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Identification of the Causal Agent of Grapevine Downy Mildew *Plasmopara viticola* Based on Quantitative PCR

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Abstract—A new method is proposed for early identification of the causal agent of grapevine downy mildew *Plasmopara viticola*, based on a real-time quantitative PCR (real-time PCR) method using the fluorescent dye SYBR Green I. Six pairs of primers were developed for real-time PCR for identification of *P. viticola*; among them, the primer pair PvITS1_2-real-s/a demonstrated the highest efficiency for early detection of grapevine downy mildew. Moreover, a positive correlation ($R = 0.86$) was demonstrated when comparing the results of real-time PCR with the primers PvITS1_2-real-s/a with data on metataxonomic analysis of the distribution of *P. viticola* among plants of Far Eastern grape species and varieties. Thus, real-time PCR with the primer pair PvITS1_2-real-s/a is a cheap and efficient method for early detection and monitoring of asymptomatic *P. viticola* infections. The developed method can be a basis for predicting epidemics of grapevine downy mildew and its control in vineyards.

Keywords: plant disease management strategies, mildew, *Vitis amurensis*, NGS

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INTRODUCTION

Plasmopara viticola (*P. viticola*, Berk. & M. A. Curtis; Berl. & De Toni) is an obligate pathogenic oomycete causing grapevine downy mildew and is one of the most dangerous pathogens for viticulture all over the world [1]. *P. viticola* affects young, green leaves and fruiting tissues of grapes, causing severe crop losses (40–90%) over short periods of time [2]. This grape pathogen is able to undergo multiple cycles of plant infection during a single growing season under favorable weather conditions, such as a high amount of precipitation and moderate temperatures. As a result of the high rate of pathogen spore formation, the epidemics of downy mildew that spread over large territories of vineyards can begin from sporadic foci of infection. Consequently, specific detection of *P. viticola* is very important for disease prediction and control.

Traditional methods for the detection of *P. viticola* oospores in grape leaves include direct microscopic observations of leaves [3–6] and indirect estimation using the "floating grape leaf disc" bioanalysis, in which oospore-containing leaf fragments are immersed in water in the presence of uninfected floating leaf discs; thus, zoospores derived from oospores cause infection in healthy leaf discs, the severity of which is proportional to the number of oospores [7].

Traditional methods of estimation do not allow quantitative determination of *P. viticola* in the infected samples, especially in the samples with a low concentration of the pathogen. At present, there are new more efficient approaches to the identification of *P. viticola* oomycete in grape tissues: (1) the spectrophotometric method for determining the viability of sporangia and zoospores of *P. viticola* oomycete [8]; (2) the nontargeted metabolomic approach (highly sensitive methods based on high-performance liquid chromatography with mass spectrometry (HPLC-MS)), in which the identification of plasma-specific lipid derivatives occurs [9]; (3) molecular methods with high specificity and sensitivity for quantitative determination of the pathogen, such as quantitative real-time PCR (real-time PCR) [10–12] and the loop-mediated isothermal amplification (LAMP) method [13]. Valsesia et al. [11] developed a multiplex real-time PCR method using TaqMan-type fluorescent probes for a relative quantitative determination of *P. viticola* DNA directly from the *Vitis vinifera* leaves, in which they used the primers selected for a region of the internal transcribed spacer 1 (*ITS1*) sequence (5.8S rDNA). Later, a similar analysis to quantify the amount of *P. viticola* DNA in infected, senescent leaves was used in the work of Ammour et al. [10]. The identification of downy mildew using LAMP was developed on the basis of the *P. viticola ITS1*

Table 1. Grape samples collected in July 2022

No.	Abbreviation	Location
		Vitis amurensis
1	Gh	Greenhouse of Federal Scientific Center of East Asian Terrestrial Biodiversity, Far East Branch, Russian Academy of Sciences, Vladivostok
2	M	Makarevich Fruit nursery, Ussuriisk
3	M-dm	*V. amurensis* with visible symptoms of *P. viticola*, Makarevich, Ussuriisk
4	S-Va	Botanical garden, Yuzhno-Sakhalinsk
5	P-1	Suburb of Vladivostok
6	P-2	Suburb of Vladivostok
7	P-3	Russkii Island, Vladivostok
8	P-4	Rikord Island, south of Primorskii krai
9	P-5	Village of Ivanovka, Primorskii krai
10	P-6	Verkhne-Ussuriisk Research Station, Federal Scientific Center of East Asian Terrestrial Biodiversity, Far East Branch, Russian Academy of Sciences
11	Kh-1	Village of Litovko, south of Khabarovsk krai
12	Kh-2	Silinskii forest, south of Khabarovsk krai
		Vitis coignetiae
13	S-1	Botanical garden, Yuzhno-Sakhalinsk
14	S-2	Kholmsk, Sakhalin Island
15	S-3	Nevelsk, Sakhalin Island
		Grape varieties from the Makarevich fruit nursery
16	Ad	*V. vinifera* × *V. amurensis* cv. Adel (hybrid No. 82-41 F^3)
17	Muk	*Vitis riparia* × *V. vinifera* cv. Mukuzani (unknown origin)
		Grape varieties from the commercial vineyard PRIM ORGANICA
18	Alfa	*Vitis labrusca* × *V. riparia* cv. Alfa,
19	Pr-St	*Vitis* Elmer Swenson 2-7-13 cv. Prairie Star

