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ARMENIAN GRAPE VARIETIES SSR FINGERPRINTING BY USING QIAXCEL DNA FRAGMENT ANALYSIS SYSTEM

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The applicability of QIAxcel System for SSR fingerprinting of grape varieties exploiting the internationally accepted 9 microsatellite markers was investigated. To optimize the allele sizing, a comparative genotyping of seven reference varieties with known sizes of microsatellite alleles, proposed by GENRES 081 project consortium, was performed. Based on the results of optimization, some Armenian table grape varieties were successfully genotyped. So, the QIAxcel System might be a useful tool for accurate genotyping and identification of plant genetic resources for scientific and commercial purposes.

Qiaxcel – microsatellites – genotyping – grape

Ուսումնասիրվել և գնահատվել է QIAxcel համակարգի կիրառելիությունը խաղողի սորտերի՝ միջազգայնորեն ընդունված 9 միկրոսատելիտային մարկերներով ուսումնասիրությունների համար: Ալելների երկարության որոշման օպտիմացման համար կատարվել է GENRES 081 ծրագրի կողմից առաջարկված խաղողի 7 ստուգիչ սորտերի համեմատական գենոտիպավորում, որոնց համար հայտնի են միկրոսատելիտային ալելների նուկլեոտիդային երկարությունները: Հիմք ընդունելով օպտիմացման արդյունքները ուսումնասիրվել են Հայաստանի խաղողի որոշ սորտեր, որոնց համար ստացվել են համապատասխան գենետիկական պրոֆիլներ: Ցույց է տրվել, որ Qiaxcel համակարգը կարող է արդյունավետ գործիք լինել բույսերի գենետիկական ռեսուրսների նույնականացման և գենոտիպային ուսումնասիրությունների համար ինչպես գիտական, այնպես էլ կոմերցիոն նպատակներով:

Qiaxcel – միկրոսատելիտներ – գենոտիպավորում – խաղող

Изучена и оценена применяемость системы QIAxcel для микросателлитного генотипироания сортов винограда с применением 9 междуноднопризнанных микросателлитных маркеров. В целях оптимизации определения длины аллелей было проведено сравнительное генотипирование семи стандартных сортов с известной длиной микросателлитных аллелей, предложенных консорциом проекта GENRES 081. Основываясь на результатах оптимизации генотипированы некоторые армянские сорта винограда. Показано, что система Qiaxcel может стать эффективным инструментом для генотипирования и идентификации генетических ресурсов растений как в научных, так и в коммерческих целях.

Qiaxcel – микросателлиты – генотипирование – виноград

Grapevine represents one of the most important products of plant domestication during the development of human civilization. It is intimately bound up with the expansion of agriculture, trade and commerce and is also important in social-religious, cultural and political aspects of many societies [4, 14].

Grapevine production, based on proper use of local genetic resources and on sustainable viticulture practices, provides a major potential source of income for farmers, especially in low income transition countries of the Caucasus and Eastern Europe, including Armenia. In 2010 the world's global grapevine area was estimated as 8.1 million hectares out of which approximately 8 million ha vineyard is mostly processed into wine, but some is destined for fresh consumption as table grapes, dried into raisins, processed into non-alcoholic juice, and distilled into spirits (http://faostat.fao.org). Currently, there are over 10,000 grape cultivars names in use worldwide [2], and their classification is often confusing because of homonyms, synonyms, scarce or incorrect historical information [13]. In addition, the different languages used in the east European countries, like Armenia, produce different spellings of the same variety name, which may cause double registering. Simultaneously accurate identification of grape varieties is most important issue for its sustainable conservation, management and use [7]. For proper identification of grape varieties in line with ampelography, the modern biomolecular approaches (DNA markers based analysis), are needed. Nowadays SSR and SNP markers are the most advanced and widely used markers in grape varieties fingerprinting and related investigation. For SSR analyses the capillary electrophoresis technology is widely used [8, 10, 14, 19]. The capillary electrophoresis is mainly combined with use of fluorescently labeled primers and performed for example ABI 3100xl DNA Sequencer (ABI 3100xl: Applied Biosystems, USA). Such kind of analyses provides high detection sensitivity [11, 17] and might be used for grape profiling. So, it is clear that the cost of the instrument and appropriate reagents are quite high and economically not effective. The QIAxcel System (Qiagen, USA) is a relatively inexpensive instrument that uses disposable micro-channel cartridges containing sieving-gel matrix with ethidium bromide (EtBr) dye to generate both gel images and allele sizes [3,12,13]. Most of small laboratories can effectively use these systems for genotyping.

