MICROSATELLITE LINKAGE MAP BASED ON F2 POPULATION FROM BULGARIAN GRAPEVINE CULTIVAR STORGOZIA

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ABSTRACT

A genetic map of Bulgarian grapevine cultivar Storgozia based on microsatellite markers was constructed. The F2 mapping population consisted of 98 progeny plants obtained by self-pollination of a newly bred wine variety Storgozia, which possesses field resistance to downy mildew, powdery mildew and grey mould, and tolerance to low temperatures. The population segregates according to disease resistance and a number of morphological characteristics.

The genetic map consisted of 92 microsatellite markers. Eighty four of them were mapped to 19 linkage groups. 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., 2006, with the exception of 5 inversions, each of them containing two adjacent markers.

The mapping project aims to develop a framework linkage map for the identification of QTLs for disease resistance and a number of morphological characteristics.

Keywords: grapevine, microsatellite linkage map, F2 segregating population

Introduction

Grapevine selection is hampered by the long life cycle of the crop and inbreeding depression. The development of new grapevine cultivars by classical selection is long-term, costly and labor-consuming process that usually takes 20-25 years. In addition, most of the agronomically important traits are quantitatively inherited, which complicates their control during the selection (7, 9). The alternative selection approach, marker assisted selection, (MAS) exploits molecular markers for the early selection of plants that harbor the traits of interest. This allows acceleration of the breeding programs and substantial reduction of related costs and space for plant growing and evaluation. The development of high density genetic maps that comprises evenly distributed in the genome markers is a useful tool for identification of quantitative trait loci (QTL) underlying the important agronomic traits and markers associated with them (21). Several genetic maps of grapevine were published during the last decade. Depending on the particular goal, the segregating populations used for mapping were generated by crosses between: vinifera cultivars (7, 8, 18, 24), vinifera and non vinifera as a source of disease resistance (4, 6, 9, 10, 14, 26) and rootstocks (15). Most of the early published maps exploited the combination of different types of markers including RAPD, SCAR, AFLP and SSRs. Among them SSRs are the most suitable for mapping studies due to their high polymorphism, even distribution in the genome, codominant Mendelian inheritance, transferability between laboratories and conservation across taxa. The first framework linkage map of V.vinifera L, based entirely on microsatellite markers was published in 2004 (18). It consists of 153 microsatellite markers mapped to 20 linkage groups. This map is recommended by the International Grape Genome Program as a reference map for numbering of linkage groups and as a basis for other mapping studies for establishment of high-density consensus map of grapevine. Additional 127 microsatellites were mapped to 19 linkage groups by Adam-Blondon et al. (1). The most complete microsatellite linkage map that integrates the segregating data from five grapevine mapping populations was developed by Doligez et al. (8). It comprises 515 loci, among them 502 SSRs mapped to 19 LG, corresponding to the chromosome number of *Vitis*. In this map LG 13 and LG18 from the map of Riaz et al. (18) were merged resulting in LG 18, while LG20 from the map of Riaz et al. (18) was designated as LG13. The 82 functional markers for R-gene candidates have been incorporated in a microsatellite map constructed by Di Gaspero et al. (6), which provides a tool for localization of the genomic regions underlying the disease resistance. The most comprehensive linkage map of grapevine to date was constructed based on 132 SSRs, 379AFLP and 483 single nucleotide polimorphism (SNP)-based markers (24). It spans 1245cM with an average distance of 1.3cM between adjacent markers.

The use of common SSRs allows the comparison of genetic maps constructed on the base of various segregating population, comparison of the localization of QTLs in different mapping populations, screening of genotypes for the presence of marker, linked to a particular locus and more precise mapping of loci of interest (1, 8).

The Bulgarian wine cultivar Storgozia was created in the last century as a result of a cross between the fungus susceptible newly bred variety Buket and the fungus resistant cultivar Villard Blanc (12). It combines resistance to three fungal diseases and high quality of the produced grape and wine. The mapping population, obtained after self-pollination of cultivar Storgozia, segregates in relation to resistance to powdery mildew, downy mildew and gray mould, and a number of agronomic traits.

In this study we present current results concerning the development of microsatellite based linkage map of the cv. Storgozia, as a first step for development of framework linkage map for identification of QTLs for disease resistance and important agronomic traits.

