УДК 634.11:575

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Использование молекулярно-генетического анализа, основанного на родословных, в селекции и в качестве эффективного метода выявления локусов количественных признаков у селекционного материала с известным происхождением

Аннотация

Молекулярно-генетический анализ родословных (*Pedigree based analysis – PBA*) является инновационным и мощным методом для обнаружения и характеристики связей между молекулярным маркером и фенотипическим признаком для дальнейшего использования в маркер вспомогательной селекции.

Статистические методы и программное обеспечение были проверены путем комплексного исследования на 27 семьях из пяти селекционных программ четырех стран [1], некоторые результаты представлены на рисунках 1 и 2. На рисунке 1 показаны позиции локусов количественных признаков (ЛКП) плотности плода после двух месяцев холодного хранения. На рисунке 2 локусы количественных признаков у 33 сортов и форм. Полученные данные могут быть использованы в селекционной работе как для подбора родительских пар для скрещивания так и для выбора целевых локусов количественных признаков. Локус картированный на группе сцепления 15 является перспективным для разработки маркера для дальнейшего использования в маркер вспомогательной селекции.

Селекционная программа по яблоне реализуемая во ВНИИСПК позволила получить целый ряд плодоносящих семей. В настоящее время ряд родителей этих

семей, а так же некоторые другие сорта были генотипированы с помощью Infinium® 20K SNP маркеров. Первые результаты дали информацию о генетическом разнообразии и родословных. Кроме того, в российских селекционных программах используются родители и предшественники общие с изучаемыми в совместном международном проекте FruitBreedomics. Все это может стать основой для сотрудничества с применением PBA методов.

Ключевые слова: маркер вспомогательная селекция, однонуклеотидные полиморфные маркеры (SNP), генотипирование, Rosaceae, *Malus domestica*

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PEDIGREE BASED ANALYSES: A POWERFUL APPROACH FOR QTL DISCOVERY IN PEDIGREED BREEDING GERMPLASM AND SUPPORT ON BREEDING DECISIONS

Abstract

Pedigree Based Analysis (PBA) is an innovative and powerful approach for the discovery and characterisation of marker-trait associations for use in marker assisted breeding. The power of PBA comes from the simultaneous analysis of multiple segregating full sib families, the use of breeding germplasm, and the use of known genetic relationships. The use of multiple families allows representation of wide genetic diversity thus increasing probability that desired genes/alleles are presented and segregating. This also allows examination of QTL performance across different genetic backgrounds, thus providing knowledge on the general applicability of results. Finally, the use of multiple families

increases the population size and thereby the statistical power. The use of breeding germplasm of ongoing breeding activities makes results directly applicable and relevant to the breeder and also reduces experimental costs since plant materials and part of the phenotypic measurements are already available. Finally, PBA provides a framework for extending the population and phenotypic data in time, thereby further strengthening statistical power.

The statistical approaches and software have been validated through an integrated study on 27 families from five breeding programs from four countries [1] from which some results are presented in figures 1 &2. Current PBA software includes FlexQTLTM for QTL discovery and characterization [1], Pedimap for the graphical presentation [2], Visual-FQ, which provides structured guidance through the analyses and FQ-haplotyper which assigns alleles to haploblocks (sets of tightly linked SNP markers).

Key words: marker assisted breeding, marker assisted selection, marker-trait associations SNP, genotyping, Rosaceae, *Malus domestica*

The above mentioned validation study in apple included only 87 SSR markers [1]. The current availability of high-throughput SNP genotyping infrastructures allows marker genotyping at much higher densities, which will contribute to higher mapping resolution and more accurate QTL characterization. FlexQTLTM has recently been adapted to such large scale SNP data. Also, FQ-haplotyper software has been developed, which builds highly informative multi-allelic haplotypes comprising consecutive lowly-informative SNPs, thus reducing dataset sizes and computation time without giving in on marker information while also increasing informativeness of single markers beyond that of SSR markers (Fig. 3). Indeed prospects for PBA on Rosaceae have dramatically increased through the availability of SNP arrays for high throughput genome wide genotyping [3, 4, 5, 6, 7], of pipelines for the processing of these SNP data into reliable genotype calls [http://compbiotoolbox.fmach.it/assist/], of strategies for composing study germplasm [9] and through the continuous decreasing prices for these arrays. To date, PBA has been embraced by several international and national research programs on apple, peach [9], cherry, strawberry and grape, and wheat and oil palm projects are expected to join soon. In apple, PBA-studies are in progress on, amongst others, fruit firmness, titratable acidity, Brix, crispness, juiciness, aroma, fruit size, fruit color, fruit over color, harvest data, and crop load within the framework of the European project FruitBreedomics [10].

