# Potential uses of microsatellites in marker-assisted selection for improved bread-making quality in wheat

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## SUMMARY

Implementation of marker assisted selection (MAS) in conventional breeding programs could allow assessment of the genetic potential of specific genotypes prior to their phenotypic evaluation. Furthermore, it could identify important trait alleles or marker-trait associations for further determination of a precise position for the loci of interest. Potential uses of microsatellite markers in molecular evaluation of bread-making quality was tested in a sample of 69 wheat genotypes that were genotyped with 3 microsatellites linked to previously mapped QTLs for loaf volume and Hagberg falling number on chromosome 3A. A total of 19 alleles were found, with an average of 6.33 alleles per loci, and average PIC value of 0.40. Specific SSR alleles were tested for association with bread-making related parameters. The association study approach, which uses statistical analysis of marker and phenotypic data, showed significant association of a specific allele at the GWM674 locus with Hagberg falling number in wheat.

**Index words:** wheat, SSRs, bread-making quality

## INTRODUCTION

The molecular basis for the role of wheat protein and non-protein components of bread-making quality (BMQ) is not fully understood. The high molecular weight (HMW), and low molecular weight (LMW) glutenin subunits account for about 60% of the variation in bread making quality. Other non-gluten factors such as amylase activity (Mansour, 1993), grain hardness (Hogg *et al.*, 2005) and other minor prolamine-like proteins (Clarke *et al.*, 2003) may account for a substantial remainder of the unexplained variation.

Several studies have found evidence of the importance of chromosome 3A in wheat. QTLs were detected for quality related traits (Gross *et al.*, 2002, Schmidt *et al.*, 2004) and for yield related traits (Shah *et al.*, 1999, Campbell *et al.*, 2003). Law *et al.* (2005) reported two highly significant QTLs for the BMQ parameters, namely loaf volume and Hagberg falling number (HFN). It was proposed that a single gene *Lvl 1*, located on the 3AL chromosome, was responsible for variation in baking quality performance. The second detected QTL, also located on 3A chromosome, could be a single gene controlling HFN.

Currently, the most common method for QTLs mapping involves generating populations derived from single crosses and estimating recombination frequencies between marker loci and the genes of interest. Despite the widespread use of this approach, marker application is being impeded through the lack of information on the polymorphism and marker trait associations in genetic material relevant to most breeding programs worldwide (Eisemann *et al.*, 2004). After the QTL detection phase, the next step towards genetic profiling of complex traits is assessment of the allelic variation at candidate loci. Marker-trait



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association studies are considered a feasible strategy for elucidation of the molecular basis of complex traits in human genetics, and in animal and plant breeding. A broad range of studies in barley (Ivandić *et al.*, 2003), potato (Gebhardt *et al.*, 2004) and wheat (Bai *et al.*, 2004, Breseghello and Sorrells, 2005) have been performed in which associations between the phenotypic and the genotypic variability were established.

This research has been conducted in order to explore potential uses of microsatellite markers in the evaluation of bread-making quality and testing the effectiveness of the association study approach for specific SSR alleles and desirable phenotypic values of five BMQ related traits.

## MATERIAL AND METHODS

The 69 wheat cultivars analysed in this study represent a subset of Novi Sad Core Collection located at Institute of Field and Vegetable Crops, Novi Sad, Serbia and Montenegro (Table 1).