Materials and Methods

Plant material

Mapping population consisted of 98 progeny plants obtained by selfpollination of the Bulgarian grapevine variety Storgosia (23). The plants from the population were grown on their own roots in the research station of the Institute of Agriculture and Seed Knowledge, Russe, Bulgaria. The female parent of Storgozia, the Bulgarian variety Buket, was obtained by cross between the ancient Bulgarian variety Mavrud and the French variety Pinot Noir. The male parent of cv. Storgozia, Villard Blanc is a complex hybrid including several Vitis species in its pedigree.

DNA extraction

Leaf samples from individual plants from the population as well as from cultivars Storgozia, Buket and Villard Blanc were collected; ground to fine powder in liquid nitrogen and DNA extraction was performed according to the protocol of Murray and Thompson (1980) (17).

Markers and amplification conditions

In total 180 SSR markers, available at the NCBI database (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov), were analyzed for heterozygosity in cv. Storgozia. The microsatellite markers belong to the following sets: VVS (22), SSRVrZAG (20), VVMD (2, 3), VMC (Vitis Microsatellite consortium), VVI (16), UDV (5), SCU (19). The primers were synthesized by Microsynth AG.

PCR reactions were performed in a volume of 20 μl containing 20ng DNA, 1μM of each primer, 200nM of each dNTP, 1.5 mM $MgCl_2$ and 1U of Taq polymerase (GE Healthcare). Amplifications were performed in GeneAmp PCR System 9700 (Applied Biosystems) using the following thermal protocol: 94^0C for 4 min followed by 36 cycles of 1min at 94^0C, 1 min at 50-60^0C (depending on the primer),1min at 72^0C and final extension of 6min at 72^0C. Fragment analysis of the obtained PCR products was carried out on an ALF Express II sequencer (GE Healthcare) and alleles were sized with the software Allele Locator 1.03 (GE Healthcare). Internal standards were produced by amplification of PUC19 fragments with sizes 100, 150, 200, 250, 300, 350, 400, 450 and 500 bp.

Linkage analysis

Genetic map was constructed using JoinMap version 3.0 (25). All markers were evaluated for the deviation from the expected Mendelian segregation with a chi-square test. The Kosambi function was used for determinantion of map distances (13). Most of the linkage groups and the relative distance between markers were defined at minimum LOD score 3.0. The obtained linkage groups were then compared to and numbered according to the consensus map of Doligez et al. (8). In some cases when the markers with a known position in the integrated map were not mapped at the corresponding LG, LOD 2.0 was applied to incorporate them in the map.

Results and Discussion

Four published *Vitis* genetic maps (1, 8, 9, 18) were used as a source of microsatelite markers for mapping. The allelic state of each microsatelite marker in cv. Storgozia was determined through comparison with the parents, cv. Buket and cv.Villard Blanc. The markers found heterozygous in cv. Storgozia were used for genotyping of the individual plants of the population. The PCR amplification conditions for some of the markers required additional optimization for their routine application (data not shown). In total 180 microsatellite markers were scored for heterozigosity in cv. Storgozia. Thirteen markers were excluded because of a stutter pattern or lack of amplification. Among the remaining 167 markers that were successfully amplified, 92 markers (55%) were heterozygous in cv. Storgozia and were used for construction of the linkage map. The portion of tested markers suitable for mapping in the 'Strogozia' population was quite lower than those utilized in other mapping studies (1, 6, 8). This is because the 'Strogozia' mapping population was generated by self-pollination, which limits the number of appropriate markers to the number of heterozygous markers in only one parent, instead of in two parents as is in the case of the pseudo-testcross strategy (11), which is commonly applied in the grapevine mapping studies.

The segregation pattern of 92 heterozygous microsatellite markers was analyzed in the mapping population of 98 progeny plants. Chi-square test showed distorted segregation ratio for 16 markers (18.4%). This value is higher than the corresponding values (7%-11%) of distorted markers found in other 'crosspollination' mapping populations used for the development of the grape genetic maps (1, 8, 18), but similar to those obtained by Troggio et al. (24) (20.3%). The obtained linkage groups (LG) were compared to the integrated grapevine map (IGM) of Doligez et al. (8) and were further numbered accordingly. Sixteen linkage groups were formed at LOD 3.0 and the markers in them remained linked at higher stringency ranging from LOD 3.0 to LOD 6.0. Markers in three linkage groups (LG 8, LG 12 and LG18) were grouped only after decreasing the LOD from 3.0 to 2.0. A total of 19 linkage groups consisting of 84 microsatellite markers were formed.

