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APPLICATION OF SSR MARKERS FOR STUDY OF GENETIC DIVERSITY OF *Venturia inaequalis* IN THE DIFFERENT TYPES OF ORCHARDS IN THE NORTH CAUCASIAN REGION

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Abstract

Apple scab caused by ascomycete fungus *Venturia inaequalis* (Cooke) G. Winter is one of the most harmful diseases of apple trees, which leads to significant economic losses in apple production in the world. North Caucasus is a region with climatic conditions favorable for *V. inaequalis*. Therefore, the creation of resistant varieties is an important target for apple breeding. Study of the genetic diversity of the pathogen is an integral part of both science-based apple breeding programs and systems of protection against the pathogen. This paper is the first report on SSR analysis of genetic diversity of *V. inaequalis* strains collected in apple orchards that differ in structure and are located geographically remotely in the Krasnodar Territory and the Republic of Adygea. To study the genetic polymorphism of the phytopathogen populations, two industrial gardens and a collection of *Malus orientalis* were surveyed in the Kuban and Caucasus foothill agro-ecological zones of the region. The genetic heterogeneity of the host plant populations at the sampling sites varied significantly, since the industrial orchards were single-cultivar plantations of the apple varieties Gala, Renet Simirenko, Golden Delicious, and Champion while in the collection garden the accessions originated from different parts of the *M. orientalis* natural area. Eight SSR markers used were 1aac4f, Viga7/116, Vitc1/2, Vitcca7/P, Vicacg8/42, Viga3/z, 1tcla, Vitc2/D. The number of alleles per locus revealed in SSR analysis of 36 monosporic isolates of *V. inaequalis* was 4 for 1aac4f, 6 for Vitc2/D, 10 for Viga7/116 and Vicacg8/42, 11 for Vitcca7/P, and 12 for Vitc1/2 and 1tcla. Upon the whole, there were 4 (1aac4f) to 12 alleles (Vitc1/2, 1tcla) for polymorphic markers, and only one allele was detected for marker Viga3/z. Despite the fact that some markers showed various distributions of identified alleles in all subpopulations, these differences were not sufficient to differentiate the subpopulations. UPGMA-analysis showed no relationship between clusterization and the geographical origin of the isolates, indicating low inter-population differences. This can indicate a free gene flow between the populations due to human activity as they are too distant from each other to allow natural transfer of spores. The obtained results suggest significant genetic diversity in the investigated set of monospore isolates. Genetic diversity was higher in the *V. inaequalis* population from the *M. orientalis* collection, indicating the effect of plant population heterogeneity on genetic polymorphism of the pathogen. In our opinion, the differences in polymorphism for some SSR markers, when compared our data and the results reported by other researchers' for European populations of *V. inaequalis*, could be due to genetic differences in populations of *V. inaequalis* from North Caucasus region and the European populations.

Keywords*:* apple scab, *Venturia inaequalis*, genetic diversity, SSR-markers, allele polymorphism, North Caucasus

Apple scab caused by ascomycete *Venturia inaequalis* (Cooke) G. Winter is one of the most harmful diseases of apple trees, which leads to significant

economic losses in apple production in the world [1]. During epiphytotics the scab may affect up to 80-100% of apple production sensitive thereto in areas with conditions favoring scab growth (basically, temperate countries including Russia, and North Caucasus region, in particular) [2]. High quality requirements to the production demand strict control over the disease. As a rule, fungicides are used for this purpose nowadays, with a number of treatments being 17-22 applied to highly-susceptible varieties in season [2].

Great attention is paid to development of resistant cultivars due to high harmfulness of scab. Analysis of genetic diversity of pathogens is important both for making breeding programs and disease control measures against pathogens. Application of artificial infection background for choosing resistant cultivars from hybrid materials will enable to intensify the breeding process. Therewith, the information on genetic diversity of pathogen will enable us to make the most heterogeneous inoculum, thereby increasing efficiency for assessing stability [3].