VNIIISPK's apple breeding program has many fruit bearing families. Currently various of its breeding parents and some other Russian cultivars are being genotyped with the Infinium® 20K SNP array [6]. First results indicated the applicability of the array on Russian germplasm, thus providing information on the available genetic diversity and pedigree records [Fig. 3, 11]. Moreover, VNIIISPK and other Russian breeding programs have parents and or progenitors in common with the FruitBreedomics study population (Fig. 3). All this could be the prelude to a multi-institution PBA study in Russia with links to European and American PBA studies.
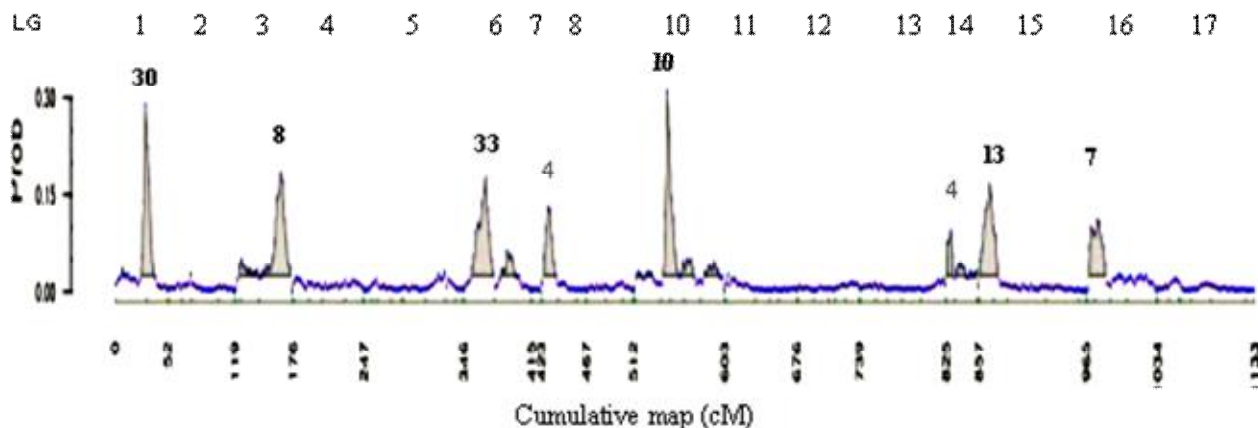
Figure 1 – Probable QTL positions for fruit firmness after two months of cold storage following a PBA analyses on 27 full sib families

