Original article

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GENETIC DIVERSITY OF *BOTRYTIS CINEREA* REVEALED BY SEQUENCING AND IDENTIFICATION OF *B. CINEREA* ISOLATES IN STRAWBERRIES OF ALMATY REGION

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ABSTRACT

This article discusses the isolation and identification of 8 isolates of strawberry gray mold pathogens from four varieties in the Almaty region: Vivara, Murano, Serofline and Albion. The morpho-cultural characteristics of the isolated *Botrytis cinerea* (*B. cinerea*) strains were studied, and the rates of radial colony growth in various nutrient media were determined. Percent identity of eight *Botrytis cinerea* isolates ranged from 95.4% to 100%. For most isolates, percent identity exceeded 99%, indicating a high degree of genetic conservatism in the *ITS* regions. An exception was isolate K12 (PI = 95.4%), which exhibited minor nucleotide variations that, however, did not affect the taxonomic classification. The isolated strains were deposited in the *GenBank* database maintained by the National Center for Biotechnology Information (NCBI): *B. cinerea K1-PV226173.1, B. cinerea K2-PV226178.1, B. cinerea K9-PV203239.1, B. cinerea K12- PV203241.1, B. cinerea K13-PV203240.1, B. cinerea K24-PV203243.1, B. cinerea K27-PV203242.1, B. cinerea K28-PV203244.1.* The constructed phylogenetic tree shows that *B. cinerea* forms a large monophyletic cluster with high intraspecific variability, which confirms its wide distribution. Other *Botrytis* species form polyphyletic and stable groups, reflecting their taxonomic isolation.

Keywords: *Botrytis cinerea, phytopathogens, gray mold, strawberry.*

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1 INTRODUCTION

The species composition of micromycetes in strawberries and other fruit and vegetable crops is determined by a number of factors, including ecological and agrotechnical conditions, as well as varietal resistance to phytopathogens. The majority of microscopic fungi are found on the surface of vegetating plants, becoming part of the epiphytic microflora complex, which leads to a decrease in the consumer characteristics of fruit and vegetable products. However, certain species of micromycetes, without exhibiting phytopathogenic properties, are capable of penetrating other plant organs through the root system, resulting in the accumulation of mycotoxins synthesized by them in the fruits. This process is not always accompanied by visible signs of spoilage, noticeable changes in sensory properties, or the appearance of decay and depigmentation. Therefore, the main negative aspect of contamination by mold fungi is the production of mycotoxins by toxigenic strains, which may precede the appearance of pronounced spoilage [1, 2]. The greatest threat to fruit-bearing strawberry plantations is posed by *B. cinerea*, *Colletotrichum acutatum*, and *Rhizopus spp*. Yield losses from these pathogens range from 30 to 96% [3, 4, 5]. *B. cinerea* is an ascomycete fungus, toxigenic, and a facultative parasite. In addition to strawberries, it infects about 400 species of plants from different families [6, 7]. The life cycle of the fungus consists of two stages: the anamorphic (conidial, occurring on plants) and the teleomorphic (ascogenous) stage, observed in culture [8-10]. In the conidial stage, mycelium is formed, which is exophytic, pigmented, septate, and produces round-elliptical smoky conidia, microconidia, and sclerotia up to 5 mm in size [11]. On sclerotia, under high humidity and low temperatures, fruiting bodies of the ascomycetous stage – apothecia – form, up to 5 mm in diameter and up to 4 mm in height. Apothecia develop as elevated, cup-shaped structures that widen upward, containing asci with ascospores in the stroma [12]. Microconidia act as spermatia in the formation of ascospores [13]. The pathogen affects the fruiting organs, leaves, and roots of strawberries [14]. A unique feature of its parasitism

is that it initially colonizes dead tissue, and then releases toxic substances that poison adjacent living cells. Gradually colonizing these living cells, it continues to spread to new tissue areas [15]. The spread and development of gray mold are facilitated by specific temperature conditions, humidity, and the presence of infectious agents. The pathogen's mycelium develops over a wide temperature range: from 2-7 to 30-34°C (optimal is 17-25°C) and high relative humidity of 95-98%. The highest parasitic activity of the pathogen occurs at a temperature of 10-20°C. It withstands temperature drops (at 12-14°C it forms sclerotia) but does not tolerate increases: at 35-37°C it dies within a day [16].