Morphological and cultural characteristics and virulence determinants [4-9] were used in early steps for analyzing genetic diversity of pathogen. However, application of DNA markers that opened a new phase in studying genetic diversity of *V. inaequalis* [10] proved to be the most efficient. In early 1990s marker analysis of *V. inaequalis* in major cases was based on three methods, i.e. RFLP (restriction fragment length polymorphism), RAPD (random amplification of polymorphic DNA) and ITS sequence analysis [10, 11]. More informative DNS-markers like SSR (simple sequence repeats) have appeared along with the development of molecular-genetic tools. They are characterized by high specificity and sensitivity, relative simplicity in operation and estimated results [10]. A number of genetic diversities of pathogen was done with their application. According to major experiment made by some scientists from France, Belgium and China *V. inaequalis* was found in Central Asia in the center of *Malus* spp. origin [12, 13]. The genetic diversities of *V. inaequalis* populations found in *Malus sieversii* in Central Asia were presented by more types than in European populations in *Malus* × *domestica* and *Malus sylvestris* [13] and indicated age of their existence. Dependence of genetic diversity of *V. inaequalis* on population age was also noted by other authors [14, 15]. High level of intrapopulation diversity and low differentiation between populations was demonstrated in many population analyses of this fungal pathogen [10, 11, 16, 17). Availability of vast panmictic populations of the pathogen at quite moderate potential of natural propagation (15-60 m) [18] is explained by high gene flow due to human activity (movement of affected seeds and fruit) [10, 13].

Expansion of geographical boundaries where genetic polymorphism is analyzed is likely to specify peculiarities characterizing both inter- and intrapopulation genetic interactions in *V. inaequalis*. Besides, analysis of genetic diversities of pathogen in both natural and man-made ecosystems of North Caucasus regions as a part of *M. orientalis* species will enable to evaluate microevolutionary processes of interactions between pathogen and host-plant. Up to now, such analyses were not conducted in the territory of Russia.

By using SSR markers we were the first in making SSR genotyping in geographically remote populations of *V. inaequalis* in North Caucasus and revealed polymorphism by a number of markers that were used at analysis of genetic structure of populations in this pathogen in other regions. Comparison between results obtained and information from literature enables us to assume that North Caucasus population of this pathogen may differ genetically from the European one.

The work includes analysis of diverse *Venturia inaequalis* isolates selected from agrophytocenoses different both by structure and localization by using SSR

markers.

Techniques. *Venturia inaequalis* sets (April-May 2015) were taken in three geographical points of two agro-ecological zones of North Caucasus regions, i.e. Prikubanskaya (No.1, Krasnodar, Vodniki Settl., ZAO Experimental Production Farm Tsentralnoe, 2nd division) and Predgornaya in the Republic of Adygea (No.2, Maikop region, Podgorny Settl., Maikop experimental station at All-Union Research Institute of Plant Breeding, collection of apple genetic resources; No.3, Abadzekhskaya village, Muskat farm household). Monospore isolates of agent were isolated from ascospores to pure culture in sterile conditions according to original procedure [19] using leaf litter with pseudothecia as per indicated protocols [20]. Agarized media were prepared by standard microbiological methods [21, 22]. Isolates in points 2 and 3 were taken from various trees of one species or cultivars (*M. orientalis* and Champion accordingly), and from various apple varieties (Gala, Renet Simirenko and Golden Delicious in point 1).

DNA was extracted as per recommendations [23].

Microsatellite DNA markers 1aac4f [10] and Viga7/116, Vitc1/2, Vitcca7/P, Vicacg8/42, Viga3/z, 1tcla, Vitc2/D [24] were used to assess genetic diversities. Mixture for PCR amplification (25 μl) contained 50-70 μg of DNA, 0.05 mM dNTPs, 0.3 μM of each primer; 2.5 μl of 10× reaction buffer, 2.5 mM $MgCl_2$, 1 U Taq DNA-polymerase. PCR (a Mastercycler gradient amplifier, Eppendorf, Germany) was performed by the following scheme: 5 min at 95 °C (initial denaturation); 10 s at 95 °C (denaturation), 30 s at 60 °C (primer annealing), 30 s at 72 °C (elongation) (30 cycles); 3 min at 72 °C. Size of amplified parts of SSR markers was identified (an automatic genetic analyzer ABI prism 3130, Applied Biosystems, USA). Data were processed with GeneMapper 4.1 application included to ABI prism 3130 software.