Table 1. Hexaploid wheat cultivars and breeding lines

| Genotype | Origin | Genotype | Origin | Genotype | Origin |
|---|---|---|---|---|---|
| Agent | USA | KG 56 | SCG | Panonija | SCG |
| Amadeus | AUT | Klein Fortin | ARG | Partizanka | SCG |
| Anastasija | SCG | Klein Toledo | ARG | Pergamino Gaboto | ARG |
| Auburn | USA | Košuta | SCG | Pesma | SCG |
| Avalon | GBR | Leones Inta | ARG | Pinzon Inta | ARG |
| Balkan | SCG | Lepenica | SCG | Pobeda | SCG |
| Bankuti 1205 | HUN | Lerma Rojo | MEX | Proteinka | SCG |
| Bezostaya 1 | RUS | Manitou Insens | CAN | Radika | FYRM |
| Cajeme 71 | MEX | Mina | SCG | Renan | FRA |
| Caldwell | USA | Mironovskaya 808 | UKR | Renesansa | SCG |
| Cappelle Desprez | FRA | Nakhodka 5 | UKR | Sava | SCG |
| Centurk | USA | Nizija | SCG | Siete Cerros | MEX |
| Chris | CAN | NS 0.1079 | SCG | Sofija | SCG |
| Cook | AUS | NS 0.1081 | SCG | Sonata | SCG |
| Dnestrovskaya 25 | RUS | NS 0.733 | SCG | Sreća | SCG |
| Evropa 90 | SCG | NS 121/98 | SCG | Sremica | SCG |
| F 53-70 | ROM | NS 164/98 | SCG | Super zlatna | CRO |
| Fawwon 77 | MEX | NS 42/00 | SCG | Suwon 92 | KOR |
| Fawwon 78 | MEX | NS 45/00 | SCG | Tavrichanka | UKR |
| Flamura 80 | ROM | NS 57/00 | SCG | Tiha | SCG |
| Garazinko | BRA | NS 82/00 | SCG | UPI 301 | IND |
| Hira | IND | NSR 2 | SCG | Vireo S | MEX |
| Jugoslavija | SCG | Odeskaya 51 | UKR | Zugoly | HUN |

Based on detailed (54 traits) evaluation of 710 wheat genotypes from 52 countries, a subset of 69 genotypes with the highest overall phenotypic variation was identified for screening with microsatellites. Thirty-one cultivars were bred in Serbia. The remaining cultivars had an international breeding background. Wheat cultivar Chinese Spring was used as control cultivar in the SSR analysis.

DNA was isolated from 0.2 g of ground seed according to Plaschke *et al.* (1995). Microsatellite markers GWM32, GWM674 and GWM720 from chromosome 3A were analysed in 20 µl PCR reaction containing: 30 ng DNA, 1xPCR buffer, 1.5 mM $MgCl_2$, 0.2 nM of each dNTP, 10 pmol of each SSR primers, and 2 units of Taq polymerase (Amersham Pharmacia). The following thermal profile was used for SSR amplification: initial denaturation for 3 min at 94° C, 45 cycles of 1 min at 94°, 2 min at 72° C, and final extension for 10 min at 72° C. Annealing of primers was 1 min at 60° C for GWM674 and GWM720, and 55° C for GWM32. PCR products were size separated by standard 0.4 mm 6% PAA denaturing gels and visualised by silver staining according to Sanguinetti *et al.* (1994). Marker fragments were scored according to their electrophoretic mobility in comparison with the Chinese Spring standard.

The following bread-making quality components were analysed: Hagberg falling number (s) determined by ICC standard method 107/1 (1999), Bread yield (g/100g flour), Loaf volume yield (ml/100g flour), Loaf volume ($cm^3$, rapeseed displacement method), and Loaf quality (scored 0-7, based on loaf volume, the spring and the break of the crust, cell size and distribution, and resilience and softness of the crumb). The values of bread-making quality components were based on 2-year average.

Statistical analysis: PIC score was calculated according to Anderson *et al.* (1993). For microsatellite markers GWM674 and GWM32 the strength of association between marker alleles and bread-making quality parameters was tested using One-Way ANOVA. Wheat genotypes were grouped according to the presence of particular microsatellite alleles (independent variables) and group mean values of analysed BMQ parameters were treated as dependent variables. In the case of microsatellite marker GWM720, where significant differences of variances for two BMQ parameters between allele-groups were found, a non-parametric ANOVA - Kruskal-Wallace H-test was applied. All tests were performed in SPSS ver.10.

## RESULTS

Nineteen alleles were found in the 69 analysed genotypes, averaging 6.33 alleles per SSR locus. GWM674 possessed a low PIC score of 0.17, with 4 alleles detected (Table 2). At GWM720, 10 alleles were found and the marker PIC value was 0.66, while at GWM32, 5 alleles were detected with a PIC value of 0.38.

Statistical analysis of marker-trait association was performed only for alleles present in 5 or more genotypes. Based on that criteria two alleles (*a* and *b*) for GWM674, two alleles (*d* and *e*) for GWM32 and three alleles (*c, g* and *i*) for GWM720 were analysed.

At the SSR locus GWM674, genotypes with allele ***a*** have had higher mean values compared to those with allele *b* for all analysed BMQ parameters (Table 3). Significant difference between group means was found only for Hagberg falling number (Table 4).