The objective of this study was the molecular-genetic analysis of gray mold pathogens in strawberries of four varieties from the Almaty region: Vivara, Murano, Serofline, and Albion. The Vivara strawberry variety is medium-sized, with dark green leaves and serrated edges. A distinctive feature of this variety is that the fruits are large or medium-sized, weighing 30-40 g. The Murano variety is characterized by a small number of intensely green leaves, large white flowers, and flower stalks located above the leaves. Murano strawberries are medium-sized, with dense, juicy, and fleshy pulp, and bright red, shiny skin. Albion fruits are large, slightly elongated, with a conical shape. The pulp is juicy, pink, and quite dense. Serofline berries are large and medium-sized (15-30 grams), elongated, bright red, juicy, with fairly dense and succulent pulp.

2 MATERIAL AND METHODS

2.1 Methods for Isolating Pure Cultures

To isolate enrichment and pure cultures, a fragment of infected strawberry approximately 4x4 mm was taken. The strawberry piece was placed in a sterile dish and kept in distilled water for 2-3 minutes. It was then rinsed for 40 seconds in 95% ethanol, followed by 3 minutes in a 10% «Dezotabs» disinfectant solution. It was rinsed for 40 seconds in 70% ethanol and then for 40 seconds in distilled water. Next, the strawberry fragment was placed in a clean, dry dish, and the infected part was cut into small pieces of 1x1 mm using sterile tweezers. To isolate fungi from infected and healthy strawberries, ready-made Chapek Dextrose Agar and Potato Dextrose Agar media were used. The incubation time for the dishes was 7 days at a temperature of 26-28°C. The grown fungal colonies were transferred into test tubes with the corresponding medium, which served as the initial material for further research. An antibiotic, ampicillin, was added to the Petri dish with the medium at a concentration of 0.01% to inhibit bacterial growth. Each Petri dish was divided into 4 sectors, with one small infected strawberry fragment placed into each sector and labeled accordingly. Incubation was conducted for 7 days at room temperature in a dark place. Subsequently, the fungal culture isolated from infected and healthy strawberries was purified. The inoculum from each batch was subjected to purity and homogeneity control (microbiological control). Micromycetes isolated from strawberries through multiple transfers were microscopically examined using a Biomed 3T microscope at 40x and 400x magnification to determine genus and species affiliation [17, 18, 19] The radial growth rate of the colonies was determined by the formula:

$$Kr = (r - r0) / \Delta t$$

Where: Kr – radial growth rate of the colony, mm/h;

r – colony radius at time t, mm;

r0 – colony radius at time t0, mm;

Δt – time elapsed from t0 to t, h [20].

2.2 Molecular Genetic Identification

A buffer solution containing 1.5 M NaCl, 10 mM EDTA, 1.5% CTAB, 0.1 M HEPES (pH – 5.3) was used for isolation. Cultures were placed in sterile 2 ml tubes with two glass beads (6 mm) and homogenized in an MM300 mixer Mill for 15 minutes at 30 Hz. Then, 350 µl of CTAB solution was added, mixed, and 700 µl of chloroform-isoamyl alcohol solution (24:1) was added, thoroughly mixed, and left to stand for 10 minutes, after which the supernatant was transferred to clean tubes. Then, 700 µl of chloroform/isoamyl alcohol solution (24:1) was added and incubated for 60 minutes at 65°C. The mixture was thoroughly mixed and centrifuged at 12,000 rpm for 10 minutes. After centrifugation, the aqueous phase was transferred to new clean tubes, and DNA was precipitated with 0.6 volumes of isopropanol. The entire volume was then transferred to columns with a silica membrane and centrifuged at 12,000 rpm for one minute. The column was washed twice with a washing buffer (80% ethanol, 10 mM Tris-HCl, pH 8.0). DNA samples were dissolved in 100 µl of single-strength TE buffer and stored at minus 20°C. DNA concentration was measured spectrophotometrically using a NanoDrop spectrophotometer at a wavelength of 280 nm.