Cluster analysis was made by UPGMA method applying PAST software [25]. Allele frequencies were calculated with GenAlEx 6.5 [26], and PIC value (Polymorphism Information Content) with Polymorphism Information Content Calculator (http://w3.georgikon.hu/pic/english/kezi.aspx) [27].

Results. A total of 36 monospore isolates were used for the analysis (in points 1, 2 and 3 — 20, 9 and 7, respectively). Seven of eight SSR markers in the analyzed set at SSR genotyping proved to be polymorphous, i.e. from 4 to 12 alleles per locus were detected in them (Table 1). Marker Viga3/z with one allele (99 bp) was excluded from statistical processing as a monomorphic one.

1. Polymorphism of SSR markers in a set of *Venturia inaequalis* isolates from various agro-ecological zones (n = 36, North Caucasus region, 2015)

Parameter	SSR marker						
	1aac4f	Viga7/116	Vitc1/2	Vitcca7/P	Vicacg8/42	1tcla	Vitc2/D
Number of alleles per locus	4	10	12	11	10	12	6
Range of part lengths, bp	107-120	138-180	181-220	168-215	203-230	116-167	213-246
PIC	0.153	0.733	0.733	0.870	0.655	0.854	0.710

N o t e. PIC — polymorphism information content.

The least allele polymorphism was detected for markers 1aac4f and Vitc2/D. A number of both concordances and reverse results are worth noting for some regions by comparing data on SSR polymorphism level obtained by us and by foreign authors. So, in Worchestershire and East Malling of Great Britain when using four SSR markers for comparing genetic diversity and structure of *V. inaequalis* population the highest polymorphism was detected by SSR marker Vitc2/D (29 alleles per locus) by selecting among 102 monospore isolates, while the value for Vitcca7/P and Vitc1/2 markers was far lower, 19 and 9 alleles per locus, accordingly [14]. In our analysis Vitcca7/P and Vitc1/2 markers may be attributed to the most polymorphous. At the same time polymorphism values of

SSR markers 1aac4f and 1tcla detected both by I. Tenzer et al. [10] and by us on the contrary correspond to each other. These scientists have assessed genetic diversity in 11 *V. inaequalis* populations from five European countries (France, Germany, Italy, the Netherlands and Switzerland). According to them 1tcla marker, as was also indicated by us, possessed one of the highest values of allelic polymorphism (26 alleles per locus), therewith the value was one of the lowest by 1aac4f marker (4 alleles per locus). A group of scientists having made 21 SSR markers for *V. inaequalis* and assessed polymorphism thereof using 44 monospore isolates from six European countries have identified 8, 18, 9 and 11 alleles per locus for Viga7/116, Vitc1/2, Vitcca7/P and Vicacg8/42, respectively, at average value of 9 alleles per locus for 21 markers [24]. High polymorphism by these markers was also indicated in our paper. At the same time the highest number of alleles (24 per locus) [24] was reported for Vitc2/D marker, while in populations analyzed by us it proved to be one of the least polymorphous (6 alleles per locus).

In our opinion, great polymorphism differences by some SSR markers detected by us in North Caucasus subpopulations of *V. inaequalis* from that of described by foreign scientists for European population of this pathogen may indicate to genetic differences between North Caucasus and European population of *V. inaequalis* due to their geographical distance.