sequence; this analysis had a high sensitivity and was able to detect the presence of less than 33 fg of *P. viticola* genomic DNA per 25 μL reaction within 30 min [13]. Thus, all methods presented were mainly used for the detection of *P. viticola* in the leaves with downy mildew symptoms and were not used for detecting *P. viticola* in asymptomatic grape leaves.

It is worth noting that the rate of manifestation of downy mildew symptoms in apparently healthy leaves after infection with *P. viticola* is still unknown. Early determination of the *P. viticola* pathogen in visually healthy grape plants will allow timely application of the methods to control the downy mildew, which is certainly a key factor in reducing the risk of crop loss, as well as limit the treatment of healthy grapevines with fungicides, which will in general reduce the environmental load of agriculture on the natural environment.

The aim of this study was to develop the most efficient primer pair for molecular quantitative determination of *P. viticola* in apparently healthy grape samples without the symptoms of downy mildew using real-time PCR based on the fluorescent dye SYBR Green I.

METHODS

Plant material and conditions of surface sterilization. In July 2022, a total of 19 grape samples were collected (Table 1). All samples (except for M-dm) appeared healthy, without the symptoms of downy mildew. We collected at least two young shoots and two leaves from each plant.

The grape samples (leaves and stems) were washed under running water with soap (Al'tsept M, Russia). Under aseptic conditions, 0.2 g of the tissue from each grape sample was weighed. Then the samples were washed in 70% alcohol for 2 min, 1 min in 10% hydrogen peroxide solution, and with sterile water five times.

DNA isolation, library preparation, and Illumina MiSeq sequencing. DNA was isolated from 0.2 g of surface–sterilized grape samples using a CTAB-spin method as described previously [14]. For next generation sequencing using the Illumina technology, the

Table 2. Primers used for real-time PCR for the diagnosis of *P. viticola* in grape samples

Primer	Primer sequence	Gene region (no. in GenBank)	Amplicon length, bp	T_m,* °C	GC**, %	Reference
PvITS1_1-real-s,	5'GGCGGTTGCAGCTAATGGAT	*ITS1* (ON183972.1)	120	61.1	55	This article
PvITS1_1-real-a	5'AGCGAAGACTTTCGTCCTCACA			61.9	50	
PvITS1_2-real-s	5'CCACGTGAACCGTTTCAACCA	*ITS1* (ON183972.1)	83	61.6	52.4	This article
PvITS1_2-real-a	5'CCATTAGCTGCAACCGCCAA			61.6	55	
Giop-F	5'TCCTGCAATTCGCATTACGT	*ITS1* (ON183972.1)	208	63.4	45	[10]
Giop-R	5'GGTTGCAGCTAATGGATTCCTA			63.3	45.5	
PvCox1_1-real-s	5'ACCTGTTCTAGCCGGTGCTATT	*Cox1* (NC_045922.1 12520–13998)	90	61.5	50	This article
PvCox1_1-real-a	5'ACCGGATCACCACCTCCAGA			62.5	60	
PvCox1_2-real-s	5'GCGTGCTCCGGGTTTAAGTT	*Cox1* (NC_045922.1 12520–13998)	105	61.2	55	This article
PvCox1_2-real-a	5'ATAGCACCGGCTAGAACAGG			59.3	55	
PvCox2_1-real-s	5'TGGTTCCGGAAAGTGATTTAGCA	*Cox2* (KP684906.1)	178	60.5	43.5	This article
PvCox2_1,2-real-a	5'TGATTTAAGCGGCCCGGACA			62.2	55	
PvCox2_2-real-s	5'CAGATGTTTTACACTCATGGGCGA	*Cox2* (KP684906.1)	73	61.4	45.8	This article
PvCox2_1,2-real-a	5'TGATTTAAGCGGCCCGGACA			62.2	55	