The purpose of this investigation was to study the applicability of QIAxcel system for SSR fingerprinting of grape varieties exploiting the internationally accepted 9 micro-satellite markers.

Materials and methods. Armenian table grape varieties ''Deghin Yerevani'', ''Vardaguyn Yerevani'', ''Parvana'', ''Marmari'', ''Sev Kishmish'' provided by Scientific Center of Viticulture, Fruit-Growing and Wine-Making in Armenia, as well as seven reference varieties (Furmint B, Sultanina, Cabernet-Sauvignon, Chardonnay B, Merlot N, Pinot noir N, Carignan N) proposed by GENRES 081 consortium were investigated.

The following 9 polymorphic SSR primers were chosen: VVS2 [20], VVMD5, 7 [5], VVMD25, 27, 28, 32 [6], ZAG62 and ZAG79 [18].

DNA was isolated from fine powdered leaf tissues frozen in refrigerator at -25C degrees and homogenized in Tissue Lyzer LT (Qiagen) to avoid cross contamination among samples. The extraction was performed according to the protocol for DNeasy Plant Mini Kit (Qiagen, Hilden, Germany).

Isolated DNA quantity and quality were evaluated using Multiskan Microplate Spectrophotometer (Thermo Scientific). In addition, 10 µl of each DNA extract were loaded and visualized on a 1.5% agarose gel.

PCR amplifications were performed by using Type it Microsatellite PCR kit (QIagen) in 25 µl reaction mixtures containing 5 µl DNA template (10 ng/µl), 12,5 µl of 2x Type-it Miltiplex PCR Master Mix (3mM MgCl2) with (Qiagen), 2.5 µl of primer Mix (2mkM of each primer), and 5 µl of sterile RNase free water. PCR Amplification was performed in gradient PCR machine (Techne, gradient thermal cycler) with the following programme 95°C for 5 min, followed by 35 cycles of 95°C for 30 seconds, 60°C for 90 seconds, 72°C for 30 seconds, and a final extension at 68°C for 10 min. For selection of optimal annealing temperature for the selected primers the gradient PCR were performed at the first steps with T – 58°C, gradient 18°C.

Gel electrophoresis (3% agarose) of 2 µL of each sample per lane was used to verify PCR amplification had occured during 30 min, under 120V. The QIAxcel System was used to determine

determine the size of the amplified alleles for each individual using the QIAxcel Advanced User Manuel (QIAGEN 2011). The QIAxcel DNA High Resolution Kit and Cartridge were utilized, along with the QX 25 bp/500 bp and pUC18/HaeIII Size Markers and the QX 15 bp/600 bp Alignment Marker, for the base calling of alleles (QIAGEN 2011). The concentration of the size marker was diluted to 30 ng/ μL (QIAGEN 2011). Data were automatically recorded and exported using BioCalculatorTM software, which provides both a gel view and an electropherogram of the separated PCR products (alleles) [21].

Results and Discussion. A comparison of the results of SSR fingerprints of grape reference varieties obtained on QIAxcel system by using QX 25 bp/500 bp and pUC18/HaeIII markers and the results provided by the Genres 081 project consortium shaw differences in size of SSR markers sizes (Tab.1). The difference in case of application of pUC18/HaeIII maker for size determination was around 10-15 bp, and in case of QX 25 bp/500 bp marker was around 5-10 bp. In table 1 the difference in allele sizes for Furmint B variety is presented. It should be noted that the QIAxcel System software calculates allele sizes based on the DNA size marker table generated from an earlier analysis on the same cartridge. However, in our experiments for each run we were running the size marker too.

