

Revealing the genetic relationship of dough mechanical properties with loaf volume using QTL analysis of mixograph traits in wheat

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INTRODUCTION

Mixograph is routinely used to test and evaluate the quality and functionality of wheat and flours related to cereal based food products. It is a direct method to relate and understand bread making quality (BMQ), which is considered as a complex trait because of the influence of different parameters of wheat grain, e.g. Gpc, kernel hardness, moisture content, starch content and dough polymer structure. Thus mixograph, which considers above parameters, can help in the appropriate selection of good BMQ lines, which in turn, if studied separately is labour intensive and time consuming.

MATERIALS AND METHODS

The Recombinant Inbred Line (RIL) population of HI977 x HD2329, comprising 105 lines was grown for two consecutive years (2004-05, 2005-06), in an augmented block design at three agroclimatically different locations. The bulked samples from each RILs were used for mixing test.

Mixing tests were conducted using a computerized 10 g Mixograph (National Mfg. Co., Lincoln, Nebraska, U.S.A.). Wheat grains were tempered to a moisture content of 14% milled, the whole grain flour was collected and kept overnight. The 10 g of whole grain flour was added with 6.2 ml of distilled water, to determine the mixing characteristics of the flour at 62% absorption according to¹ and mixed for 8 min with a mixing head speed of 88 rpm. The data point was recorded at 10 points per second (pps). The complete population (with 5 repeating checks), was analysed without repeat except for every 11th sample to estimate the repeatability. The mixogram data were analysed with the software Mixsmart v. 3.40 and 44 parameters were recorded, of which 9 parameters were considered for QTL analysis.

The results from the Mixsmart® software were investigated by constructing a curve with two envelopes and one midline. The loaf volume (Lv) estimation and

QTL mapping was performed as per the procedure detailed in our earlier report².

Strong correlation between midline and top envelope parameters were observed, however best repeatability was observed with midline parameters (Martinant et al. 1998). The mixograms analyzed with Mixsmart ver. 3.4 software generated 44 mixograph parameters. To enhance the feasibility of analysis, the 44 traits from 6 data sets (3 locations x 2 years) were correlated together and cluster diagrams were constructed to identify closely related traits. Among the 44 mixograph traits, following 9 traits representing overall picture of the dough development, such as optimum dough development, break down and consistency were selected for QTL mapping:

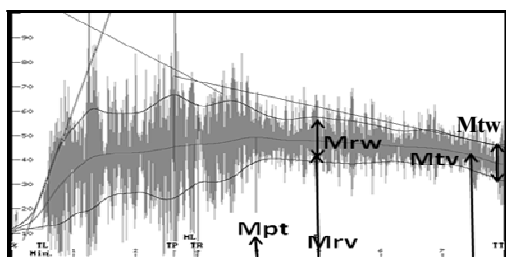
- 1) Midline peak time (Mpt): The time in minutes required for optimum dough development.
- 2) Midline right value (Mrv): The height of the peak at 1 min after MPT.
- 3) Midline curve tail value (Mtv): It indicates the total breakdown or loss of strength during mixing³.
- 3) Band width: The width of the mixogram curve and the angle of descent indicate the tolerance of the dough to over mixing.
- 4) Midline right width (Mrw): Width of the peak at 1 min after Mpt. It exhibits dough tolerance during over mixing.
- 5) Midline curve tail width (Mtw): Width of the peak at the end of the mixing period (8 Min.). It denotes the consistency of the dough at the end of the mixing⁴.
- 6) Midline right integral (Mri): It is a total area under the mixogram midline curve from the starting point to one min after the peak time⁵.
- 7) Midline curve tail area (Mti): It is an area under the curve till the end of the mixing process⁶.
- 8) Envelope peak integral (Epi): Total area under the mixogram envelope curve calculated from the starting point to envelope peak time.
- 9) Weakening slope (Ws): It indicates the rate of breakdown while mixing and calculated as the

difference of curve height at peak time and curve height at tail (Mtv) ⁴.

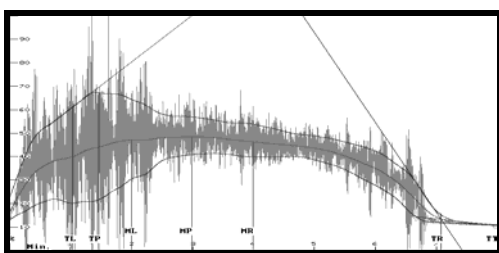
QTL MAPPING

The Win QTL cartographer ver. 2.5⁷ was used for Composite interval mapping as described in our earlier report².

