low Mr gluten subunit locus on chromosome 1B of durum wheat. *J. Cereal Sci.,* 21: 209-213.

Nachit, M.M., Baum, M., Impilia, A. and Ketata, H. (1995a). Studies on some grain quality traits in durum wheat grown in Mediterranean environments. In: *Procceedings of the Seminar on Durum Wheat Quality in the Mediterranean Region,* Di Fonzo, N., Kaan, F. and Nachit, M.M. (eds), Zaragoza (Spain), 17-19 November. *Options Mediterraneenes, Mediterranean Seminars,* No. 22, pp. 181-188.

Nachit, M.M., Sorrels, M., Labhilili, M., Baum, M., Asbati, A., Azrak, M. and Baalabaki, M. (1995b). Durum wheat improvement, grain quality. *Cereal Improvement Program. Ann. Report,* 1995, pp. 102-105.

Payne, P.I. (1987) Genetics of wheat storage proteins and the effect of allelic variation on breadmaking quality. *Annu. Rev. Plant Physiol.,* 38: 141-153.

Payne, P.I., Holt, L., Jackson, E. and Law, C.N. (1984). Wheat storage proteins: Their genetics and their potential for manipulation by plant breeding. *Phil. Trans. R. Soc. Lond.,* 304: 359-371.

Pogna, N.E., Autran, J.C., Mellini, F., Lafiandra, D. and Feillet, P. (1990). Chromosome 1B-encoded gliadins and glutenin subunits in durum wheat: Genetics and relationship to gluten strength. *J. Cereal Science,* 11: 15-34.

Tinker, N.A. and Mather, D.E. (1995). *MQTL: Software for simplified composite interval mapping of QTL in multiple environments.* J quantitative trait loci (http://probe.nalusda.gov:8000/otherdocs/jqtl/2).