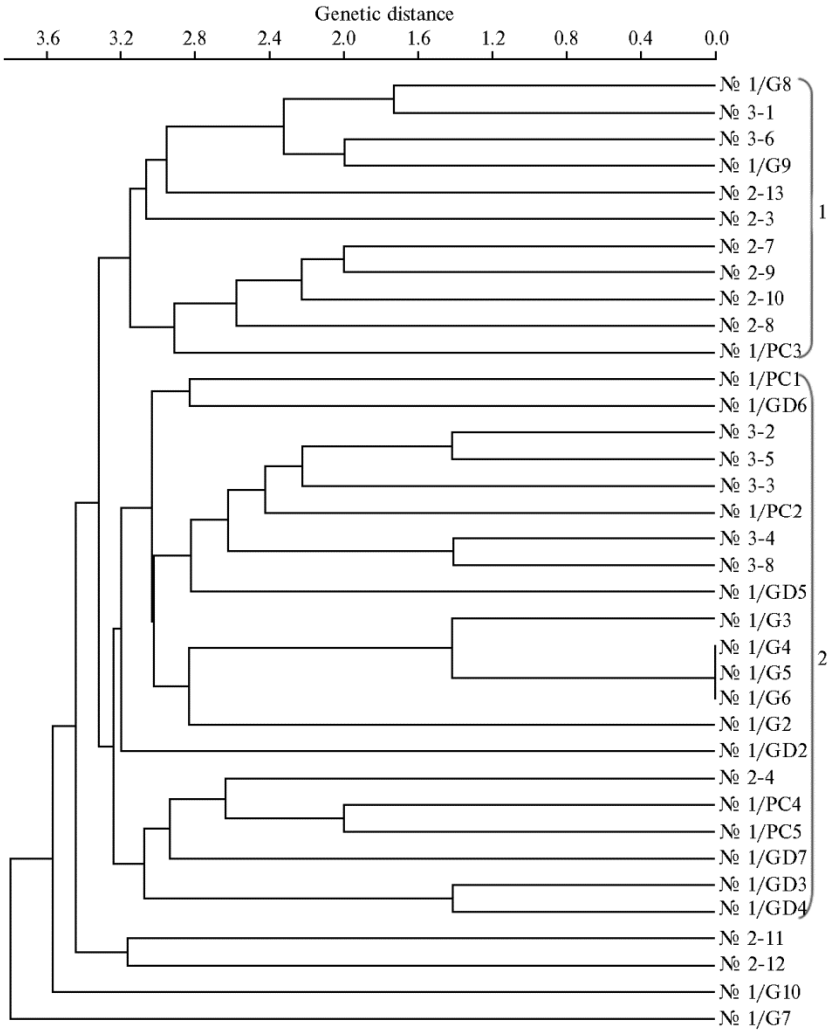
Comparison of allele frequency of SSR markers in set of monoisolates is given in Table 2 for assessing differences between three subpopulations.

2. Allele frequency detected by SSR genotyping of *Venturia inaequalis* isolates in three populations of pathogen from various agro-ecological zones (North Caucasus region, 2015)

SSR marker	No. 1 (n = 20)	No. 2 (n = 9)	No. 3 (n = 7)
1aac4f	107 (0.950); 120 (0.050)	107 (0.778); 109 (0.111); 112 (0.111)	107 (1.000)
Viga7/116	143 (0.025); 164 (0.175); 166 (0.050); 168 (0.350); 170 (0.125); 172 (0.075); 174 (0.125); 180 (0.075)	138 (0.111); 141 (0.111); 164 (0.444); 168 (0.333)	143 (0.143); 164 (0.286); 168 (0.571)
Vitc1/2	183 (0.175); 187 (0.025); 190 (0.100); 192 (0.200); 193 (0.050); 195 (0.300); 213 (0.050); 215 (0.100)	181 (0.111); 190 (0.222); 192 (0.222); 210 (0.333); 220 (0.111)	190 (0.286); 192 (0.286); 194 (0.429)
Vitcca7/P	0 (0.050); 168 (0.125); 194 (0.175); 196 (0.050); 200 (0.075); 202 (0.250); 204 (0.050); 210 (0.125); 215 (0.100)	170 (0.111); 196 (0.222); 198 (0.222); 200 (0.111); 202 (0.111); 204 (0.111); 215 (0.111)	194 (0.286); 198 (0.429); 200 (0.143); 202 (0.143)
Vicacg8/42	205 (0.025); 210 (0.575); 212 (0.050); 216 (0.100); 218 (0.050); 222 (0.100); 224 (0.050); 228 (0.050)	203 (0.222); 205 (0.333); 210 (0.222); 216 (0.111); 230 (0.111)	210 (0.857); 216 (0.143)
1tcla	116 (0.050); 120 (0.175); 129 (0.050); 131 (0.200); 135 (0.150); 141 (0.375)	133 (0.222); 137 (0.111); 139 (0.111); 143 (0.333); 145 (0.111); 167 (0.111)	120 (0.429); 133 (0.286); 135 (0.286)
Vitc2/D	213 (0.200); 232 (0.200); 234 (0.400); 236 (0.150); 244 (0.050)	232 (0.667); 234 (0.111); 236 (0.111); 246 (0.111)	234 (0.286); 236 (0.714)

