

IMPROVING WHEAT YIELDS USING ASSOCIATION MAPPING

Steve Quarrie¹, Dejan Dodig², Boris Kobiljski³, Vesna Kandić², Jasna Savić⁴, Dragana Rančić⁴, Sofija Pekić Quarrie⁴

Abstract

Increasing plant biomass is a major breeding target, and molecular marker technologies can help breeders meet the challenge of increasing yields.

We used association mapping with 96 bread wheats prepared by the IFVC Novi Sad, representing 20 countries around the world, that had been screened for marker alleles at 47 simple-sequence-repeat (SSR) loci. Genotypes were trialled in the field in Zaječar each year from 2002 to 2006, with varying water and assimilate availability treatments. Association mapping was carried out using ANOVA of phenotype means for each allele at each SSR marker locus.

Linear regressions of individual genotype biomass against experiment/treatment mean biomass over all 96 genotypes were used to interpolate genotype biomass at site biomass yields of 15 t/ha. Genotypes producing the best biomass did not always give the best yields. Thus, Phoenix gave the fourth highest biomass, but was ranked 32 for yield.

Association mapping identified 19 marker loci significantly associated with biomass. However, none of the ten highest biomass genotypes was associated with the highest biomass allele at each of those loci. Therefore, biomass (and thereby yield) could be improved in new genotypes by replacing low-biomass alleles in existing high biomass genotypes with high-biomass alleles from other high-biomass genotypes.

Several of these high-biomass genotypes have been crossed to combine more favorable biomass alleles, and 27 progeny at the F4 generation grown in 2010-2011. A few of these showed both very high biomass/stem and yield/ear. They are currently being screened with SSR molecular markers.

Keywords: wheat, biomass, yield, molecular marker, breeding

Introduction

To meet the challenges of rising demands for food worldwide, total wheat production will need to increase faster than at any time previously. Although climate change will contribute some of this increase, mainly through rising CO₂ concentrations (Ewert *et al.*, 2005), most of the increase in wheat production is predicted to come from technological developments: improved agronomy and improved genetic yield potential (Ewert *et al.*, 2005). Increasing yield means increasing either harvest index (HI, the proportion of biomass converted into harvestable grains) or total plant above-ground biomass. Austin (1980) calculated a theoretical maximum HI of around 0.62, and modern high-yielding varieties are already approaching this theoretical limit (Foulkes *et al.*, 2007). Therefore, most breeding effort is focused on increasing efficiency of converting the sun's energy into biomass and channelling biomass into grain yield. Thus, increasing plant biomass is a major breeding target.

¹ Visiting Professor, School of Biology, Newcastle University, NE1 7RU, UK

² Maize Research Institute "Zemun Polje", R. Serbia

³ Institute of Field and Vegetable crops, Novi Sad, R. Serbia

⁴ Faculty of Agriculture, Belgrade - Zemun, R. Serbia

Molecular marker technologies are essential to help breeders meet the challenge of increasing yields (Reynolds and Borlaug, 2006; Gupta *et al.*, 2010). Although quantitative trait locus analysis with populations derived from crossing two parents has been used for many years to locate genes for traits of interest to breeders, it has the disadvantage of testing only two alleles at each locus (Neumann *et al.*, 2011). Association mapping overcomes this limitation as it uses the full range of alleles available in breeders' genetic resources to study marker-trait associations, and is now widely in use for bread wheat (e.g. Crossa *et al.*, 2007; Neumann *et al.*, 2011 and references cited therein).

We present here an example of the use of association mapping to identify opportunities to improve biomass and, thereby, yield of bread wheat based on trials carried out in Serbia.

Material and methods

Ninety-six bread wheat (*Triticum aestivum* L.) genotypes prepared by the Institute of Field and Vegetable Crops (IFVC), Novi Sad, Serbia, representing 20 countries around the world, were used in this study (see Dodig *et al.*, 2010 for details of the genotypes). Genotypes were chosen on the basis of contrasting expression for one or other of 12 traits of agronomic importance such as flowering time, grains per spike, tillering capacity, grain-fill rate and seed size (Quarrie *et al.*, 2003).

The 96 genotypes were trialled at the Centre for Agricultural and Technological Research, Zaječar under varying water and assimilate availability treatments each year from 2002 to 2006 with two replicate plots. In 2002, 2003 and 2004, drought stress was given by a rain-out plot shelter (Dodig *et al.*, 2002), and other plots were either rainfed or irrigated to maintain soil water content near field capacity. Terminal drought stress in 2005 and 2006 was simulated by cutting off all leaves soon after anthesis. Control plants were left intact. Further details of sowing density, plot sizes and agronomic practices are given in Dodig *et al.* (2010).