* T_m, melting temperature of primers; GC, percentage of guanine (G) and cytosine (C) in the primer.

first technical replicate of DNA samples was sent to a commercial organization Syntol (Russia) as described previously [15], while the second technical replicate of the samples was used for real-time PCR. To determine the quality and quantity of DNA, it was analyzed using a Nanodrop-1000 device (Nanodrop, United States) and a Quantus quantum fluorometer (Promega, United States). The libraries were carefully prepared for sequencing in strict compliance with the protocol outlined in the manual *Library Preparation for 16S Metagenomic Sequencing* (part no. 15 044 223 Rev. B; Illumina, United States). The primers ITS1f (5'CTTGGTCATTTAGAGGAAGTAA) and ITS2 (5'GCTGCGTTCTTCATCGATGC) were used to amplify the *ITS1* rDNA regions in fungi and fungus-like organisms in all samples. Nextera® XT Index Kit reagents (Illumina, United States) were used for indexing amplicons. The library pool was sequenced on the Illumina MiSeq platform using the MiSeq Reagent Kit v2 (Illumina, United States). The amplicon sequences were read from both ends (250 bp).

Next generation sequencing data were successfully deposited in the database of the National Center for Biotechnology Information (NCBI, United States) under accession numbers PRJNA980748 and PRJNA998468 and in the database of the Laboratory of Biotechnology of the Federal Science Center of the East Asia Terrestrial Biodiversity, Far East Branch, Russian Academy of Sciences, Russia (https://biosoil.ru/downloads/biotech/Metagenoms/) [15].

Preliminary processing of data obtained and taxonomic identification of *ITS1* sequences were performed as described in Nityagovskii et al. [15].

Primer design and real-time quantitative PCR (real-time PCR). For quantitative analysis of the presence of *P. viticola* in the collected samples, six specific primer pairs were designed according to bioinformatics analysis and literature data. The primer design was carried out using a Primer-BLAST instrument [16] with the following parameters: amplicon length 70–180 bp, melting temperature (Tm) 59–62°C, GC content 50–60%, targeting only *P. viticola* sequences. Three specific primer pairs PvITS1_1-real-s/a, PvITS1_2-real-s/a (developed in this article) and Giop [10] intended for amplification of *ITS1* in *P. viticola* (GenBank ON183972.1); two specific primer pairs PvCox1_1-real-s/a, PvCox1_2-real-s/a for amplification of DNA region of the cytochrome C oxidase subunit I (Cox1) (GenBank NC_045922.1, region from 12520 bp to 13 998 bp); two specific primer pairs PvCox2_1-real-s/a and PvCox2_2-real-s/a for the cytochrome C oxidase subunit II (Cox2) (GenBank KP684906.1) were used. For the detection of *P. viticola*, the real-time PCR method with the *ITS1*, *Cox1*, and *Cox2* genes as targets was used. Due to these unique DNA sequences, the required accuracy of *P. viticola* determination was reached [17, 18]. The primers used for real-time PCR are given in Table 2.

Real-time PCR was conducted using additional DNA samples (second technical replicate) that were

previously isolated from the same plants at the same time, but not sent for the next generation sequencing (1–5 samples for each grape plant). DNA was amplified in 20 μL of the reaction mixture containing 1 × Taq buffer with 2.5 mM $MgCl_2$, 250 μM dNTPs, 0.25 μM of each primer, 1 × SybrGreen I, 1 unit of Taq DNA polymerase activity, and 1 μL of DNA. The reagents for real-time PCR were used from Evrogen (Russia). The synthesis of gene-specific primers was ordered in Evrogen (Russia). Amplification was carried out under the following conditions: 2 min at 95°C, then 50 cycles of 95°C for 10 s and 62°C for 25 s.

For real-time PCR, the DT Prime detecting amplifier (NPO DNA-Tekhnologiya, Russia) with a RealTime_PCR v7.3 software was used. The mode "quantitative analysis with standards" was used to calculate the amplification results. This mode of the analysis allows us to determine the amount of the desired DNA fragment in the sample using calibration samples (standards, DNA dilution from the Mildew sample). In the presence of calibration, the RealTime_PCR v7.3 program automatically constructs a calibration line and determines the concentration in the samples analyzed.