Tab. 1. Comparison allele sizes genereated by 9 SSR primers for reference variety Furmint B

The name of reference variety	SSR markers	SSR markers sizes determined by GENRES 081 consortium	SSR markers sizes determined by Qiaxcel system, using pUC18/HaeIII size marker	SSR markers sizes determined by Qiaxcel system, using QX 25/500 bp size marker
Furmint B	VVMD5	223 238	240 248	229 242
	VVMD7	239 249	250 261	245 257
	VVMD25	238 240	249 255	243 250
	VVMD27	176 191	185 202	182 200
	VVMD28	227 247	238 285	235 253
	VVMD32	263 271	274 285	269 280
	VVS2	131 151	145 163	136 160
	VrZAG62	188 204	201 215	194 210
	VrZAG79	238 250	252 265	245 255

The widely used for SSR genotyping ABI instruments use an internal standard in each sample and allelic samples are calculated individually for each capillary. That is why the resolution and accuracy of size determination is around 1-2 base pairs. The application of QIAxcel system for evaluation of genetic diversity of flowering dogwood (Cornus florida L.) [9], for molecular characterization of wild species and miniature roses [1] was shown that only limitation is the low resolution of allele determination in comparison with ABI systems. In all mentioned cases the authors are using unmodified protocols of size determination by QIAxcel system provided by manufacturer.

In our experiments to increase the accuracy of size determination we have chosen QX 25/500 bp size marker as the range of DNA fragments is mostly correspond to the expected sizes of the SSR alleles. We have included the size marker in all of the samples for the QIAxcel System, and as result the calculated allelic sizes were within 1 or 2 bp. So the modification of the protocol increased the accuracy of size determination by Qiaxcel system.

By using the optimized allele scoring protocol for Qiaxcel system, we have performed genetic fingerprinting of some Armenian table grape varieties. According to the results obtained it is possible to differentiate the varieties with 9 SSR markers by using Qiaxcel system (tab.2).

Tab. 2. Genetic fingerprints of table grapes by 9 SSR markers

Variety	SSR markers size (bp)								
	VVMD5	**VVMD7**	**VVMD25**	**VVMD27**	**VVMD28**	**VVMD32**	**VVS2**	**VrZAG62**	**VrZAG79**
Deghin Yerevani	227 232	230 244	228 237	162 177	203 231	236 237	126 133	170 170	233 242
Vardaguyn Yerevani	227 227	236 250	228 238	162 177	202 231	236 236	156 135	173 173	232 245
Parvana	229 234	234 244	241 241	165 180	232 232	243 266	130 135	175 175	240 246
Marmari	232 232	240 241	229 239	178 180	220 232	238 238	141 133	171 171	235 244
Sev Kishmish	228 228	249 253	237 241	164 180	205 235	238 238	140 161	179 193	235 240

The QIAxcel System (fig.1) automatically generated tables of allele size, the resolution and allele size determination might be optimized, it is less expensive and less labor intensive in comparison with ABI systems.

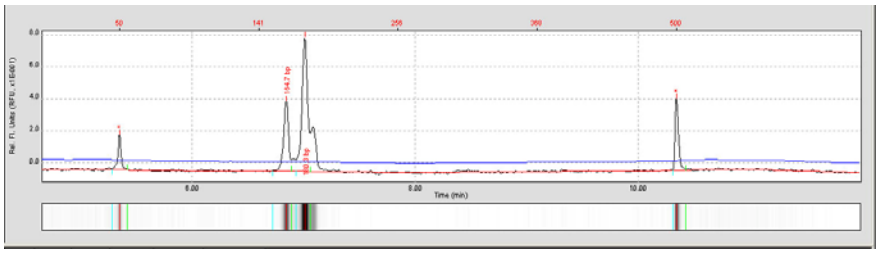

Fig.1. The graphs of the allele sizes of Deghin Yerevani grape variety with SSR primers VVMD 27 (upper) and VrZAG262 (lower) presented by QIAxcel System genetic analyzer

QIAxcel system is much more effective than conventional electrophoreses in agarose and polyacrylamide gels. It could automatically analyze 96 samples without the need for manual sample and gel preparations. So, it can be useful tool not only for accurate genotyping assays, but also for large scale population analysis, when allele sizes are converted to binary (1, 0) output before analysis. QIAxcel System might be useful tool for accurate genotyping and variety identification of microbial, plant and animal genetic resources for scientific and commercial purposes.

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