At final harvest, yield, yield components and above-ground biomass were recorded to give, in total, 26 year x treatment x replicate values for each trait.

The 96 genotypes were sampled for DNA extraction and screened for marker alleles at 47 simple-sequence-repeat (SSR) loci using an automatic ABI 377 Sequencer at the John Innes Centre, Norwich, UK, as described in Dodig *et al.* (2010). Association mapping was done using one-way ANOVA of phenotype means for each allele at each SSR marker locus.

Crosses were made between selected genotypes on the basis of both initial marker locus-trait associations and traditional phenotypic breeding criteria (plant height, flowering time, spike characteristics, disease resistance). Progenies of crosses were taken through to F4 seeds, keeping only those lines that were phenotypically adapted on the basis of medium height, early/medium flowering date, large spikes, large grains and good disease resistance. In total 45 genotypes (27 F4 families, 16 genotypes representing other breeding material and the two Serbian Commission wheat standards Pobeda and Renesansa) were trialled at the Maize Research Institute, Zemun Polje, Serbia in 2010/11 and yield, its components and above-ground biomass were recorded.

Results and discussion

Linear regressions of individual genotype above-ground biomass m^{-2} against mean biomass m^{-2} over all 96 genotypes for the 26 years x treatments x replicates showed a wide range in regression slopes (Fig. 1). To simplify association mapping, these fitted regressions were used to interpolate genotype biomass yield at harvest at a high biomass site (1500 g m^{-2} : 15 t ha^{-1})

and a low biomass site (5 t ha⁻¹). Results are presented here for only the 15 t ha⁻¹ high biomass site. A similar analysis for yield (interpolating genotype yields at a high yielding site of 7 t ha⁻¹) showed that genotypes producing the best biomass did not always give the best yields. Thus, although Ivanka gave both the third highest biomass and yield (19.8 and 10.0 t ha⁻¹ respectively) and NS46/90 the tenth largest biomass and fourth highest yield (19.0 and 9.8 t ha⁻¹ respectively), Phoenix gave the fourth highest biomass (19.6 t ha⁻¹), but only 7.8 t ha⁻¹ yield (rank 32).

Linear regressions of yield against biomass (Fig. 2) also showed considerable variability, reflecting varying proportions of biomass being converted into yield (HI). This clearly demonstrated the high biomass potentials for the genotypes Phoenix and NS46/90, but

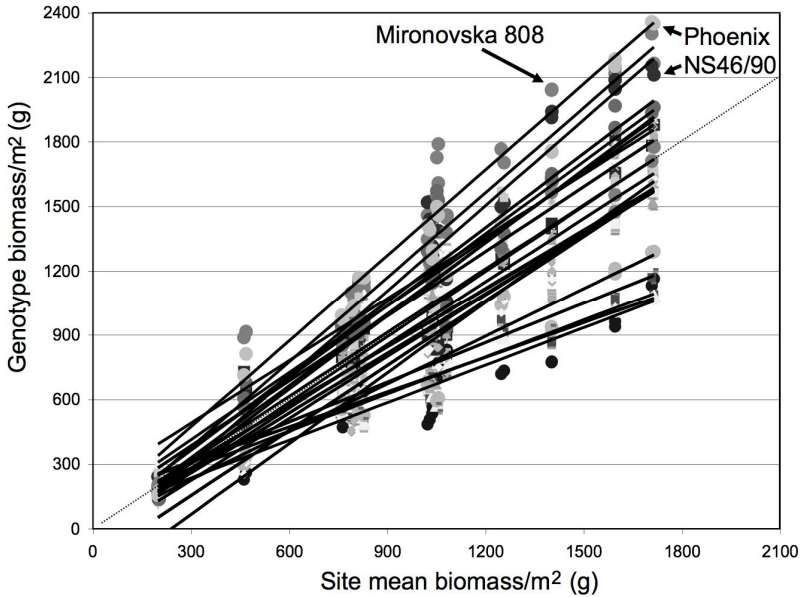


Figure 1. Plots of selected genotype biomass m⁻² against site x treatment x replicate biomass m⁻², showing the range of genotype responses to site biomass production. Fitted linear regression slopes are shown. The dotted line is the mean regression slope for 96 genotypes.