N o t e. No. 1 — Krasnodar, Vodniki Settl., ZAO Experimental Production Farm Tsentralnoe, 2nd division (Prikubanskaya agro-ecological zone); No. 2 — Maikop region, Podgorny Settl., Maikop experimental station at All-Union Research Institute of Plant Breeding, collection of apple genetic resources, No. 3 — Abadzekhskaya village, Muskat farm household 3 (the Republic of Adygea, Predgornaya agro-ecological zone). Allele size is given in bp, allele frequency is indicated in brackets.

Majority of alleles detected in subpopulation 1 from Prikubanskaya agro-climatic zone is due to small sample size, i.e. 20 monospore isolates. It should be mentioned availability of alleles with maximum value of this indicator simultaneously in two or three subpopulations (allele 107 bp by marker 1aac4f, allele 168 bp by markerViga7/116 and allele 210 bp by marker Vicacg8/42). This fact may testify to small interpopulation differences in spite of



UPGMA-dendrogram characterizing high level of genetic similarities between monospore isolates of *Venturia inaequalis* from different agro-ecological zones: No. 1 — Krasnodar, Vodniki Settl., ZAO Experimental Production Farm Tsentralnoe, 2nd division (Prikubanskaya agro-ecological zone); No. 2 — Maikop region, Podgorny Settl., Maikop experimental station at All-Union Research Institute of Plant Breeding, collection of apple genetic resources, No. 3, Abadzekhskaya village, Muskat farm household 3 (the Republic of Adygea, Predgornaya agro-ecological zone) (North Caucasus region, 2015).

the presence of unique alleles in each sampling that in majority cases appeared occasionally. A group of monospore isolates from collection of *Malus orientalis* sets in Maikop experimental station at All-Union Research Institute of Plant Breeding is of interest. For some SSR markers (1aac4f, 1tcla and Vitc2/D), a number of alleles in this group consisting of 9 isolates has matched or exceeded the same in population No. 1 presented by 20 isolates. It may be considered as higher genetic diversity of *V. inaequalis* population to be found on *M. orientalis* plants from a collection of genetic resources well preserving a wide spectrum of specific sets of *Malus* species, as well as of apple tree varieties (point No.2). It should be mentioned that our results agree with data by O.N. Barsukova [5], who has compared diversity of pathogen in this collection and in the region under morpho-cultural and virulent characteristics. The author concludes that the diversity of pathogen found in wildlife host species in natural growing conditions and in collection planting is higher than in populations having formed

in cultivated species [5].

We have made a cluster analysis for assessing level of genetic similarity in monospore isolates from sampling to be studied (Fig.). Under clusterization results two main clusters and four sets (Nos. 2-11, 2-12, 1/G10, 1/G7), may be identified to be referred to three separate branches. Distribution of isolates by clusters is obviously not to correspond to their geographical origin. Besides, distribution of sets in cluster 1 did not depend on cultivar where the pathogen was isolated. In spite of the fact that a group of sets (Nos. 1/G3, 1/G4, 1/G5, 1/G6, 1/G2) taken from Gala in garden varieties of ZAO Experimental Production Farm Tsentralnoe was formed in cluster 2, three other sets from this variety were included to other clusters. Sets Nos.1/G4, 1/G5 и 1/G6 are likely to be presented by clones as they were united at minimum genetic distance (due to identical allelic set by studied SSR markers). Groups of monospore isolates from Golden Delicious (No. 1/GD) and Renet Simirenko (No. 1/RS) varieties formed no separate clusters either.