Table 2. Allele frequency at GWM720, GWM674 and GWM32 loci

| Allele | Number of genotypes | Allele frequency | Allele | Number of genotypes | Allele frequency |
|---|---|---|---|---|---|
| GWM720 | | | GWM674 | | |
| a | 1 | 0.014 | a | 62 | 0.900 |
| b | 2 | 0.030 | b | 5 | 0.072 |
| c | 36 | 0.521 | c | 1 | 0.014 |
| d | 1 | 0.014 | d | 1 | 0.014 |
| e | 2 | 0.030 | GWM32 | | |
| f | 1 | 0.014 | b | 3 | 0.043 |
| g | 9 | 0.130 | c | 4 | 0.058 |
| h | 4 | 0.058 | d | 5 | 0.072 |
| i | 11 | 0.159 | e | 54 | 0.783 |
| k | 2 | 0.030 | f | 3 | 0.043 |

Statistical analysis of GWM32 alleles and BMQ parameters showed that genotypes with allele ***e*** possessed higher mean values compared to the ones with allele *d*, for all analysed BMQ traits (Table 3), but no significant differences between marker classes were observed (Table 4).

Table 3. Basic statistics for analysed BMQ parameters in regard to used SSRs

| Locus | allele | N | HFN | | Bread yield | | Loaf vol. yield | | Loaf volume | | Loaf quality | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| **GWM 674** | ***a*** | 62 | 356.6 | 99.4 | 139.5 | 2.7 | 519.5 | 75.8 | 1288.5 | 171.57 | 4.5 | 1.7 |
| | ***b*** | 5 | 256.0 | 56.4 | 137.7 | 1.7 | 509.1 | 67.0 | 1276.0 | 142.9 | 3.8 | 1.4 |
| **GWM 32** | ***d*** | 5 | 296.8 | 118.9 | 138.12 | 2.4 | 509.3 | 96.3 | 1271.0 | 220.2 | 3.9 | 2.5 |
| | ***e*** | 54 | 353.7 | 100.6 | 139.38 | 2.9 | 518.9 | 73.5 | 1288.5 | 166.2 | 4.4 | 1.7 |
| **GWM 720** | ***c*** | 36 | 365.8 | 92.2 | 140.1 | 2.4 | 538.6 | 70.2 | 1330.4 | 164.7 | 4.9 | 1.3 |
| | ***g*** | 9 | 338.4 | 84.4 | 138.6 | 2.0 | 480.1 | 74.7 | 1201.7 | 167.6 | 3.7 | 2.2 |
| | ***i*** | 11 | 309.5 | 113.6 | 138.3 | 3.8 | 496.2 | 85.8 | 1239.1 | 185.9 | 3.4 | 2.1 |

Table 4. Basic statistics (ANOVA) for analysed BMQ parameters in regard to GWM674 and GWM32

| BMQ parameters | **GWM 674** | | | **GWM 32** | | |
|---|---|---|---|---|---|---|
| | df | F | Sig. | df | F | Sig. |
| HFN | 1, 65 | 4.941* | 0.030 | 1, 57 | 1.426 | 0.237 |
| Loaf volume | 1, 65 | 0.025 | 0.875 | 1, 57 | 0.048 | 0.827 |
| Bread yield | 1, 65 | 2.188 | 0.144 | 1, 57 | 0.895 | 0.348 |
| Loaf vol. yield | 1, 65 | 0.088 | 0.767 | 1, 57 | 0.074 | 0.786 |
| Loaf quality | 1, 65 | 0.725 | 0.398 | 1, 57 | 0.321 | 0.573 |

For GWM720 Levene's test showed that variances of allele-groups were non-homogenous (Table 5), and sample sizes were unequal (Table 3) indicating that a non-parametric ANOVA would be appropriate to test the strength of association between marker alleles and bread-making quality parameters. Results of non-parametric Kruskal-Wallis H test determine that allele-groups do not differ in mean ranks for the criterion variables (Table 6). Based on presented results it could be concluded that no significant association were found between any particular allele in GWM720 locus and any BMQ parameter.