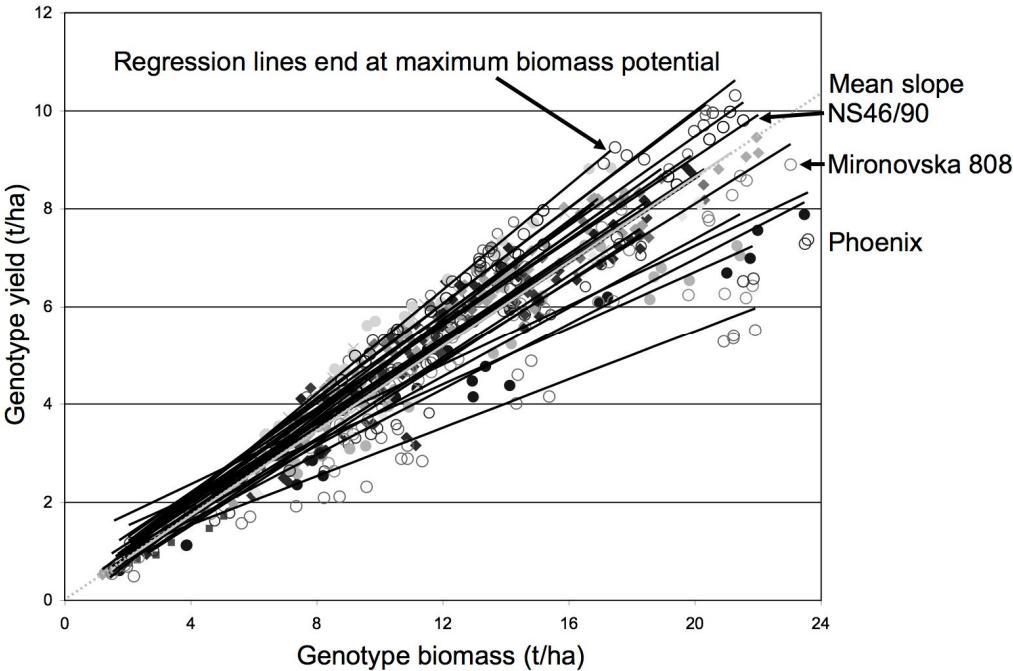


Figure 2. Plots of above-ground biomass against yield for selected genotypes showing the range amongst them. Fitted regressions are shown low HI for the variety Phoenix and high HI for NS46/90. The variety Mironovska 808 also had a high biomass potential, but with an intermediate HI (Fig. 2). Thus the two-fold challenge for breeders to continue increasing wheat yields is to increase biomass (moving maximum biomass potential to the right in Fig. 2) and also to convert as much of that biomass as possible into yield by maximising HI (moving in the vertical axis in Fig. 2).

Genetic analysis of above-ground biomass production would identify marker loci and alleles associated with high above-ground biomass. Thus, association mapping using one-way ANOVA identified 19 marker loci significantly associated with biomass (Fig. 3). Sixteen of those marker loci were also significantly associated with effects on yield, demonstrating the close relationship between biomass and yield. However, none of the ten highest biomass genotypes was associated with the highest biomass allele at each of those loci. For example, the highest biomass genotype at a site biomass of 15 t ha⁻¹, Lambriego Inia (21.2 t ha⁻¹), was associated with a low biomass allele (mean biomass 13.9 t ha⁻¹) for marker gwm11 on chromosome 1BS. Similarly, Mironovska 808 had relatively low biomass alleles for markers gwm155 (3AL), gwm190 (5DS) and psp3071 (6AS), and NS46/90 had below average biomass alleles at gwm190 (5DS) and gwm325 (6DS). Therefore, biomass (and thereby yield) could be improved in new genotypes by replacing low-biomass alleles in existing high biomass genotypes with high-biomass alleles from other high-biomass genotypes.