DNA isolated from the *V. vinifera* grape leaf with the traits of downy mildew (which was confirmed by specialists from the Chaika Federal Scientific Center of Agricultural Biotechnology of the Far East, Russia) was used as the standard. This sample (Mildew) was obtained in August 2023 from the Far East Experimental Station of the branch of the Federal Research Center Vavilov All-Russia Institute of Plant Genetic Resources (Russia).

DNA from the Mildew sample was diluted with sterile water till the concentrations of 20, 4, 0.8, and 0.16 ng/μL and 1 μL used was used per reaction as calibrators in real-time PCR. Real-time PCR was conducted in a similar way using both the primers to the regions of *P. viticola* sequences (Table 2) and the primers to the "grape housekeeping" genes *VaGAPDH* and *VaActin* [19]. The values of the amplification level of *P. viticola* sequences obtained in this way were divided by the values obtained for the *VaGAPDH* and *VaActin* genes from the appropriate samples. Then data were then presented as the mean ± standard error (SE) and calculated using one-way analysis of variance (ANOVA) with subsequent Tukey HSD multiple comparison test conducted using the R package Stats (https://www.r-project.org, cited March 6, 2024) [20]. The value $p < 0.05$ was considered statistically significant. The Pearson correlation plot between real-time PCR data and metataxonomic analysis data was obtained using the R package ggpubr (cited March 6, 2024) [21].

Sixteen technical replicates (eight real-time PCR reactions normalized to the *VaGAPDH* gene region and eight real-time PCR reactions to the *VaActin*) were used for quantitative estimation of amplification of the sequences studied.

RESULTS AND DISCUSSION

Analysis of the efficiency of using real-time PCR with SYBR Green I for diagnosis of *Plasmopara viticola* in the grape. Based on literature data and using bioinformatics analysis, six pairs of primers were developed for the detection of *P. viticola* in the grape samples. When analyzing the efficiency of real-time PCR, only three specific primer pairs (PvITS1_1-real-s/a, PvITS1_2-real-s/a, and PvCox1_1-real-s/a) had an efficiency close to 100% (Table 3).

Comparison of real-time PCR data with previously obtained next generation sequencing data on the relative representation of *P. viticola* in grape samples. The samples collected from the same plants, but not sent for next generation sequencing were analyzed for the presence of the pathogen by real-time PCR with SYBR Green I using the selected primer pairs. The obtained real-time PCR data were compared with previously obtained data on next generation sequencing [15] (Fig. 1). Using the real-time PCR method, a relatively high average pathogen representation (5.5–61%) (comparable with data obtained as a result of next generation sequencing) was detected in three plants (P-4, S-1, M-dm). According to real-time PCR data, the relative level of the number of amplicons in the samples, in which grape DNA with visible symptoms of grape downy mildew (M-dm) was used, was higher (except for PvCox1_1) as compared with other plant samples without visible symptoms (Fig. 1a). According to real-time PCR data, the relative amplification level of *P. viticola* amplicons using the primers PvITS1_2-real-s/a and PvCox1_1-real-s/a in 15 samples, in which DNA of grape samples Gh-1, P-1, P-2, P-3, P-5, P-6, Kh-1, Kh-2, S-Va, S-2, S-3, Ad, Muk, Alfa, and Pr-St was used as a matrix, did not differ from the negative control. Out of them, the level of real-time PCR amplification in 11 samples (Gh-1, P-1, P-2, P-3, P-5, P-6, Kh-1, S-Va, S-2, Alfa, and Pr-St) corresponded to the absence or low average representation of the pathogen (0–1%) according to a metataxonomic analysis. In four samples (Kh-2, S-3, Ad, Muk), the results of real-time PCR were inconsistent with the next generation sequencing method. These results could be associated with the extremely uneven distribution of the pathogen within the plant, since we carried out real-time PCR on reserve grape samples that were not sent for next generation sequencing (Fig. 1).

The average level of real-time PCR amplification using the primers PvITS1_1-real-s/a, PvITS1_2-real-s/a, and PvCox1_1-real-s/a in a backup copy of grape DNA samples had a high correlation with the average relative representation of *P. viticola ITS1* according to next generation sequencing data (Fig. 2). The level of the Pearson correlation coefficient between the estimations of the average relative ampli-

Table 3. Efficiency of primers used for real-time PCR for the diagnosis of *P. viticola* in grape samples