The starts and ends of chromosomes are indicated by dashed vertical lines. Peaks present the most probable position of QTLs. The probability of the presence of a true QTL on a linkage group (LG) is reflected by Bayes Factors (BFs). The 2lnBFs for the presence of one over no QTL are presented on top of each peak. A value greater than 5 and 10 indicate strong and decisive evidence respectively. This analysis gave no strong evidence for the presence of two QTL on a single LG. These data thus give at least strong evidence for the presence of six QTL. Data are taken from Bink et al. 2014 [1] for their additive QTL model with the Poisson prior distribution E(NQTL) = 5. ("Q5_R0").
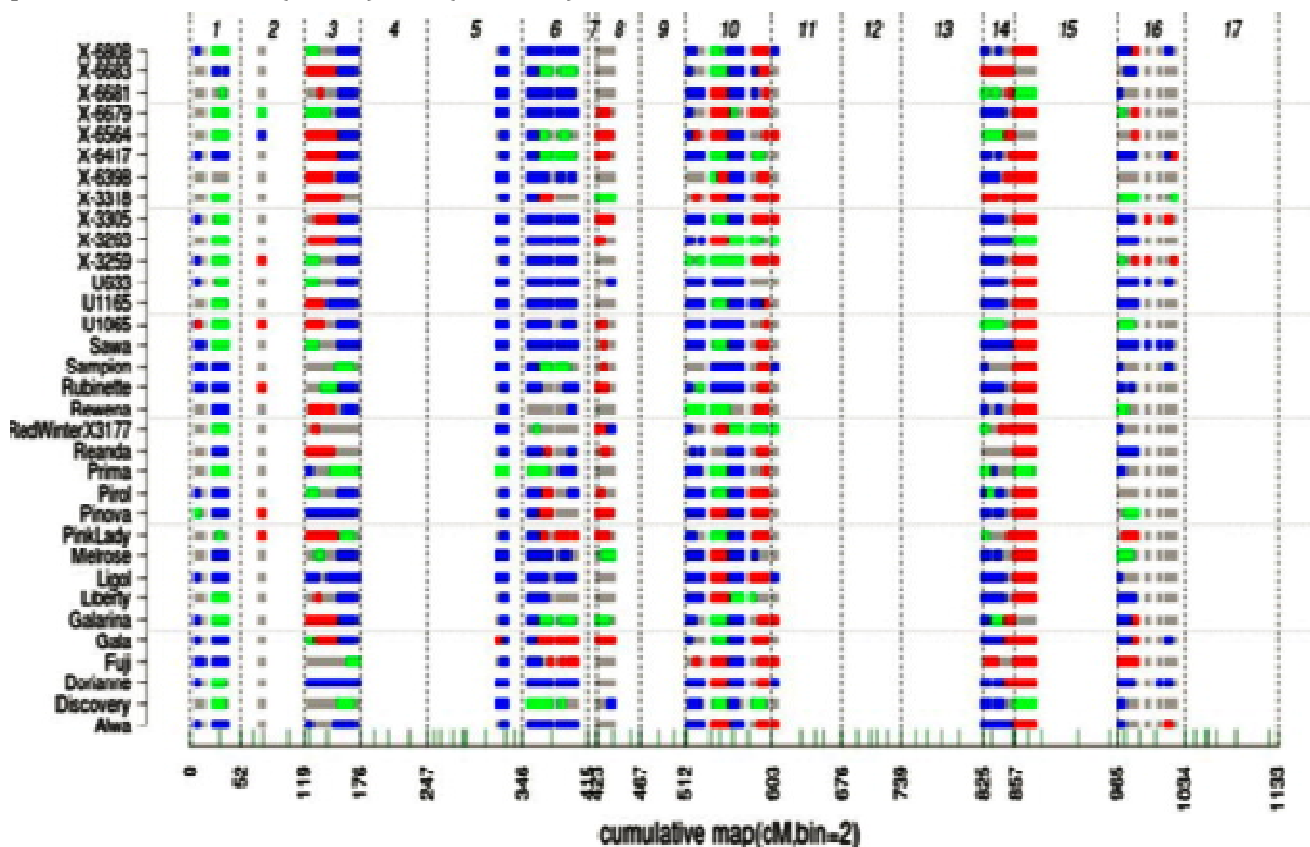

Figure 2 – Estimates of QTL genotype for the 33 parents of the full sib families

Estimates are plotted for those chromosome regions with at least strong (2lnBF10 ≥ 5) for QTL presence (as presented in Fig. 1). The starts and ends of chromosomes are indicated by dashed vertical lines, and marker positions are indicated by inner ticks at the horizontal axis.

The blue, green and red colors indicate positive evidence for QTL genotypes qq(−−), Qq(+−) and QQ(++), respectively. The grey color indicates ignorable evidence for any genotype).