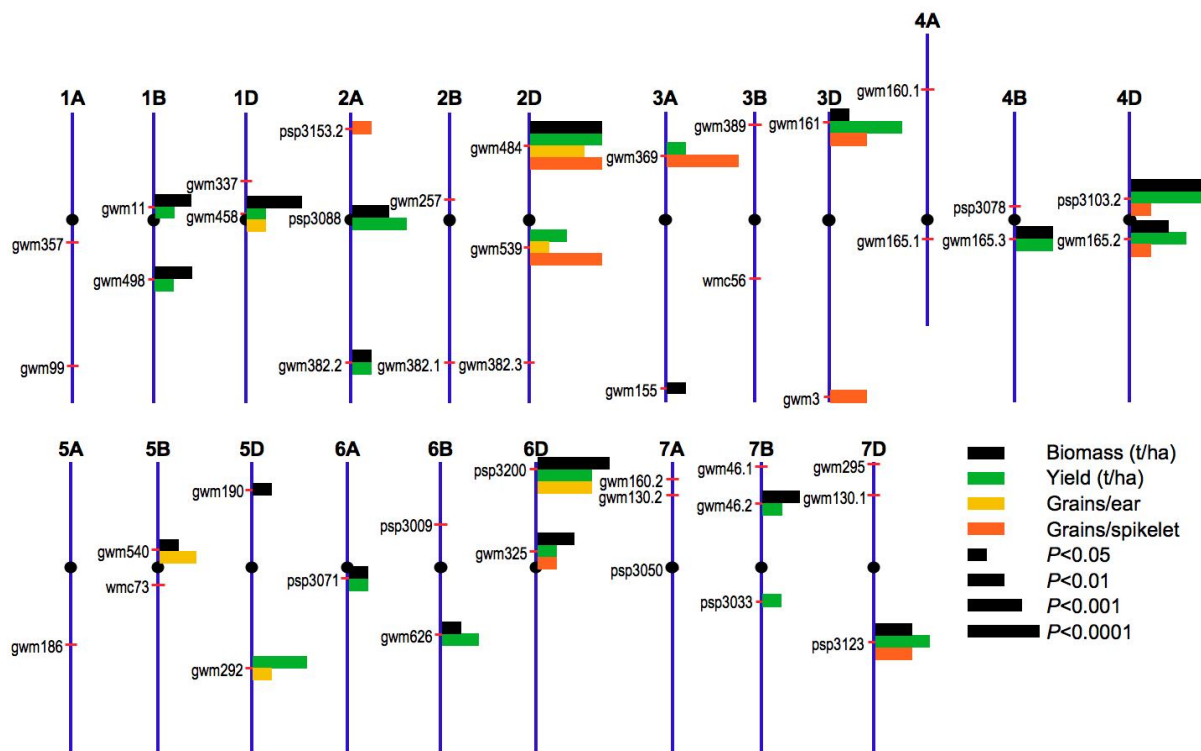


Figure 3. Location of SSR marker loci and their significant associations with biomass and yield traits

Figure 3 also illustrates contributions of the individual yield components grains per ear and grains per spikelet (a measure of floret fertility) to overall yield. Eleven of the 20 significant marker locus associations with yield coincided with significant marker associations with either grains per ear and/or grains per spikelet. The highly-significant

associations between allele size and all four traits at the locus *gwm484* is probably due to tight linkage of *gwm484* with the *Ppd-D1* photoperiod sensitivity gene on 2DS (Hanocq *et al.*, 2004). This gene is known to play a major role in adapting wheat to its environment through optimizing flowering time (Worland, 1996). Other significant loci for yield were also associated with spikelet number per ear and thousand grain weight (data not presented).

Crosses amongst several of these high-biomass genotypes were made to combine additional favorable biomass alleles. After selecting the progenies for typical breeding traits, 27 F4 families were grown in 2010-2011, together with 16 other genotypes in the wheat breeding programme and two Commission wheat standards. On the basis of individual stems sampled for a range of traits, the relationship between stem biomass and ear grain yield (Fig. 4) showed that four genotypes combining the largest stem biomass with high grain weight came from the F4 selections (labeled in Fig. 4). Of the 27 F4 families, 17 had above-average biomass per stem and 10 below-average biomass per stem, whereas the additional 16 genotypes from the wheat breeding programme and two Commission standards showed only six with above-average and 12 with below-average biomass per stem. These 45 genotypes have been sampled for DNA extraction to establish which of them have recombined marker alleles for high biomass.

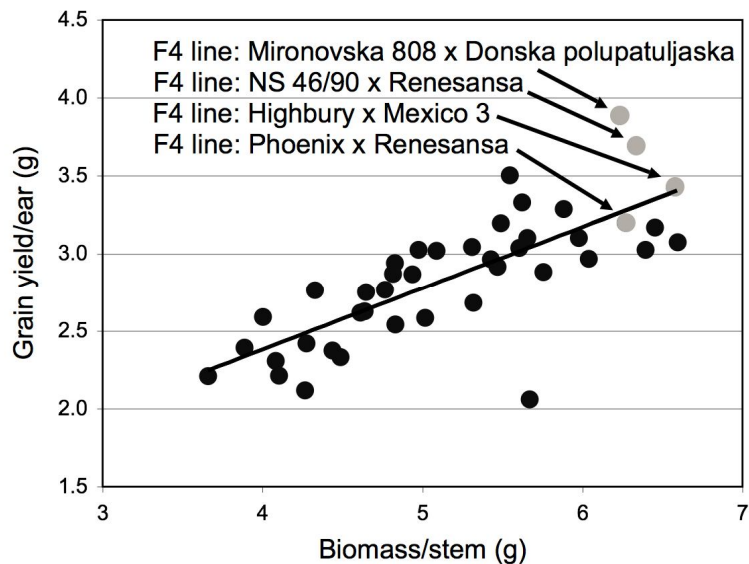


Figure 4. Association between stem biomass and grain yield per ear for 45 genotypes trialled in the Maize Research Institute, Zemun Polje 2010-2011.