RESULTS



HI977 – Good bread making



HD2329 – Poor bread making

Fig. 1 Mixogram showing the strong (HI 977) and weak (HD 2329) dough characteristics

The mixogram of the parents HI977 and HD2329 has been represented in Fig. 1. Based on the nature of curves the overall dough strength was of HI977 comparatively higher than HD2329. Transgressive segregants were identified for all mixograph traits with wide range, suggesting that both the parents carry both favourable and unfavourable alleles. The mixing peak time (Mpt) was also higher in HI977 than HD2329. Furthermore, correlation between Lv and 9 mixograph traits were performed and observed to be insignificant (at $P \leq 0.05$) for all the locations (data not shown).

About 169 QTLs were identified for mixograph, 21 QTLs were detected on 6B, followed by 18 on 1B, 3A & 5B chromosomes, 17 on 6D, 13 on 2B and 10 on 2A & 5D chromosomes. The mixograph trait Ws had maximum QTLs (27) followed by Mpt (22), Mrw (22), Mtw (18), Epi (18), Mri (18), Mtv (18), Mrv (16) and Mti (10) (data not shown).

QTL CLUSTERS FORMED BY QTLs AFFECTING YIELD, DOUGH RHEOLOGY AND BMQ TRAITS

A total of 45 QTL clusters were detected on 14 chromosomes of which 6 clusters were identified on chromosomes 1B followed by 5 (5B), 4 (1D, 2B, 3A, 6B, 6D), 3 (1A, 5A, 5D) and 1 (2A, 3D, 4B, 4D, 6A).

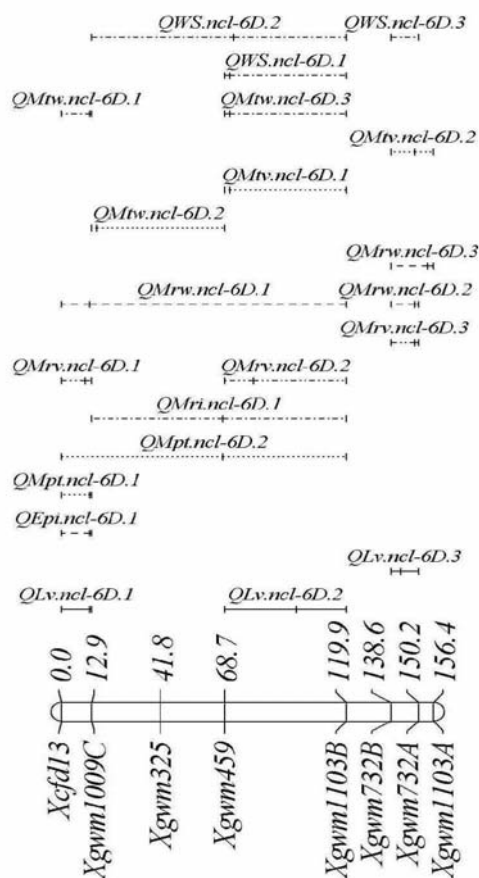


Fig. 2 Chromosome 6D, showing QTLs of Lv, Epi, Mrv, Mri, Mrw, Mtw, Mtv and Ws forming a clusters

A cluster on chromosome 6D had a maximum of 12 QTLs (Fig. 2), while 10 QTLs were identified in the cluster of 2A chromosome. Mixograph traits often collocated together, rather than with other traits and three traits Ws, Mrv and Mrw clustered often with Lv trait (Fig. 2). In chromosome 2A *QLv.ncl-2A.1* collocated with Epi, Mpt, Mri, Mrv, Mtw, Mti and Ws and in chromosome 6D *QLv.ncl-6D.1* with Epi, Mpt, Mrv and Mrw.

DISCUSSION

Dough development is the outcome of adding mechanical energy (strain) while mixing water with flour. It is a complex and dynamic process and is influenced by several flour components including proteins, starch, and lipids that vary among cultivars and even among batches of flour from the same

cultivar⁸. However, majority of dough mixing traits are influenced by complex traits governed by QTLs.