Data are taken from Bink et al. 2014 [1] for their additive QTL model with the Poisson prior distribution E(NQTL) = 5 ("Q5_R0"). This QTL genotype information can be used in breeding decisions, like for the choice of parents for new crosses and for the QTL to target. The QTL on LG15 seems to be homozygous for the favourable allele for most parents. This may reflect high selection intensity and thereby the importance of this QTL. Also, due to the high level of homozygosity, development and use of markers may not be very cost efficient, especially in case of single marker tests.
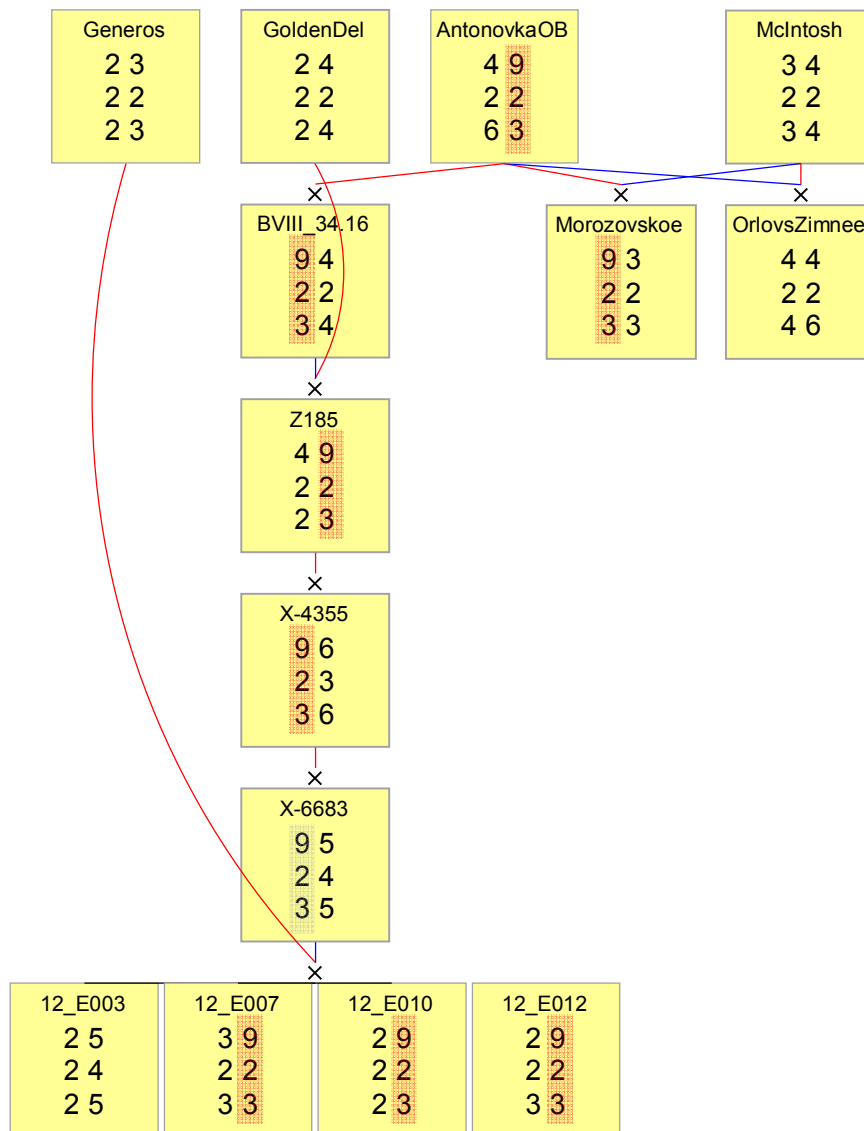

Figure 3 – Flow of haploblock alleles over six successive generations from the founder Antonovka Obyknovennaya to four progenies that are part of the QTL mapping study of Fig. 1 & 2 and that are also part of a larger mapping population of 45 individuals, and to two Russian cultivars. Female parentage is indicated by pink and male parentage by blue lines. The two columns within a box indicate homologous chromosome segments. The two numbers within a row indicate the two alleles within a haploblock locus. The three presented haploblock-loci cover the first 4cM of linkage group 12 of apple and comprise nine, seven and ten SNP markers respectively

Antonovka's haplotype 9-2-3 was passed to the German Breeding selection B VIII 34.16 [11] to the breeding selection Z185 of Wageningen UR, The Netherlands, to two breeding selections of INRA-Angers, France, and finally segregates in a full-sib family of INRA-Angers. Actually, a full LG12-chromosome of Antonovka OB was passed to this family (data not shown), due to which QTL results for this chromosome could be transferable to Russian germplasm. Other Russian breeding germplasm that are in the pedigree of mapping populations of the FruitBreedomics [10] are Borowitsky (= Charlamowsky), and R12740-7A. On the other hand, Russian breeding programs used western cultivars that are now studied in FruitBreedomics like McIntosh and Wealthy.

Finally, the presented marker data also confirm the pedigree records of Morozovskoe and Orlovskoe Zimnee.

Acknowledgements

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