TOWARD MARKER ASSISTED SELECTION FOR FUNGAL DISEASE RESISTANCE IN GRAPEVINE

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ABSTRACT
Grapevine is a crop of major economic importance in Bulgaria where viticulture and winemaking have been traditional since ancient times. Fungal diseases, which severely affect the yield, the cost and the quality of the grapevine production, are currently controlled by hard fungicidal treatments. The development of high quality grapevine cultivars with increased resistance towards main fungal pathogens through MAS is a proper way to reduce both the cost of grapevine production and the environmental risk. The present mini review is focused on the current knowledge concerning genetic factors related to fungal disease resistance in grapevine. It provides information on QTLs/markers for fungal disease resistance, as well as on homologues of the resistance related genes, identified in genetic and physical maps, and in the grape genome sequence. Further we present the research activities in AgroBioInstitute aimed at the establishment of the MAS for fungal disease resistance in grapevine trough utilizing Bulgarian grapevine genetic resources. The research program related to the identification of QTLs, molecular and metabolite markers for fungal disease resistance, as well as the selection of grapevine breeding lines bearing resistance to downy mildew and powdery mildew, is outlined. The program was established in the frame the NSF funded project DO02-105 “Centre for sustainable development of plant and animal genomics”.

Keywords: grapevine, MAS, QTLS, molecular markers, genetic linkage map, fungal disease resistance, downy mildew, powdery mildew, resistance genes analogs


Introduction
Grapevine is the most economically important and widely cultivated fruit crop in the world, used as a source for a variety of products for food, wine and pharmaceutical industry. The application of agrochemicals for plant protection in viticulture leads to a substantial increase of the production costs, ecological problems and reduction of the quality of grape and wine. The elevating requirements of EU concerning the quality of food and wine, as well as for sustainable agriculture, enforces the development of alternative management strategies in viticulture for production of high quality grapevine with reduced environmental risk.

Powdery mildew, downy mildew and grey mould, caused by the fungal pathogens Uncinula necator, Plasmopora viticola, and Botritis cinerea, respectively, are a major risk for viticulture and can substantially reduce the yield (50-70%). Currently, fungal diseases are controlled by the application of fungicides, where the fungicidal treatments can reach up to 14 per year. Reduction of the level of applied fungicides could be achieved by development of high quality grapevine cultivars with increased resistance towards fungal pathogens. The development of a new grapevine cultivar is a long term and costly process that usually takes 25 years. V. vinifera cultivars are not resistant to fungal pathogens. Such a resistance can be introgressed in V. vinifera from American and Asian Vitis species, through interspecific breeding. However, due to the low grape quality of these species, a number of additional crosses with V. vinifera cultivars have to be included in breeding programs to achieve the high quality of V. vinifera. This leads to a substantial increase of the cost and time spent for the development of a new resistant variety.

The application of marker assisted selection (MAS) enables us to overcome to a great extent the difficulties in the selection of grapevine that are related to the long generation cycle of this crop, the size of plants and plantations, the high clonal heterozigosity and inbreeding depression. The application of molecular markers associated with genes/loci conferring resistance allows to trace the introgression of the corresponding genes/loci in plants at an early stage of development and thus to accelerate the breeding by early selection of the plants harbouring this trait. This approach is of great relevance for achievement of pyramiding resistance, where markers distinguishing different source of resistance have to be used (2).