Results presented here using simple one-way ANOVA should be regarded with caution, as the analyses did not take account of population structure. Thus, further more detailed analysis will be required using specialist software, such as TASSEL (Yu *et al.*, 2006), which takes account of population structure and genetic relatedness amongst the genotypes. Nevertheless, the results presented here demonstrate a method by which molecular marker technologies can contribute to meeting the breeders' challenge of increasing wheat yield through capturing more of the sun's energy in the form of greater plant biomass.

Conclusion

The work presented here has demonstrated opportunities to increase yield by targeting crosses between high biomass genotypes having complementary marker alleles associated with high biomass. Although this work illustrates the principles of using molecular marker technologies to help breeders increase wheat biomass production, and thereby yield, ideally a larger collection of genotypes giving more reliable estimates of allele frequencies and a much greater marker density, representing the 21 bread wheat chromosomes, would be required to ensure that marker loci were tightly linked to the functional genes. With continued developments in ease of use and reductions in cost per analysis, marker technologies are now widely adopted by the major breeding organizations as an essential and cost-effective tool to increase the productivity and efficiency of selecting improved wheat varieties.

Acknowledgements

Dr B Kobiljski is grateful to the Royal Society, UK for a fellowship to visit the John Innes Centre for molecular marker analyses. Dr D Dodig, V Kandić, Prof J Savić, Dr D Rančić and Prof S Pekić Quarrie are grateful to the Serbian Ministry of Education and Science for funding part of this research under project TR31005.

References

- Austin, RB. (1980) Physiological limitations to cereals yields and ways of reducing them by breeding. In: Opportunities for Increasing Crop Yields (Eds RG Hurd, PV Biscoe & C Dennis), pp. 3–19. Pitman, Boston: Association of Applied Biology.
- Crossa J, Burgueno J, Dreisigacker S, Vargas M, Herrera-Foessel SA, Lillemo M, Singh RP, Trethowan R, Warburton M, Franco J, Reynolds M, Crouch JH, Ortiz R (2007) Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. *Genetics* 177: 1889–1913.
- Dodig D, Quarrie SA, Stanković S, Milijić S, Denčić S (2002) Characterising wheat genetic resources for responses to drought stress. In 'Proceedings of International Conference on Drought Mitigation and Prevention of Land Desertification'. Bled, Slovenia, 21–25 April 2002. (CD-ROM)
- Dodig D, Zorić M, Kobiljski B, Surlan-Momirović G, Quarrie S (2010) Assessing drought tolerance and regional patterns of genetic diversity among spring and winter bread wheat using SSRs and phenotypic data. *Crop and Pasture Science* 61: 812–824.
- Ewert F, Rounsevell MDA, Reginster I, Metzger MJ, Leemans R (2005) Future scenarios of European agricultural land use I. Estimating changes in crop productivity. *Agriculture, Ecosystems and Environment* 107: 101–116.
- Foulkes MJ, Snape JW, Shearman VJ, Reynolds MP, Gaju O, Sylvester-Bradley R (2007) Genetic progress in yield potential in wheat: recent advances and future prospects. *Journal of Agricultural Science* 145: 17–29.
- Gupta PK, Langridge P, Mir RR (2010) Marker-assisted wheat breeding: present status and future possibilities. *Mol Breeding* 26: 145–161.
- Hanocq E, Niarquin M, Heumez E, Rousset M, Le Gouis J (2004) Detection and mapping of QTL for earliness components in a bread wheat recombinant inbred lines population. *Theor. Appl. Genet.* 110: 106–115.
- Reynolds MP, Borlaug NE (2006) Centenary review. Applying innovations and new technologies for international collaborative wheat improvement. *Journal of Agricultural*

- Science 144: 95-110.
- Quarrie SA, Dodig D, Pekić S, Kirby J, Kobiljski B (2003) Prospects for marker-assisted selection of improved drought responses in wheat. *Bulgarian Journal of Plant Physiology Special Issue*, 83–95.
- Worland AJ (1996) The influence of flowering time genes on environmental adaptability in European wheats. *Euphytica* 89: 49-57.
- Yu J, Pressoir G, Briggs WH, Vroh Bi I, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Nielsen DM, Holland JB et al. (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nature Genetics* 38: 203–208.