2.3 Amplification of the ITS Region

PCR reaction was performed with primers *ITS* (5-CAACCTTCAAAATGCGTGAG-3) and (5-AGATGGGTTGCTGAGCTTCA-3) [21] in a total volume of 30 µl. The PCR mixture contained 15 ng of DNA, 1 unit of Taq DNA Polymerase (Fermentas), 0.2 mM of each dNTP, 10x KCl buffer (Fermentas), 2.5 mM MgCl2, and 10 pmol of each primer. The PCR amplification program included initial denaturation at 95°C for 6 minutes; 35 cycles: 94°C for 30 seconds, 52°C for 30 seconds, 72°C for 1 minute; final elongation for 9 minutes at 72°C. The PCR program was carried out using a SimpliAmp Thermal Cycler (Applied Biosystems).

2.4 Determination of Nucleotide Sequence

PCR product purification from unbound primers was conducted enzymatically using Exonuclease I (Fermentas) and Shrimp Alkaline Phosphatase (Fermentas). Sequencing reactions were performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions, followed by fragment separation on an automatic genetic analyzer 3730xl DNA Analyzer (Applied Biosystems).

2.5 Nucleotide Sequence Analysis

Nucleotide sequences were analyzed and assembled into a consensus sequence using SeqMan software (DNA Star). Terminal fragments (primer nucleotide sequences and fragments with low-quality scores) were removed. The obtained sequences were identified in GeneBank using the BLAST algorithm. Additionally, phylogenetic trees were constructed with sequences deposited in the international GeneBank database [22, 23].

2.6 Bioinformatic Analysis of Obtained Nucleotide Sequences

Figure 1 – Infected strawberries with pathogenic fungi: A – damage to the leaves and berries of the Vivara variety; B – lesions on the berries of the Murano variety; C – pathology of infected strawberry berries. Arrows indicate the affected areas.

Bioinformatic analysis of the obtained nucleotide sequences was performed using the molecular evolution statistical analysis and phylogenetic tree construction program - MEGA 12. Multiple alignments were conducted using MUSCLE, part of the MEGA software package. Evolutionary history and divergence between sequences were inferred using the maximum likelihood method and the Tamura-Nei model.

2.7 Statistical analysis

All experiments were repeated three times to ensure the reliability of the results. Statistical analysis of the results was performed using the statistical test ANOVA [24].

3 RESULTS AND DISCUSSION

3.1 Sample collection

During this study, 8 isolates of *Botrytis cinerea* (gray mold) were isolated from 48 strawberry samples grown in the Almaty region (4 variety): Vivara, Serofline, Albion and Murano. It was found that Vivaro and Murano varieties had a higher degree of gray rot damage compared to Serofline and Albion varieties during the strawberry harvest (the data was recorded using photographic documentation) Figure 1. This can be explained by the fact that the Serofline and Albion varieties have a higher resistance to phytopathogenic microorganisms, including *Botrytis cinerea*, which reduces the likelihood of infection of these varieties [25]. It is known that species *Botrytis cinerea* is the causal agent of the disease worldwide as gray mold. This pathogen has a wide host range, infecting more than 1000 species, mainly dicotyledonous, including several economically important crops such as strawberry, grape, kiwifruit, tomato, lettuce and ornamental plants such as rose, lily, tulip and chrysanthemum and eucalyptus [26].

Gray mold developed most intensively at the end of strawberry flowering – before harvesting, due to multiple generations of conidia and apothecia contributing to the formation of secondary inoculum of the pathogen. The end of flowering and the beginning of strawberry fruit ripening occurs at the end of May and early June in the Almaty region. Harvesting begins in the first decade of June. Air temperature ranged from 21 to 25.1°C, and 33-50% of infected fruits were recorded. In infected fruits, seeds either did not form or formed with reduced sowing and technological qualities. As the pathogen expanded, it often covered a large part of the plants. On decaying tissue, an ash-gray coating formed – sporulating mycelium. It is also worth noting that, at first glance, external signs and even the sporulating coating of gray and dry rot pathogens are similar, but they are easily distinguishable under microscopy. As the pathogen expanded, it often covered a large part of the plants (Figure 1).