Primer name	Efficiency of real-time PCR, %	C_t for standards I and IV	C_t for Nc
PvITS1_1-real-s PvITS1_1-real-a	98 ± 4	15.8–22.8	–
PvITS1_2-real-s PvITS1_2-real-a	102 ± 6	15.2–22.2	27.8
Giop-F Giop-R	154 ± 14	35.7–42.8	43.8
PvCox1_1-real-s PvCox1_1-real-a	107 ± 7	16.8–23.5	33.8
PvCox1_2-real-s PvCox1_2-real-a	124 ± 11	17.9–24.9	28.6
PvCox2_1-real-s PvCox2_1,2-real-a	85 ± 6	23.0–29.7	34.6
PvCox2_2-real-s PvCox2_1,2-real-a	112 ± 7	19.2–25.6	33.4

Ct, threshold cycle; standards I–IV, series of fivefold DNA dilutions (20, 4, 0.8, and 0.16 ng per sample) from *V. vinifera* leaves with downy mildew symptoms; Nc, negative control.

fication level according to real-time PCR data and the average relative representation of *P. viticola* in NGS samples was the highest for amplification using the primers PvITS1_2-real-s/a ($R = 0.86$, $p < 0.001$) (Fig. 2).

Specific detection of phytopathogens of agricultural crops is very important for predicting the disease foci and its control. A more sensitive and cheap diagnostic method using real-time PCR based on the fluorescent SYBR Green I dye was proposed in this article, and six pairs of real-time PCR primers were developed (Table 2). According to the results of real-time PCR efficiency, PvITS1_1-real-s/a, PvITS1_2-real-s/a, and PvCox1_1-real-s/a were the most efficient primers (Table 3). In addition, the Pearson correlation coefficient was the highest when the primers PvITS1_2-real-s/a were used (Fig. 2). Thus, real-time PCR data confirmed the results of next generation sequencing, in which the highest representation of *P. viticola* (in addition to grape samples with visible symptoms of downy mildew) was detected in the grape samples collected on Rikord Island and in the botanical garden on Sakhalin Island (Fig. 1). In addition, the analysis of the cost of detecting *P. viticola* in the grape samples using real-time PCR, metagenomic analysis, and LAMP was performed. Taking into account the costs of the required consumables purchased on the Russian market, the estimated costs of analyzing a single grape sample using real-time PCR was 500 rubles, while the use of Illumina-based next generation sequencing was 6000 rubles. Previously, it was reported that the cost of the LAMP method was approximately 650 rubles per sample [22]. Consequently, the developed primers for early detection of *P. viticola* represent a very cost-effective alternative to the NGS and LAMP methods.

The quantitative method developed in this study (based on the SYBR Green I modification of real-time PCR) also has great advantages as compared with traditional visual analyses, and in vitro analyses require a relatively long time, but allow us to determine the amount of the pathogen in the grape rapidly and with a high accuracy. The use of the method for early diagnostic of *P. viticola* in the vineyards of the Russian Far East, where relatively high humidity and moderate temperatures create favorable conditions for the reproduction of this oomycete [23], can allow us to detect this pathogen before the emergence of the first symptoms, which will in general decrease the severity of the epidemic during the vegetation period. It is advisable to apply the proposed method a couple of weeks before a potential mildew outbreak and, first of all, to analyze the grape varieties susceptible to downy mildew. For a more accurate diagnosis, it is recommended to use two leaves and two young shoots of the grape per grapevine in order to exclude uneven pathogen distribution inside the plant. Moreover, we encountered the fact that many infected plants had no symptoms of mildew. The symptoms of downy mildew are also similar to other grape diseases (for example, oidium), but these diseases require different methods of treatment. In addition, information about the absence of *P. viticola* in asymptomatic plants will allow us to avoid the additional preventive treatment with chemical reagents, which will also have a positive effect on the crop quality and will be economically justified.