The QTLs for mixograph traits were analysed and plotted on respective chromosomes. Some of the traits formed QTLs clusters, with 11 QTLs in a cluster were identified on chromosome 2A between the markers *Xgwm830* and *Xgwm249A*. These QTLs colocalized with *Lv*, *Sv*, *Tw*, *Epi*, *Mpt*, *Mri*, *Mrv*, *Mrw*, *Mti*, *Mtv* and *Ws*. The QTLs of *Lv* and *Ws* were consistent at more than one location and all the QTLs in this interval had positive additive effect except *Ws*, which had negative additive effect. The colocalization of mixograph traits along with *Lv*, stressed the role of these component traits in governing *Lv*. On Chromosome 5D, within the interval of *Xgwm736A* and *Xgwm1016*, ten QTLs comprising *Lv*, *Mpt*, *Mrv*, *Mtw*, *Mrw* and *Ws* were observed. Among these QTLs, only *Mtw* (*QMtw.ncl-5D.1*) was the consistent one. On chromosome 6B, eight QTLs were identified in the interval of *Xbarcl178* - *Xgwm132C* including *Lv* (*QLv.ncl-6B.1*), *Epi*, *Mri*, *Mrv*, *Mtv*, *Mtw* and *Ws*. Among these only *Ws* (*QWs.ncl-6B.1*) was observed to be consistent.

The same type of cluster was observed on chromosome 6D within the interval of *Xcfd13-Xgwm1009C*, where in QTLs for 7 traits were detected, namely *Lv*, *Epi*, *Mpt*, *Mrv* and *Mtw*. Among these QTLs, *QEpi.ncl-6D.1*, *QMtw.ncl-6D.1*, *QWs.ncl-6D.1* and *QLv.ncl-6D.1* were consistent in more than one location. The graphical picture of the chromosome 6D, along with the traits, *Lv*, *Epi*, *Mpt*, *Mrw* and *Mtw* was depicted in the Fig. 2.

Though the correlation between *Lv* and other mixograph traits were not significant, QTL mapping revealed the genetic relationship between these traits through QTL clusters. Such a combination helps in marker assisted selection using the markers flanking this interval. Saturating this region with more markers would help in identifying close flanking markers for these QTL and pyramiding of these QTL clusters⁹ will result in overall improvement of other traits.

REFERENCES

1. Pon CR, Lukow OM, Buckley DJ (1989) A multichannel, computer based system for analysing dough rheology. *Journal of Texture Studies* 19:343–360
2. Elangovan M, Rai R, Dholakia BB, Lagu MD, Tiwari R, Gupta RK, Rao VS, Röder MS, Gupta VS (2008) Molecular genetic mapping of quantitative trait loci associated with Loaf volume in hexaploid wheat (*Triticum aestivum*). *Journal of Cereal science* 47:587-598
3. Martinant JP, Nicolas Y, Bouguennec A, Popineau Y, Saulnier L, Branlard G (1998) Relationships between mixograph parameters and indices of

wheat grain quality. *Journal of Cereal Science* 27: 179–189

4. Ohm JB, Chung OK, Deyoe CW (1998) Single-kernel characteristics of hard winter wheats in relation to milling and baking quality. *Cereal Chemistry* 71:156–161
5. Nelson JC, Andreescu C, Breseghello F, Finney PL, Gualberto DG, Bergman CJ, Pena RJ, Perretant MR, Leroy P, Qualset CO, Sorrells ME Quantitative trait locus analysis of wheat quality traits. *Euphytica* 149:145–159
6. Breseghello F, Sorrells ME (2005) Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L) cultivars. *Genetics* 177:1165–1177
7. Basten CJ, Weir BS, Zeng ZB (2000) QTL Cartographer Version 2.5 A reference manual and tutorial for QTL mapping. Department of Statistics, North Carolina State University, Raleigh, NC
8. Blechl A, Lin J, Nguyen S, Chan R, Anderson OD, Dupont FM (2007) Transgenic wheats with elevated levels of Dx5 and/or Dy10 high-molecular-weight glutenin subunits yield doughs with increased mixing strength and tolerance. *Journal of Cereal Science* 45:172-183
9. Ashikari M, Sakakibara H, Lin S, Yamamoto T, Takashi T, Nishimura A, Angeles ER, Qian A, Kitano H, Matsuoka M (2005) Cytokinin oxidase regulates rice grain production. *Science* 309:741-745