On decaying tissue, an ash-gray coating formed – sporulating mycelium. Conidiophores are straight, multicellular, with a thick shell, 257.4 μ in length, broad at the base, tapering towards the apex, almost colorless, branching into short «branches,» which in turn produce more «branches,» where unicellular spores form – colorless, ovoid, or spherical, measuring 12.7×13.7 μ. The presented parameters correspond to literature data.

3.2 Morphological Description of the Culture

Eight isolates of *B. cinerea* were obtained in pure culture for the study of species' cultural characteristics (Figure 2). Its morphology includes several key characteristics that help in its identification and understanding of its life cycle. The mycelium is multicellular, branched, and colorless, with thin threads (hyphae). The mycelium usually consists of colorless, smooth hyphae that form a dense network penetrating the plant tissues (Figure 1). Mycelial cells are often septate, meaning they have partitions separating the cellular sections. Sclerotia do not form. On Chapek medium, conidia appear on the fourth day of growth, with a sporulation intensity of 154.6 × 10^3 units/cm^2. As the culture ages, individual sclerotia with a diameter of 2-3 mm are formed.

The table 1 shows that *Botrytis* grows favorably on Potato dox agar nutrient medium compared to Chapek dox agar.

Table 1 – Growth dynamics of *B. cinerea* isolates.

Nutrient media	Colony diameter			Colony growth rate		
	2-days	4-days	6-days	2-days	4-days	6-days
Chapek dextrose agar	2,5±0,06	5,74±0,15	6,86±0,25	0,51±0,01	0,93±0,02	0,19±0,02
Potato dextrose agar	3±0,5	6,9±0,15	8,86±0,25	0,7±0,01	0,9±0,01	0,2±0,01