Identification of QTLs/markers for resistance and resistance gene homologues in grapevine
Marker assisted selection launched for the development of cultivars that are resistant to diseases has been highly advanced by the achievements of grape genomics, such as construction of a number of genetic linkage maps, BAC libraries, physical maps and the competed sequencing of the whole grape genome. During the last decade, efforts have been made to develop genetic linkage maps and to identify markers associated with...
loci controlling quantitative traits (quantitative trait loci, QTLs) (6, 12, 15, 16, 38). The construction of maps was assisted by the establishment of large collections of molecular markers such as simple sequence repeats (SSRs) and expressed sequence tags (ESTs), and more recently by the utilization of single nucleotide polymorphisms (SNPs) (NCBI, National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/) (24, 29, 30, 35, 36). Several genetic linkage maps, primarily based on SSRs, have been constructed during the last several years (1, 8, 11, 26, 31, 38). SNPs are considered the most frequent polymorphisms in eukaryotic genomes. They were found to be highly abundant in the grapevine genome with an average value of frequency of 4.0 per kilobase across the grape genetic map (36). The implementation of SNP based markers, derived from ESTs and BESs (BAC end sequences) allowed the construction of high dense genetic maps (35) and to map expressed genes (32). A recently published reference integrated map that is based on segregating data of three crosses, including five elite cultivars of V. vinifera (Cabernet Sauvignon, Syrah, Pinot Noir, Grenache and Riesling), is the most dense linkage map to date. It comprises of 1134 markers (BESs and ESTs derived SNPs, SSRs and AFLP) spanning 1443 cM with a mean distance between adjacent markers of 1.27 cM (37). The established genetic maps present a source for markers for other mapping projects and are useful tool for identification of QTLs/markers, for anchoring BAC clones, and for map-based cloning of candidate genes related to disease resistance.

QTL analysis is a widely applied approach for identification of loci underlying disease resistance in the frame of genetic linkage map, but it is not effective enough for identification of loci with a minor effect concerning pathogen resistance (23). Through the use of this approach, one major and one minor QTL with a strong effect on the resistance to downy mildew were identified and mapped on LG (linkage group) 18 and LG 4, respectively in the integrated “Regent” x “Lemberger” genetic map. The corresponding microsatellite markers closely linked to these loci were obtained in cultivar Regent. In the same map, one major QTL conferring resistance to powdery mildew was mapped on LG 15 (15, 38). Further, two SCAR markers associated with the major QTL, related to powdery mildew resistance in cultivar Regent, were developed and their correlation with the resistance was validated in a different genetic background (2).

The integration of functional markers in the genetic maps, such as resistance gene analogs (RGA), SNPs derived from ESTs and their co-localization with QTLs conferring resistance is currently an appropriate way for identifying candidate genes involved in the disease resistance.

Resistance gene analogs are a big family of resistant genes that are involved in a defence mechanism of diverse plant species against a broad range of pathogens. They are thought to be involved in the pathogen recognition that activates signal transduction that switches on the defence mechanism. Members of this gene family were first identified in grape by Di Gaspero and Cipriani (9, 10). The information, concerning their genomic organization, co-localization with QTLs for disease resistance and their potential for development of markers linked to the resistance loci in grape is provided by genetic map studies, physical maps and the grape genome sequence. RGAs are found organized in clusters, often tightly linked to the previously mapped loci for resistance (3, 7, 8, 28, 36, 38).

Two RGA-derived markers were found co-located with the major QTL locus (LG 18) conferring resistance to downy mildew, thus supporting its putative function (38). Further, CC-NBS-LRR candidate resistance gene family, expressed after inoculation with P. viticola, was isolated, characterized and mapped on LG 10 of the cultivar Regent resistant to powdery and downy mildew (23).

Resistance gene analogs, linked to the powdery mildew Run 1 locus, introgressed from Muscadinia Rotorundifolia, were identified and mapped in a pseudo-backcross population BC5 x Cabernet Sauvignon (7). The position of Run 1 locus was refined in a comprehensive genetic and physical map and 20 new markers were identified that are closely linked to this locus. Data was obtained that Run 1 locus contains two NBS-LRR gene families (3).

Further, 82 markers for R-gene candidates were localized in a consensus linkage map of grapevine based on two segregating populations (8). Most of RGA markers (83%) were found in clusters into few regions in seven LGs: 3, 7, 9, 12, 13, 18 and 19 and 43% of the mapped RGAs were localized in LGs 12 and 18. The major clusters were obtained to consist of one type of RGA, CC-NBS-LRR in LGs 13 and 19, TIR-NBS-LRR in LG 18, while a mix of clusters of both types of RGAs was localized on LG 12. RGAs were found in the surrounding of the locus for resistance to powdery mildew previously mapped by Barker et al. and Donald et al. (3, 7), and co-located with a previously identified major QTLs for downy mildew (2, 15, 33). The presence of NBS-LRR genes was determined in both susceptible vinifera and resistant non-vinifera genotypes. However, the high level of allelic variation of RGA genes found between non-vinifera and of vinifera grapes, suggested a significant divergence of the alleles between resistant and susceptible phenotypes that could be a bases for a resistant trait (8).