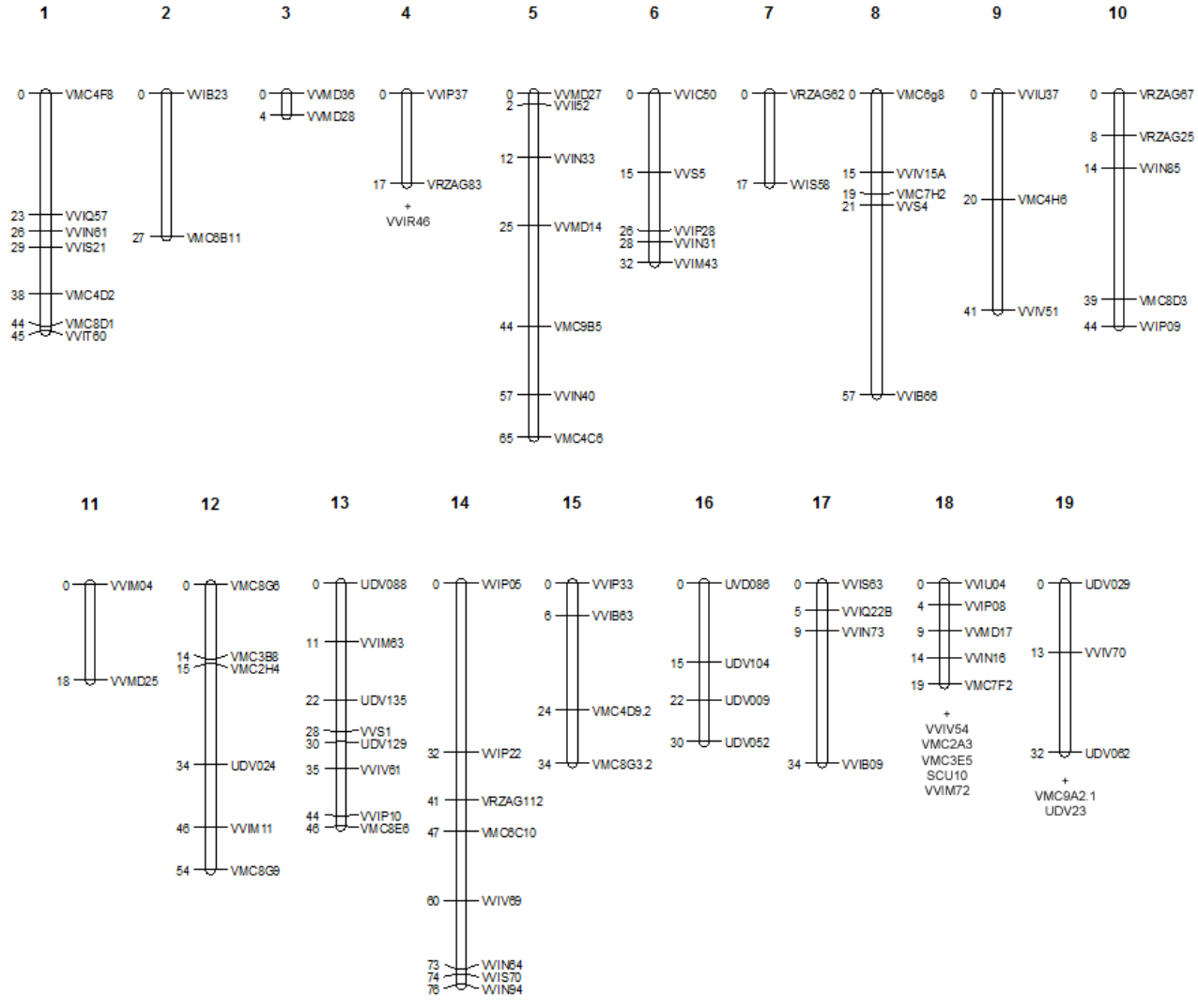

Fig.1. Linkage map of cultivar Storgozia. The linkage groups are numbered according to Doligez et al. (8). Distances are shown in cM Kosambi. The unmapped markers are shown under the corresponding LGs. Tzvetanka D. Hvarleva[1], Krasimir E. Russanov[1], Anastasia T. Bakalova[1], Miroslava K. Zhiponova[1], Galina J. Djakova[2], Atanas I. Atanassov[1], Ivan I. Atanassov[1].