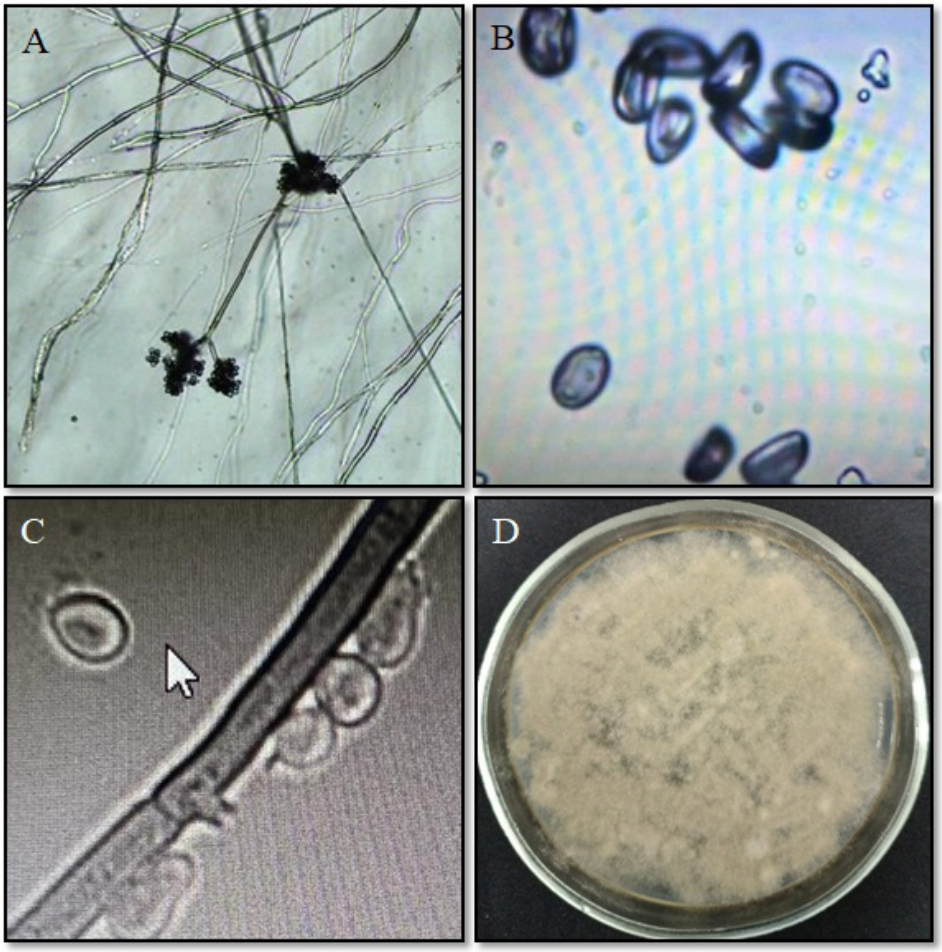

Figure 2 – Microscopic and morphological characterististics of isolated *B. cinerea* from Vivara, Murano, Serofline and Albion strawberries: A – mycelium (40×), B and C - conidia (400×) from *B. cinerea K1-PV226173.1* strains were inoculated on the Potato dextrose agar, D - colony growth features of *B. cinerea K1-PV226173.1* strain.

The diameter of the colonies on Potato dox agar medium is 8.86mm on the 6th day, while 6.86mm on Chapek dox agar. The colony growth rate is 0.2mm/hour.

3.3 Molecular Genetic Identification

Phytopathogenic organisms have a negative impact on seedlings grown in agriculture. Molecular genetic analysis is one of the most highly effective methods of diagnosing phytopathogenic organisms. This method consists of determining the types of diseases that are difficult to identify by morphological characteristics, as well as their detection at an early stage of pathogenesis. In the result of molecular identification 8 isolates of *B. cinerea* were determined from 48 strawberry samples grown in the Almaty region: Vivara, Serofline, Albion and Murano. The table 2 demonstrates the results of the identification of eight *B. cinerea* isolates using morphological and molecular methods. To assess the reliability of the molecular data, additional parameters are provided: percent identity, E-value, and query coverage. The percent identity ranged from 95.4% to 100%. For most isolates, the percent identity exceeded 99%, indicating a high degree of genetic conservatism in the *ITS* regions. The exception was isolate K12 (PI = 95.4%), which showed slight nucleotide variations that, however, did not affect the taxonomic classification.

The E-value for all isolates, except K12, was 0.0, confirming the exceptional accuracy of the match. For isolate K12,

Table 2 – Comparative analysis of morphological and molecular identification of *B. cinerea* isolates.

Isolate	Morphological Match	Molecular Match (*ITS*)	PI (%)	EV	QC (%)
K1	*B. cinerea*	*B. cinerea*	99.8	0.0	100
K2	*B. cinerea*	*B. cinerea*	99.7	0.0	100
K9	*B. cinerea*	*B. cinerea*	99	0.0	100
K12	*B. cinerea*	*B. cinerea*	95.4	5e-165	95
K13	*B. cinerea*	*B. cinerea*	99.8	0.0	100
K24	*B. cinerea*	*B. cinerea*	100	0.0	100
K27	*B. cinerea*	*B. cinerea*	100	0.0	100
Abbreviation: PI – Percent Identity, EV – E-value, QC – Query Coverage.					

Table 3 – Details of the isolates used in the phylogenetic analysis in this study.

Species	Strain/Isolate	Host	Origin	GenBank accession numbers
Botrytis byssoidea	ICMP10259	*Allium cepa*	NA	JX399176.1
	ICMP5601	*Allium cepa*	NA	JX399181.1
Botrytis caroliniana	CH_WS_11	*Danthonia californica*	Whetstone, USA	MT548671.1
	pm11	NA	NA	PQ037778.1
Botrytis cinerea	K1	*Fragaria ananassa*	Almaty, Kazakhstan	PV226173.1
	K2	*Fragaria ananassa*	Almaty, Kazakhstan	PV226178.1
	K9	*Fragaria ananassa*	Almaty, Kazakhstan	PV203239.1
	K12	*Fragaria ananassa*	Almaty, Kazakhstan	PV203241.1
	K13	*Fragaria ananassa*	China	PV203240.1
	K24	*Fragaria ananassa*	China	PV203243.1
	K27	*Fragaria ananassa*		PV203242.1
	K28	*Fragaria ananassa*		PV203244.1
	DY-28	NA	Lueyang, China	OR574197.1
	HNSMJ-2	*Cardamine hupingshanensis*	China	MW820311.1
	HNSMJ-3	*Cardamine hupingshanensis*	China	KY810530.1
Botrytis fabae	Bo.fabae2	NA	NA	PP758476.1
	CBS 109.57	NA	Netherlands	PP838558
Botrytis fabiopsis	BroadbeanBC-30	*Vicia faba*	China	EU563126.1
Botrytis galanthina	CBS 138.60	NA	Netherlands	MH857925.1
	CBS 127.37	NA	Netherlands	MH855851.1
Botrytis hyacinthii	1471069		NA	FJ710809.1
Botrytis polyblastis	CBS287.38	NA	NA	AJ716291.1
Botrytis porri	CBS 190.26	NA	NA	MH854885.1
	CBS 190.26	NA	NA	MH866378.1
Botrytis squamosa	L123	NA	NA	MK681210.1
	L122	NA	NA	MK681209.1
Botrytis tulipae	BT9901	NA	NA	AM235313.1
Sclerotinia sclerotiorum	F11	NA	China	PV052996.1