The grapevine is the first fruit tree, whose genome was completely sequenced by French-Italian Consortium (17). The whole genome sequence of cultivar Pinot Noir clone ENTAV115 (36) (IASMA Genomics) resulted in identification of SNPs (around 2 000 000 SNPs were discovered, 1 751 176 were mapped to chromosomes and 86.7% were identified in anchored genes) and genes (in total 29 585 genes were predicted, of which 96% were assigned to LGs and 79% were annotated), thus providing the opportunity to identify the candidate genes involved in different traits. Homologous genes for host and non host disease resistance are found to be highly abundant in the grape genome. 341 NBS genes were identified in the genome of Pinot Noir, of which 233
that contain LRR domain, were grouped in 5 major clades: 1) mainly TIR-NBS-LRR and their truncated structure; 2) and 3) mainly CC-NBS-LRR; 4) mainly NBS-LRR; and 5) CC-NBS-LRR. Truncated NBS were obtained in LGs 12 and 13. The NBS genes were found organized in clusters and subclusters, where one cluster can contain genes from one or from different clades. TIR-NBS-LRR genes were found to be preferentially located in LG 18, while CC-NBS-LRR in LGs 9 and 13. The mapping of NBS genes allowed to reveal the co-localization of several clusters of NBS genes and previously identified QTLs for resistance to downy in LG12 and 18, and to powdery mildew in LG14 and 15 (2, 6, 15).

Besides of the NBS genes, genes encoding pathogenesis-related proteins (PRs), powdery mildew non-host resistance-related genes and genes similar to MLO genes that confer resistance to mildew in barley were also located in the genome of Pinot Noir (36).

Homologues of genes for host, non-host resistance and signalling pathway, were also localized on the physical map of Cabernet Sauvignon (28). Grapevine analogs to NBS-LRR class were found to be present in all LGs, although LGs 12, 13 and 18 were most abundant in NBS-LRR, while LG 14 contained more RLK genes. Twenty seven homologues to non-host resistance and disease resistance signalling genes were obtained to be evenly distributed among grapevine LG, although sequences homologues to the genes for signalling pathways were more frequently found in LGs 14, 15 and 19. Sequence homologous to MLO1 gene in barley that confers resistance to powdery mildew was assigned to LG 5. Several NBS-LRR genes were obtained to be co-mapped with a major QTLs or genes for disease resistance (2, 3, 8, 38), while the lack of co-location was found between reported in the literature QTLs for disease resistance and genes involved in non-host resistance and signalling pathways (28).

The vast amount of accumulated data concerning the identified QTLs/markers, related to disease resistance, the localization of homologues of the resistance related genes in genetic and physical maps, and in the grape genome sequence, as well as the developed methods for SNPs discovery, opens the opportunity for the identification of candidate disease resistance genes and gene/allele specific markers. All this can be exploited in MAS for development of disease resistant grapevine cultivars.

Current status of capacity for establishment of MAS for disease resistance in Bulgaria

Grapevine is a crop of major economic importance in Bulgaria where the viticulture and the winemaking have been a traditional activity since ancient times. Due to the diverse climatic and soil conditions in Bulgaria and its location at the crossroad between Europe and Asia, the genetic diversity of grapevines in Bulgaria is high. Nowadays, the grape genetic pool consists of unique old native, locally bred and widespread European cultivars (V. vinifera ssp. sativa) as well as populations of wild grapes (V. vinifera ssp. sylvestris), most of them characterized with microsatellite markers (13, 14, 19, 20, 21). This genetic variability presents an important source of valuable genes that can be used in breeding programs for development of cultivars with improved agronomic characteristics.