Table 5. Levene's test of homogeneity of variances for GWM 674, GWM 32 and GWM720

| BMQ parameters | Levene statistics | df1 | df2 | Sig. |
|---|---|---|---|---|
| GWM674 | | | | |
| HFN | 2.942 | 1 | 65 | 0.091 |
| Loaf vol. | 0.327 | 1 | 65 | 0.569 |
| Bread yield | 0.943 | 1 | 65 | 0.335 |
| Loaf vol. yield | 0.185 | 1 | 65 | 0.669 |
| Loaf quality | 0.107 | 1 | 65 | 0.744 |
| GWM32 | | | | |
| HFN | 0.154 | 1 | 57 | 0.697 |
| Loaf vol. | 0.785 | 1 | 57 | 0.379 |
| Bread yield | 0.253 | 1 | 57 | 0.617 |
| Loaf vol. yield | 0.754 | 1 | 57 | 0.389 |
| Loaf quality | 2.588 | 1 | 57 | 0.113 |
| GWM720 | | | | |
| HFN | 0.703 | 2 | 53 | 0.499 |
| Loaf vol. | 0.457 | 2 | 53 | 0.636 |
| Bread yield | 3.206* | 2 | 53 | 0.048 |
| Loaf vol. yield | 0.962 | 2 | 53 | 0.389 |
| Loaf quality | 6.976** | 2 | 53 | 0.002 |

Table 6. Kruskal-Wallis test statistics for GWM720[a]

| | HFN | Loaf vol. | Bread yield | Loaf vol. yield | Loaf quality |
|---|---|---|---|---|---|
| Chi-Square | 3.057 | 4.589 | 4.027 | 4.483 | 4.749 |
| df | 2 | 2 | 2 | 2 | 2 |
| Asymp. Sig. | 0.217 | 0.101 | 0.134 | 0.106 | 0.093 |

[a] grouping variable: allele

## DISCUSSION

Based on chromosomal maps of chromosome 3A, Law *et al.* (2005) previously identified two highly significant QTLs for loaf volume and Hagberg falling number, (Cappelle Desprez x Bezostaya 1) flanked by 7 microsatellite markers. These results showed that a QTL for loaf volume is located on the long arm of chromosome 3A, approximately 30-40 cM from the centromere, while a QTL for Hagberg falling number is located in the centromeric region of 3A chromosome.

The microsatellite map of chromosome 3A (Law *et al.*, 2005) places marker GWM720 at the proximal region of 3AL. Of the three microsatellites analysed, this marker is nearest to the suggested QTL region for loaf volume. In this study GWM720 allele ***c*** had the highest frequency and genotypes with allele ***c*** possessed higher mean values for all analysed BMQ parameters. Unfortunately, results of statistical analysis do not confirm a significant association between any particular allele at this locus and any baking quality parameter. The reason for this could be in either the strong effect of GxE interaction on BMQ parameters or could be the result of unsuitable genetic background of the analysed genotypes in respect to BMQ performance in Serbian environment.

Marker GWM674 is located at the centromere region of 3AL, while GWM32 is located at the centromere region of 3AS (Roder *et al.*, 1998). The absence of allele GWM32 association and the presence of significant differences in marker-trait association for allele GWM674 and Hagberg falling number, could imply QTL for analysed trait to be located at centromere region of 3AL, as also pointed out by Law *et al.* (2005). Even so, this fact should be reconsidered because marker GWM674 showed low allelic variability (4 alleles), only two of which could be statistically analyzed.

Although for marker GWM32 statistically significant differences were not found for quality parameters, it should be pointed out the genotypes that possess allele ***e*** also possess higher values for all analysed quality parameters. If the frequencies of named target alleles of all three GWM markers are compared, it is clear they are present in the highest frequencies in analysed loci (allele ***c*** 52% at GWM720, allele ***a*** 90% at GWM674 and allele ***e*** 78% at GWM32). High frequencies of specific alleles could indicate genomic regions responsible for expression of the mentioned quality traits. Since analysed genotypes are commercial cultivars or advanced lines it seems that, in different countries, these regions have been under strong selection pressure during the breeding process. In this content, the statement of Simko (2004) that an associative study approach most likely detect only alleles with high frequencies, is proven to be correct.

Our future strategy is based on identification of the desirable regions, which, in respect to BMQ, could gain benefits in breeding for these traits. We consciously accept to, among all alleles, select even "false positive" ones, assuming the frequency of "real positive" ones could and would bring about benefits to our wheat breeding program targeting excellent BMQ. A similar approach, which proved to be very informative and useful, has already been determined to be useful in molecular breeding for improved grain yield in wheat (Kobiljski *et.al.*, 2005).

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