the E-value was 5e-165, remaining within the statistically significant range (≤1e-50), and further confirming the reliability of the result. Query coverage reached 100% for all isolates except K12 (95%), which could be related to technical artifacts during sequencing or the presence of polymorphisms in the *ITS* region.

3.3 Phylogenetic Analysis of Botrytis Species

Figure 3 presents a phylogenetic tree constructed using the maximum likelihood method with 1,000 bootstrap replications. The tree illustrates the relationships among Botrytis isolates, as well as with *Sclerotinia sclerotiorum*, which is shown as an outgroup.

The tree clearly separates species into clades with high bootstrap support (81-100%), confirming their genetic uniqueness. It is known that *B. cinerea* has high genetic variability, which has already been associated with hosts and geographical origin [27, 28]. We also found genetic variability among the isolated isolates of Almaty strawberries. In our study *B. cinerea* forms a large monophyletic cluster with pronounced intraspecific variability, namely PV203241.1 with the reference sequence OR574197.1 with 93% support, characteristic of adaptive and widespread species (table 3). The tree was rooted with outgroup of *Sclerotinia sclerotiorum*. The obtained data, based on the analysis of the standard *ITS* genetic marker, align with the existing taxonomy of the genus and underscore the need to expand sampling for rare species and include additional markers to refine evolutionary relationships.

CONCLUSION

In this study 8 isolates of *B. cinerea* (gray mold) were isolated from 48 strawberry samples grown in the Almaty region (4 varieties): Vivara, Serofline, Albion and Murano. The *Botrytis sp.* isolates are sporulating and heterogeneous in colony growth rate and sporulation intensity when cultured on various nutrient media. Percent identity of eight *B. cinerea* isolates ranged from 95.4% to 100%. For most isolates, percent identity exceeded 99%, indicating a high degree of genetic conservatism in the *ITS* regions. An exception was isolate K12 (PI = 95.4%), which exhibited minor nucleotide variations that, however, did not affect the taxonomic classification. The phylogenetic tree shows that *B. cinerea* forms a large monophyletic cluster with high intraspecific variabil-