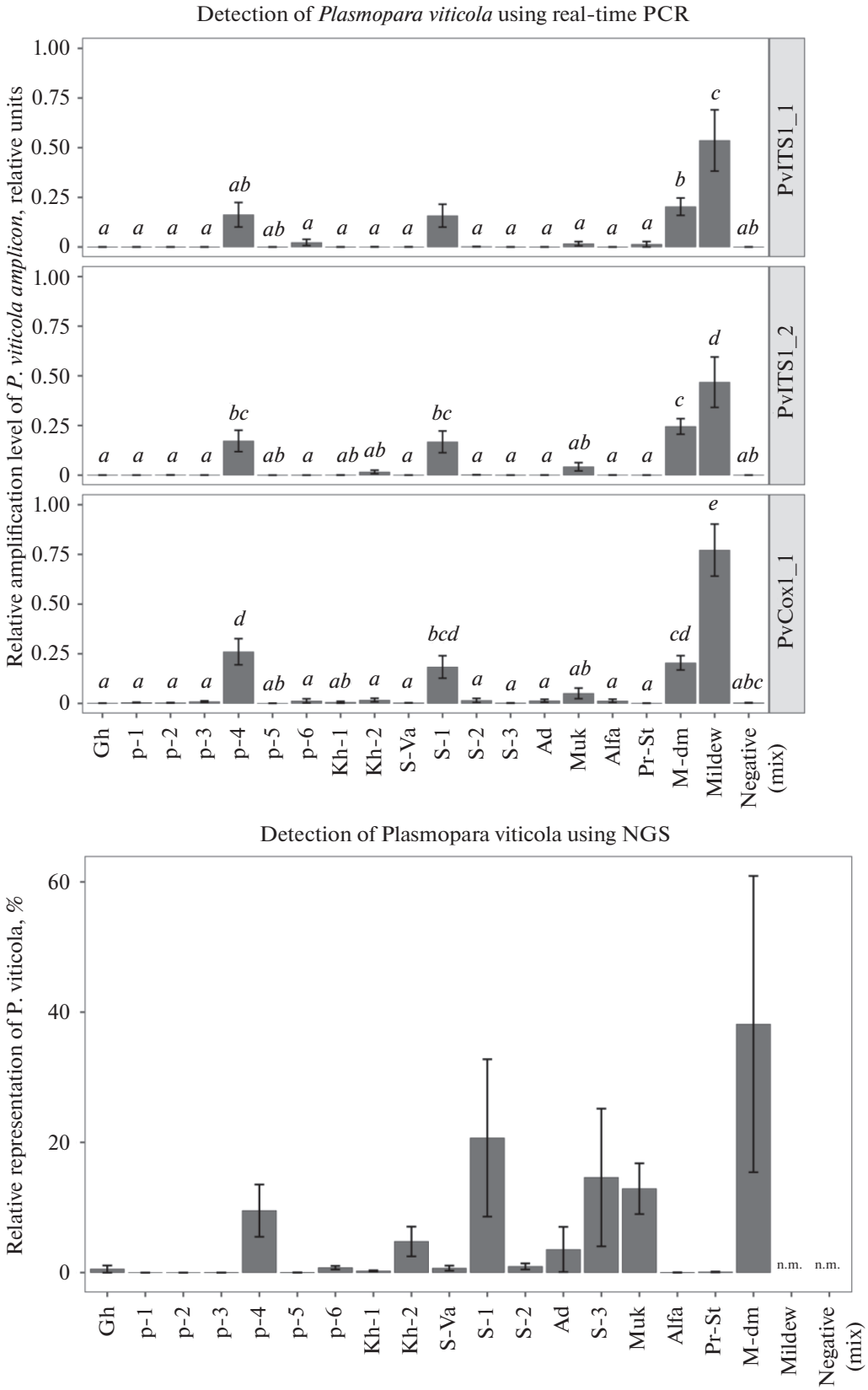

Fig. 1. (a) Quantitative determination of amplification of PvITS1_1, PvITS1_2, and PvCox1_1 sequence regions in grape DNA samples conducted by real-time PCR method; (b) relative representation of *P. viticola* in NGS samples. The origin of all samples is indicated in Table 1. Nc, real-time PCR reaction without grape DNA. n.m., not measured. Data are presented as the mean value ± SE (pooled data by leaf and stem samples of the same plant). The mean values of each digit followed by the same letter did not differ when using one-way analysis of variance (ANOVA) with subsequent Tukey multiple comparison test.