Therefore, high indicators of genetic diversity in the analyzed sampling of monospore isolates of *V. inaequalis* at low interpopulation differences may testify to a free gene flow between the populations studied stipulated (as they are too distant from each other to allow natural transfer of spores) by human activity that is agreed with information from references [10, 11, 15]. SSR markers have revealed genetic diversities between populations of pathogen having been formed in agrophytocenoses different in structure: allelic polymorphism of SSR locus in *V. inaequalis* was higher in heterogenetic collection trees (sampling point No. 2) than in single-cultivar planting of industrial orchards (likely due to far more high variety of host plant). High level of genetic diversity in phytopathogen population is known to prevent from domination of single supervirulent and aggressive biotypes thereby decreasing probability of epiphytoties occurrence [28]. This fact is proving the approach based on application of plantings with mixed varieties.

It should be noted that there are some differences in the results of assessing polymorphism both in our research of North Caucasus population and in papers where European *V. inaequalis* populations are considered. The reason for this is likely to be vast genetic distances between *V. inaequalis* populations in the indicated regions which may testify both to limitation of gene flow from Europe to North Caucasus and to independent formation of pathogen population in North Caucasus region. One of the factors for such formation may be explained that North Caucasus is situated within the area of *M. orientalis* species, i.e. host plant for *V. inaequalis*.

Thus, the obtained results suggest significant genetic diversity in the investigated set of monospore isolates of *Venturia inaequalis*. Clusterization of isolates does not depend on geographical origin thereof, thereby indicating low inter-population differences. At the same time clusterization is closely related with planting type (single-cultivar orchards or heterogeneous collection varieties) but not always depends on cultivar where isolates were collected. Polymorphism observed for some SSR markers corresponds to that described on European populations of pathogen, but there are also some differences that in our opinion could be due to peculiarities of formation of North Caucasus pathogen population.

REFERENCES

1. MacHardy W.E. *Apple scab: biology, epidemiology, and management*. The American Phytopathological Society, St. Paul, MN, 1996.