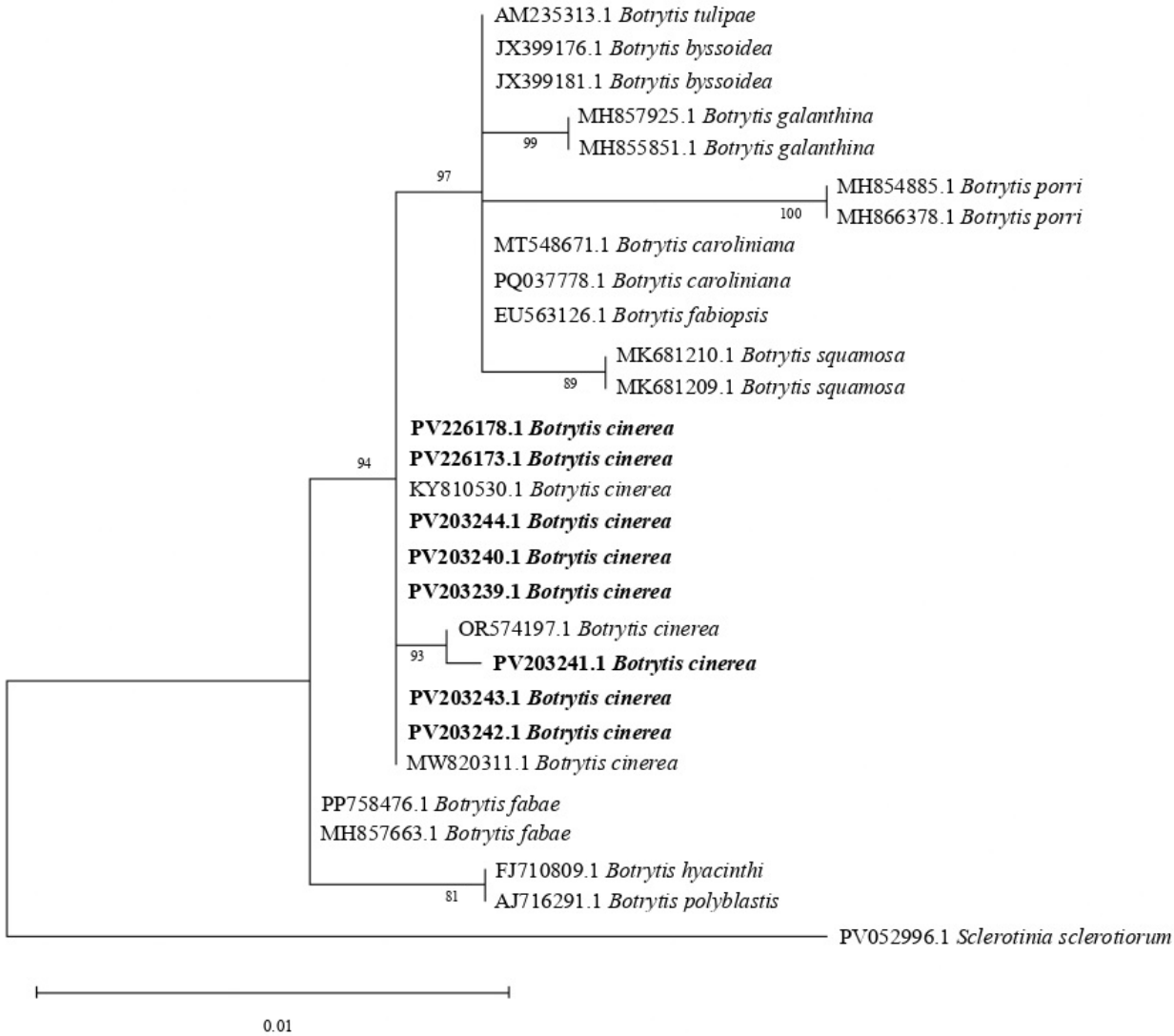

Figure 3 – The phylogram of *Botrytis* species constructed using maximum likelihood analyse based on *ITS* region. The tree was constructed using 1000 bootstrap replications, divergence 0.01. Isolates obtained in this study are shown in bold.

ity, confirming its widespread distribution. Other *Botrytis* species are divided into polyphyletic and stable groups, reflecting their taxonomic distinctness. The obtained results are of great importance, as a comprehensive study of *B.cinerea*, which is highly pathogenic and widespread, causes enormous damage to strawberry plantations, contributing to the prevention of its spread and the organization of control measures against it.

AUTHOR CONTRIBUTIONS

T.Zh.A.: responsible for developing the concept of the study. N.S.K.: wrote the main part of the text. Zh.A.Zh.: conducted experiments or modeling. K.Zh.I.: participated in interpreting the data. I.A.B.: was involved in collecting and analyzing data. O.A.K.: editing the text. C.E.: editing the text.

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CONFLICT OF INTEREST

There are no conflicts of interest to declare.