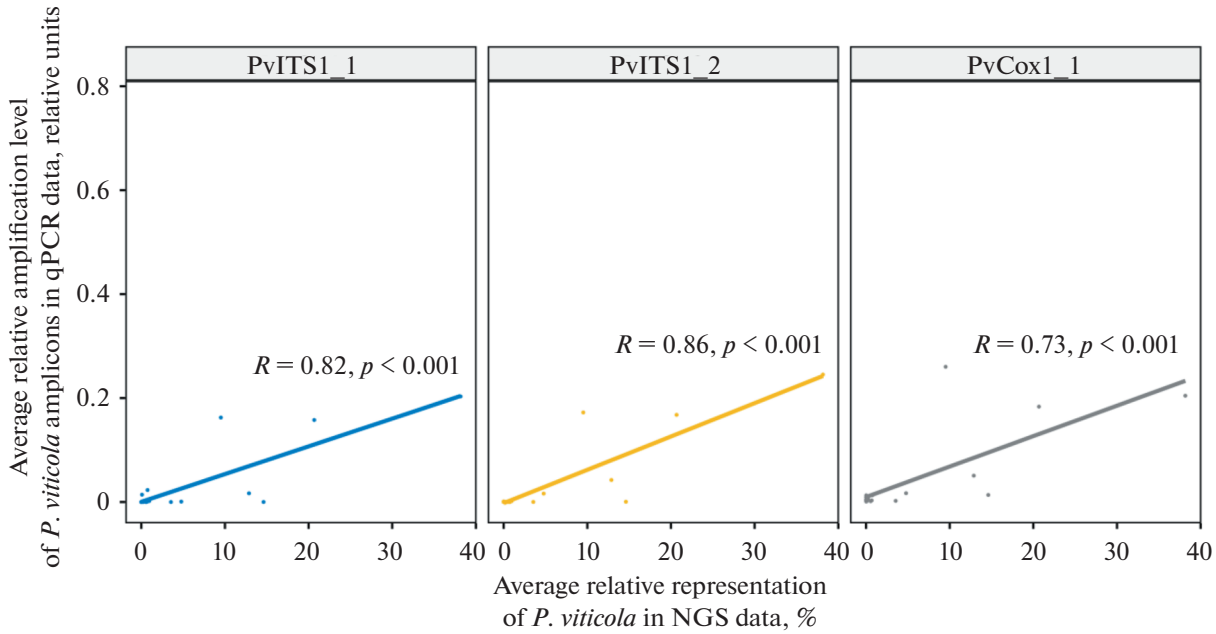

Fig. 2. Determination of the Pearson correlation coefficient of the relationship between the estimations of the relative amplification level according to real-time PCR data (relative units) and the relative representation of *P. viticola ITS1* amplicons in NGS samples: 1, 2, 3, linear regression lines for PvITS1_1, PvITS1_2, and PvCox1_1 amplicons, respectively.

Thus, the use of the developed method for early detection of *P. viticola* based on real-time PCR can provide useful information for fungicide application guidelines and potentially increase the efficiency of downy mildew control in the vineyards.

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In this work, a relatively cheap and efficient method for early diagnostics of the causal agent of grapevine downy mildew *P. viticola* was developed based on the quantitative real-time PCR method (SYBR Green I modification). The efficiency of this method was confirmed by previously obtained data on next generation sequencing using the Illumina technology, in which a linear dependence of the correlation of data obtained was found. The application of this method can be useful as a diagnostic tool for the identification of *P. viticola* and control of downy mildew in commercial vineyards.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human or animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

REFERENCES

1. Koledenkova, K., Esmaeel, Q., Jacquard, C., Nowak, J., Clément, Ch., and Ait Barka, E., *Front. Microbiol.*, 2022, vol. 13, p. 889472. https://doi.org/10.3389/fmicb.2022.889472
2. Toffolatti, S.L., Russo, G., Campia, P., Bianco, P.A., Borsa, P., Coatti, M., Torriani, S.F., and Sierotzki, H., *Pest Manage. Sci.*, 2018, vol. 74, no. 12, pp. 2822–2834. https://doi.org/10.1002/ps.5072
3. Toffolatti, S.L., Serrati, L., Sierotzki, H., Gisi, U., and Vercesi, A., *Pest Manage. Sci.*, 2007, vol. 63, no. 2, pp. 194–201. https://doi.org/10.1002/ps.1327
4. Burruano, S., *Mycologist*, 2000, vol. 14, no. 4, pp. 179–182. https://doi.org/10.1016/s0269-915x(00)80040-3
5. Díez-Navajas, A.M., Greif, Ch., Poutaraud, A., and Merdinoglu, D., *Micron*, 2007, vol. 38, no. 6, pp. 680–683. https://doi.org/10.1016/j.micron.2006.09.009
6. Vercesi, A., Sirtori, C., Vavassori, A., Setti, E., and Liberati, D., *Med. Biol. Eng. Comput.*, 2000, vol. 38, no. 1, pp. 109–112.
7. Vercesi, A., Toffolatti, S.L., Zocchi, G., Guglielmann, R., and Ironi, L., *Eur. J. Plant Pathol.*, 2010, vol. 128, no. 1, pp. 113–126.