The project, launched to the development of grapevine cultivars, resistant to the main fungal pathogens, through MAS is a part of the recently initiated in ABI project DO02-105 “Centre for Sustainable Development of Plant and Animal Genomics”. It aims to initiate the grape molecular breeding in Bulgaria by 1) the establishment of markers associated with resistance to fungal diseases and 2) selection and the development of breeding lines harbouring resistance by utilizing Bulgarian grapevine genetic resources. Among more than hundreds of cultivars that are bred in Bulgaria mainly in the last century, there are only several that are considered resistant to the main fungus diseases. In most of these varieties, the resistance was introgressed from Villard Blanc, the variety known to bear resistance to Uncinula necator and Plasmopora viticola. These varieties present an available source for the development of new resistant cultivar with improved quality through MAS. One of them, the Bulgarian wine cultivar Storgozia was created in the last century by the cross between the susceptible to fungus and newly bred variety Buket (Mavrud x Pinot Noir) (22) and the fungus resistant cultivar Villard Blanc. It combines field resistance to three fungal pathogens, Plasmopara viticola, Uncinula necator and Botritis Cinerea, and high quality of the produced grape and wine. Storgozia is also tolerant to low temperatures. The F2 population, that is obtained after selfpollination of the cultivar Storgozia, consisting of 98 plants, segregates in relation to resistance to powdery mildew, downy mildew and gray mould, and a number of agronomic traits (34). It is being used for the development of a framework linkage map of cultivar Storgozia aimed at identification of QTLs that confer disease resistance to the above mention fungi, and molecular and metabolic markers associated with the resistance. The constructed microsatellite based genetic linkage map of cultivar Storgozia consists of 92 microsatellite markers, eighty four of them mapped to 19 linkage groups, corresponding to the haploid chromosome number of grapevine. The mapped SSRs span 692 cM with an average distance of 10.7 cM between the markers. The order of markers in the constructed linkage map of cultivar Storgozia was consistent with the integrated map of Doligez et al. (11), with the exception of 5 inversions, each of them containing two adjacent markers (18). The established map is currently saturated with SSRs.

The plants from the segregating population, grown on their own roots in the field, were evaluated for resistance to powdery, downy mildew and grey mold without treatment with fungicides. The obtained preliminary data for QTLs for resistance to downy mildew will be verified following the infection of the replicated plants, of this population, in controlled conditions. The current implementation of functional markers, such as EST and RGA, in the map of Storgozia will...
allow a search for co-localization of markers with the obtained QTLs for resistance. Presently, in addition to the saturation of the established map with SSRs and RGAs, we put efforts on mapping SNPs markers, corresponding to the selected genes for disease resistance and other agronomic traits utilizing information from public databases and grapevine genome sequence data. In parallel, GC/MS metabolite profiling of contrast phenotypes prior and after inoculation with *P. viticola* in controlled conditions will be performed in order to select metabolite markers associated with the resistance. Several biomarkers, associated with resistance of cultivar Storgozia to downy mildew have been recently identified (4, 5).

The accumulated data for markers associated with resistance to fungal pathogens will be utilized for selection of valuable breeding lines homozygous for the particular alleles, transferred from Villard Blanc. Presently, three plants from the population were selected that possess a complex field resistance to *Uncinula Necator, Plasmopora Viticola, Botritis Sinerea*, which can be used for further utilization in breeding programs. This will enable the selection, on the base of different contrasts phenotypes prior and after inoculation with *P. viticola* sequence data. In parallel, GC/MS metabolite profiling of information from public databases and grapevine genome for disease resistance and other agronomic traits utilizing mapping SnPs markers, corresponding to the selected genes in controlled conditions will be performed in order to select metabolite markers associated with the resistance. Several biomarkers, associated with resistance of cultivar Storgozia to downy mildew have been recently identified (4, 5).

The SNP alleles from Villard Blanc, identified in Storgozia, will be investigated in several Bulgarian cultivars derived from Villard Blanc. Once identified, they will be further analyzed in segregating populations that are developed from these cultivars. This will enable the selection, on the base of different cultivars, of plants bearing resistance to fungal pathogens, which can be used for further utilization in breeding programs.

The expected outcomes from this project: identification of molecular and metabolite markers for disease resistance and the selections of grapevine breeding lines bearing resistance; will be relevant for the establishment of MAS in Bulgaria. Further, the identification of QTLs for important agronomic traits, as well as the application of markers associated with berry and wine quality will assist for the development of resistance cultivars with improved quality.

REFERENCES