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ГЕНЕТИЧЕСКОЕ РАЗНООБРАЗИЕ *BOTRYTIS CINEREA*, ВЫЯВЛЕННОЕ ПУТЕМ СЕКВЕНИРОВАНИЯ, И ИДЕНТИФИКАЦИЯ ИЗОЛЯТОВ *B. CINEREA* В КЛУБНИКАХ АЛМАТИНСКОЙ ОБЛАСТИ

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АБСТРАКТ

В данной статье рассматривается выделение и идентификация 8 изолятов возбудителей серой гнили клубники из четырех сортов в Алматинской области: Vivara, Murano, Serofline и Albion. Исследованы морфо-культуральные особенности выделенных штаммов *Botrytis cinerea* (*B. cinerea*), определена скорость радиального роста колоний в различных питательных средах. Процент идентичности восьми изолятов гриба *B. cinerea* варьировал от 95.4% до 100%. Для большинства процент идентичности превышал 99%, что указывает на высокую степень генетической консервативности *ITS*-регионов. Исключение составил изолят K12 (PI = 95.4%), демонстрирующий незначительные нуклеотидные вариации, которые, однако, не повлияли на таксономическую классификацию. Выделенные штаммы были депонированы в базе данных GenBank, поддерживаемой Национальным центром биотехнологической информации (NCBI): *B. cinerea K1-PV.73.1, B. cinerea K2-PV226178.1, B. cinerea K9-PV203239.1, B. cinerea K12-PV203241.1, B. cinerea K13-PV203240.1, B. cinerea K24-PV203243.1, B. cinerea K27-PV203242.1, B. cinerea K28-PV203244.1*. Построенное филогенетическое дерево показывает, что *B. cinerea* образует большой монофилетический кластер с высокой внутривидовой изменчивостью, что подтверждает его широкое распространение. Другие виды *Botrytis* образуют полифилетические и стабильные группы, что отражает их таксономическую обособленность.

Ключевые слова: *Botrytis cinerea, фитопатогены, серая гниль, клубника.*

АЛМАТЫ ОБЛЫСЫНДАҒЫ ҚҰЛПЫНАЙЛАРДАҒЫ *B. CINEREA* ИЗОЛЯТТАРЫН АНЫҚТАУ ЖӘНЕ СЕКВЕНИРЛЕУ АРҚЫЛЫ *BOTRYTIS CINEREA*-НЫҢ ГЕНЕТИКАЛЫҚ ӘРТҮРЛІЛІГІН АНЫҚТАУ

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АҢДАТПАСЫ

Бұл мақалада Алматы облысындағы құлпынайдың төрт сортынан: Вивара, Мурано, Серофлайн және Альбион сұр зең қоздырғыштарының 8 изоляттарын бөліп алу және анықтау қарастырылған. Оқшауланған *Botrytis cinerea* (*B. cinerea*) штамдарының морфо-культуралдық ерекшеліктері зерттеліп, әртүрлі қоректік орталарда колониялардың радиалды өсу қарқыны анықталды. *B. cinerea* саңырауқұлақтарының сегіз изолятының пайыздық сәйкестігі 95,4%-дан 100%-ға дейін өзгерді. Көпшілік үшін сәйкестілік пайызы 99%-дан асты, бұл *ITS* аймақтарының генетикалық сақталуының жоғары дәрежесін көрсетеді. Ерекшелік нуклеотидтердің шамалы ауытқуларын көрсететін K12 изоляты болды (PI = 95,4%), бірақ олар таксономиялық жіктеуге әсер етпеді. Оқшауланған штаммдар Ұлттық биотехнологиялық ақпарат орталығы (NCBI) жүргізетін *GenBank* дерекқорында сақталған: *B. cinerea K1-PV.73.1, B. cinerea K2-PV226178.1, B. cinerea K9-PV203239.1, B. cinerea K12-PV203241.1, B. cinerea K13-PV203240.1, B. cinerea K24-PV203243.1, B. cinerea K27-PV203242.1, B. cinerea K28-PV203244.1.* Құрылған филогенетикалық ағаш *B. cinerea* жоғары түр ішілік өзгергіштігі бар үлкен монофилді кластерді құрайтынын көрсетеді, бұл оның кең таралуын растайды. Ботритистің басқа түрлері полифилетикалық және тұрақты топтар құрайды, бұл олардың таксономиялық оқшауланын көрсетеді.

Түйін сөздер: *Botrytis cinerea, фитопатогендер, сұр шірік, құлпынай.*