8. Hong, Ch. and Scherm, H., *J. Phytopathol.*, 2020, vol. 168, no. 5, pp. 297–302. https://doi.org/10.1111/jph.12892
9. Negrel, L., Halter, D., Wiedemann-Merdinoglu, S., Rustenholz, C., Merdinoglu, D., Hugueney, P., and Baltenweck, R., *Front. Plant Sci.*, 2018, vol. 9, no. 9, p. 360. https://doi.org/10.3389/fpls.2018.00360
10. Si Ammour, M., Bove, F., Toffolatti, S.L., and Rossi, V., *Front. Plant Sci.*, 2020, vol. 11, p. 1202. https://doi.org/10.3389/fpls.2020.01202
11. Valsesia, G., Gobbin, D., Patocchi, A., Vecchione, A., Pertot, I., and Gessler, C., *Phytopathology*, 2005, vol. 95, no. 6, pp. 672–678. https://doi.org/10.1094/phyto-95-0672
12. Yang, L., Chu, B., Deng, J., Yuan, K., Sun, Q., Jiang, C., and Ma, Z., *Phytopathol. Res.*, 2023, vol. 5, no. 1, p. 19. https://doi.org/10.1186/s42483-023-00178-w
13. Kong, X., Qin, W., Huang, X., Kong, F., Schoen, C.D., Feng, J., Wang, Z., and Zhang, H., *Sci. Rep.*, 2016, vol. 6, no. 1, p. 28935. https://doi.org/10.1038/srep28935
14. Kiselev, K.V., Nityagovsky, N.N., and Aleynova, O.A., *Appl. Biochem. Microbiol.*, 2023, vol. 59, no. 3, pp. 361–367.
15. Nityagovsky, N.N., Ananev, A.A., Suprun, A.R., Ogneva, Z.V., Dneprovskaya, A.A., Tyunin, A.P., Dubrovina, A.S., Kiselev, K.V., Sanina, N.M., and Aleynova, O.A., *Horticulturae*, 2024, vol. 10, no. 4, p. 326. https://doi.org/10.3390/horticulturae10040326
16. Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., and Madden, T.L., *BMC Bioinf.*, 2012, vol. 13, no. 1, p. 134. https://doi.org/10.1186/1471-2105-13-134
17. Robideau, G.P., De Cock, A.W.A.M., Coffey, M.D., Voglmayr, H., Brouwer, H., Bala, K., Chitty, D.W., Désaulniers, N., Eggertson, Q.A., Gachon, C.M.M., Hu, Ch., Küpper, F.C., Rintoul, T.L., Sarhan, E., Verstappen, E.C.P., Zhang, Yo., Bonants, P.J.M., Ristaino, J.B., and André Lévesque, C., *Mol. Ecol. Resour.*, 2011, vol. 11, no. 6, pp. 1002–1011. https://doi.org/10.1111/j.1755-0998.2011.03041.x
18. Choi, Yo., Beakes, G., Glockling, S., Kruse, J., Nam, B., Nigrelli, L., Ploch, S., Shin, H., Shivas, R.G., Telle, S., Voglmayr, H., and Thines, M., *Mol. Ecol. Resour.*, 2015, vol. 15, no. 6, pp. 1275–1288. https://doi.org/10.1111/1755-0998.12398
19. Kiselev, K.V., Aleynova, O.A., Grigorchuk, V.P., and Dubrovina, A.S., *Planta*, 2017, vol. 245, no. 1, pp. 151–159. https://doi.org/10.1007/s00425-016-2598-z
20. R Core Team, R Foundation for Statistical Computing. 2021. https://www.r-project.org/.
21. Kassambara, A., ggpubr: "ggplot2" Based Publication Ready Plots, R package, 2023. https://rpkgs.datanovia.com/ggpubr/.
22. Lou, D., Meurer, M., Ovchinnikova, S., Burk, R., Denzler, A., Herbst, K., Papaioannou, I.A., Duan, Yu., Jacobs, M.L., Witte, V., Ürge, D., Kirrmaier, D., Krogemann, M., Gubicza, K., Boerner, K., Bundschuh, Ch., Weidner, N.M., Merle, U., Knorr, B., Welker, A., Denkinger, C.M., Schnitzler, P., Kräusslich, H., Dao Thi, V.L., De Allegri, M., Nguyen, H.T., Deckert, A., Anders, S., and Knop, M., *EMBO Rep.*, 2023, vol. 24, no. 5, p. e57162. https://doi.org/10.15252/embr.202357162
23. Mouafo-Tchinda, R.A., Beaulieu, C., Fall, M.L., and Carisse, O., *Can. J. Plant Pathol.*, 2021, vol. 43, no. 1, pp. 73–87. https://doi.org/10.1080/07060661.2020.1758958

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