2. Nasonov A. I., Suprun I.I. *Mikologiya i fitopatologiya*, 2015, 49(5): 275-285 (in Russ.).
3. Sedov E.N., Zhdanov V.V., Serova Z.M., Makarkina M.A. Apple breeding for scab resistance as a development of N.I. Vavilov's and I.V. Michurin's ideas. *Agricultural Biology*, 2013, 1: 42-52 (in Russ.).
4. Fedorova R.N. *Parsha yabloni* [Scab of apple trees]. Leningrad, 1977 (in Russ.).
5. Barsukova O.N. *Mikologiya i fitopatologiya*, 1985, 19(6): 499-502 (in Russ.).
6. Barsukova O.N. *Mikologiya i fitopatologiya*, 1983, 17(5): 395-403 (in Russ.).
7. Dorozhkin N.A., Bondar' L.V., Konovalova N.A. *Mikologiya i fitopatologiya*, 1979, 13(5): 401-404 (in Russ.).
8. Tsikaridze O.N., Turtseladze Z.R., Lezhava I.L., Mikaberidze M.S. *Soobshcheniya AN GSSR*, 1983, 3(1): 101-104 (in Russ.).
9. Zhdanov V.V., Sedov E.N. *Selektsiya yabloni na ustoichivost' k parshe* [Breeding apple trees for resistance to scab]. Tula, 1991 (in Russ.).
10. Tenzer I., Degli-Ivanissevich S., Morgante M., Gessler C. Identification of microsatellite markers and their application to population genetics of *Venturia inaequalis*. *Phytopathology*, 1999, 89(9): 748-753.
11. Tenzer I., Gessler C. Subdivision and genetic structure of four populations of *Venturia inaequalis* in Switzerland. *Eur. J. Plant Pathol.*, 1997, 103(6): 565-571.
12. Gladieux P., Zhang X.G., Afoufa-Bastien D., Sanhueza R. V., Sbaghi M., Le Cam B. On the origin and spread of the scab disease of apple: out of Sentral Asia. *PLoS ONE*, 2008, 3(1): 1455 (doi: 10.1371/journal.pone.0001455).
13. Gladieux P., Zhang X.G., Roldan-Ruiz I.R., Caffier V., Leroy T., Devaux M., Glabeke S.V., Coart E., Cam B.L. Evolution of the population structure of *Venturia inaequalis*, the apple scab fungus, associated with the domestication of its host. *Mol. Ecol.*, 2010, 19(4): 658-674 (doi: 10.1111/j.1365-294X.2009.04498.x).
14. Xu X.-M., Yang J.-R., Thakur V., Roberts A.L., Barbara D.J. Population variation of apple scab (*Venturia inaequalis*) isolates from Asia and Europe. *Plant Dis.*, 2008, 92: 247-252 (doi: 10.1094/PDIS-92-2-0247).
15. Xu X., Yang J., Thakur V., Roberts A., Barbara D.J. Population variation of apple scab (*Venturia inaequalis*) within mixed orchards in the UK. *Eur. J. Plant Pathol.*, 2013, 135(1): 97-104 (doi: 10.1007/s10658-012-0068-4).
16. Khajuria Y.P., Kaul S., Dhar M.K. Molecular characterization of *Venturia inaequalis* causing apple scab in Kashmir. *Open Access Scientific Reports*, 2012, 1: 339 (doi: 10.4172/scientificreports.339).
17. Padder B.A., Sofi T.A., Ahmad M., Shah M.-Ul-D., Hamid A., Saleem S., Ahanger F.A. Virulence and molecular diversity of *Venturia inaequalis* in commercial apple growing regions in Kashmir. *J. Phytopathol.*, 2013, 161: 271-279 (doi: 10.1111/jph.12061).
18. Leroy T., Lemaire C., Dunemann F., Le Cam B. The genetic structure of a *Venturia inaequalis* population in a heterogeneous host population composed of different *Malus* species. *BMC Evol. Biol.*, 2013, 13(1): 64 (doi: 10.1186/1471-2148-13-64).
19. Nasonov A.I., Yakuba G.V., Suprun I.I. *Mikologiya i fitopatologiya*, 2016, 50(2): 131-132 (in Russ.).
20. Smol'yakova V.M., Yakuba G.V. *Diagnostika, uchet i prognoz parshi yabloni na Severnom Kavkaze. Nauchno-metodicheskie rekomendatsii* [Apple scab detection, records and forecasting in the North Caucasus. Scientific and methodical recommendations]. Krasnodar, 2003 (in Russ.).
21. Tepper E.Z., Shil'nikova V.K., Pereverzeva G.I. *Praktikum po mikrobiologii* [Workshop on microbiology]. Moscow, 1979 (in Russ.).
22. Bilai V.I. *Metody eksperimental'noi mikologii* [Methods of experimental mycology]. Kiev, 1982 (in Russ.).
23. Cenis J.L. Rapid extraction of fungal DNA for PCR amplification. *Nucleic Acids Res.*, 1992, 20: 2380.
24. Guérin F., Franck P., Loiseau A., Devaux M., Le Cam B. Isolation of 21 new polymorphic microsatellite loci in the phytopathogenic fungus *Venturia inaequalis*. *Mol. Ecol. Notes*, 2004, 4(2): 268-270 (doi: 10.1111/j.1471-8286.2004.00637.x).
25. Hammer O., Harper D.A.T., Ryan P.D. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 2001, 4(1): 9.
26. Peakall R., Smouse P.E. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research — an update. *Bioinformatics*, 2012, 28(19): 2537-2539 (doi: 10.1111/j.1471-8286.2005.01155.x).
27. Nagy S., Poczai P., Cernák I., Gorji AM., Hegedűs G., Taller J. PIC calc: an online program to calculate polymorphic information content for molecular genetic studies. *Biochem. Genet.*, 2012, 50: 670-672 (doi: 10.1080/09712119.2015.1124328).
28. D'yakov Yu.T. *Populyatsionnaya biologiya fitopatogennykh gribov* [Population biology of phytopathogenic fungi]. Moscow, 1998 (in Russ.).