Eight microsatellite markers present in the integrated grapevine map remained unlinked to the above linkage groups: VVIR46 (LG4 of IGM); VVIV 54, VMC2A3, VMC3E5, SCU10 and VVIN72 (LG18 of IGM); VMC9A2 and UDV023 (LG19 of IGM). In all cases the lack of grouping of these markers is due to the large gap, more than 40 cM, between the unmapped marker and the last mapped marker on the corresponding LG. The further analysis of segregation of these markers shows linkage of VVIV 54, VMC2A3, VMC3E5 (located on LG18 of IGM) and separate linkage of VMC9A2 and UDV023 (located on LG19 of IGM) at LOD 3.0 and higher. Such linkage of ungrouped markers further confirms the transferability of the grape map data and point out the necessity of further saturation of the established map. One of the grouped microsatelite markers VVIV51 has not been mapped in any of the published to date grape genetic maps. In the present study it was mapped 21 cM away from marker VMC4H6 on LG9 at LOD 7.0, which demonstrates high probability for correct LG assignment of this marker.

In total the mapped microsatelite markers in the current study span 692 cM with an average distance of 10.7cM between the adjacent markers.

The order of the grouped markers was consistent with the integrated grapevine genetic map of Dolingez et al.(8), with exception of 5 inversions in LG1 (VVIT60/VMC8D1), LG8 (VMC7H2/VVS4), LG12 (VMC3B8/VMC2H4), LG15 (VVIP33/VVIB63) and LG19 (UDV029/VVIV70). The observed inversions could be due to the presence of non vinifera genetic background in cv. Storgozia, which may influence the rate of recombination. The male parent of cv. Storgozia (Villard Blanc) is a complex hybrid which has several Vitis species in its pedigree. The sampling bias and low number of individuals in the population could also be an explanation for the observed inversions.

Grapevine is a vegetatively propagated crop which is highly heterozygous and with a low tolerance to inbreeding. For this reason, almost all published grapevine linkage maps have been developed by application of the pseudo-testcross strategy (11) on the full-sib populations obtained by crosses between two heterozygous and distantly related parents (6, 8). To date the only grapevine genetic map based on F2 population obtained by self-pollination of the Riesling variety was published by Adam-Blondon et al. (1). It is widely assumed that higher level of genome-wide homozygosity in grapevine is difficult or impossible to be achieved due to inbreeding depression. In this study we apply a genome-wide set of microsatelite markers to characterize a grapevine F2 population derived from self-pollination of cv. Strogozia. Cultivar Storgozia originates from a cross between the newly bred Bulgarian cultivar Buket and cultivar Villard Blanc. The female parent of cv. Storgozia (cv. Buket), originates from the cross between the ancient Bulgarian variety Mavrud and the French variety Pinot Noir. Cultivar Villard Blanc has a complex genome structure derived from successive inter-specific crosses between V. vinifera and American Vitis species. Thus the expected elevated heterozygousity of cv.Strogozia suggests a lower level of inbreeding depression. The present data does not show the presence of clusters of distorted markers, as was reported by Troggio et al. (24) and by Lowe and Walker (15). Further increasing of the map density is required for the correct genome-wide evaluation of such regions related to the inbreeding depression. Presently in addition to the further saturation of the established map with SSRs and RGA (resistance gene analogs) markers we put efforts on mapping of SNP markers corresponding to a set of selected genes for disease resistance and agronomic traits based on data from the sequencing of the grapevine genome. Step-vice increasing of the size of the studied F2 population of cv. Strogozia will further enforce the mapping studies.

Conclusions

In this study we used a F2 segregating population for construction of microsatellite based linkage map of Bulgarian cultivar Storgozia. This variety possesses a field resistance to three economically important for the country fungal diseases (downy mildew, powdery mildew and grey mould) and correspondingly the mapping population obtained after its selfpollination segregates for the response to these pathogens. The established in this study microsatellite framework linkage map offers an important background for mapping of QTLs related to fungal disease resistance and selection of breeding lines for respective breeding programs. The obtained genetic map is consistent with the consensus grapevine linkage map. The use of common microsatellite markers allows a cross-reference between different linkage maps of grapevine, which in a great degree benefits the studies related to the identification and precise mapping of loci underlying important agronomic traits in grapevine. Parallel mapping of other characteristics like berry size, yield, color, ripening, etc will largely facilitate the breeding process. Such investigations are in particular interest for development of MAS in the country and generating a genotypes combining high quality of grapes and resistance to different diseases.

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