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**RESISTANCE OF *PHASEOLUS VULGARIS* L.
ACCESSIONS TO RACES 2
AND 7 OF *COLLETOTRICHUM LINDEMUTHIANUM*
AS A BASIS FOR AGROBIODIVERSITY CONSERVATION**

The common bean (*Phaseolus vulgaris* L.) is an important grain legume crop with high nutritional, economic and genetic value. Anthracnose, caused by *Colletotrichum lindemuthianum*, is one of the most destructive diseases of this crop, and the high race variability of the pathogen complicates the selection of resistant breeding material. The aim of this study was to evaluate the resistance of 10 bean accessions and 2 control samples to races 2 and 7 of *C. lindemuthianum* under greenhouse conditions. The scientific and practical significance of the work lies in identifying accessions suitable for breeding, hybridisation and the conservation of bean agrobiodiversity. Seeds provided by Michigan State University were used, while MDRK and MICH served as control samples. Inoculation was carried out separately for each pathogen race, and the degree of plant infection was assessed visually using the CIAT 1–9 rating scale. The results showed that most of the tested accessions demonstrated resistance to anthracnose; the control samples MDRK and MICH were susceptible, whereas Beluga showed only mild symptoms. The value of the study is that it confirms the importance of multi-race screening in phytopathological evaluation. The practical significance of the results is the selection of resistant genotypes for further breeding work.

Keywords: Common bean, anthracnose, *Colletotrichum lindemuthianum*, resistance, agrobiodiversity.

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***Phaseolus vulgaris* L. үлгілерінің *Colletotrichum lindemuthianum* 2 және
7 нәсілдеріне төзімділігі агробиоалуантүрлілікті сақтаудың негізі ретінде**

Кәдімгі үрме бұршақ (*Phaseolus vulgaris* L.) тағамдық, шаруашылық және генетикалық маңызы жоғары дәнді-бұршақты дақылдардың бірі болып табылады. Бұл дақыл үшін ең қауіпті аурулардың бірі – *Colletotrichum lindemuthianum* саңырауқұлағы қоздыратын антракноз, ал қоздырғыштың нәсілдік өзгергіштігі төзімді бастапқы материалды дұрыс таңдауды қиындатады. Зерттеудің мақсаты жылыжай жағдайында үрме бұршақтың 10 үлгісі мен 2 бақылау үлгісінің *C. lindemuthianum*-ның 2 және 7 нәсілдеріне төзімділігін бағалау болды. Жұмыстың ғылыми және практикалық маңызы селекцияға, будандастыруға және агробиоалуантүрлілікті сақтауға жарамды төзімді формаларды анықтаумен байланысты. Зерттеуде Michigan State University ұсынған тұқымдық материал пайдаланылды, ал MDRK және MICH бақылау үлгілері ретінде алынды. Инокуляция патогеннің әрбір нәсілі бойынша жеке жүргізіліп, өсімдіктердің зақымдану дәрежесі CIAT 1–9 балдық шкаласы бойынша визуалды бағаланды. Нәтижесінде үлгілердің басым бөлігі антракнозға төзімділік көрсетті; MDRK және MICH бақылау үлгілері сезімтал болды, ал Beluga сортында тек әлсіз симптомдар байқалды. Зерттеудің құндылығы материалды бірнеше нәсіл бойынша бағалау қажеттілігін дәлелдеуде. Нәтижелердің практикалық маңызы төзімді генотиптерді кейінгі селекциялық жұмыстарға ұсынумен айқындалады.

Түйін сөздер: кәдімгі үрме бұршақ, антракноз, *Colletotrichum lindemuthianum*, төзімділік, агробиоалуантүрлілік.

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Устойчивость образцов *Phaseolus vulgaris* L. к расам 2 и 7 *Colletotrichum lindemuthianum* как основа сохранения агробиоразнообразия

Фасоль обыкновенная (*Phaseolus vulgaris* L.) является важной зернобобовой культурой, имеющей пищевое, хозяйственное и генетическое значение. Одной из наиболее опасных болезней культуры является антракноз, вызываемый *Colletotrichum lindemuthianum*, высокая расовая изменчивость которого затрудняет подбор устойчивого исходного материала. Целью исследования была оценка устойчивости 10 образцов фасоли и 2 контрольных образцов к расам 2 и 7 *C. lindemuthianum* в тепличных условиях. Научная и практическая значимость работы связана с выявлением форм, пригодных для селекции, гибридизации и сохранения агробиоразнообразия фасоли. В исследовании использовали семена, предоставленные Michigan State University; MDRK и MICH применяли как контрольные образцы. Инокуляцию проводили отдельно для каждой расы патогена, после чего степень поражения растений оценивали визуально по шкале CIAT 1–9. Установлено, что большинство исследованных образцов проявили устойчивость к антракнозу; контрольные MDRK и MICH были восприимчивыми, а Beluga показала только слабые симптомы. Ценность исследования состоит в подтверждении необходимости многорасовой оценки материала. Практическое значение результатов заключается в отборе устойчивых генотипов для дальнейшей селекционной работы.

Ключевые слова: фасоль обыкновенная, антракноз, *Colletotrichum lindemuthianum*, устойчивость, агробиоразнообразие.

Abbreviations and symbols

CIAT – International Center for Tropical Agriculture; MSU – Michigan State University; R – resistant; S – susceptible.

Introduction

The common bean (*Phaseolus vulgaris* L.) occupies an important place in global agriculture as a valuable legume crop, serving as a source of plant protein, dietary fibre, vitamins, minerals, and other bioactive compounds [6].

Alongside its food value, the common bean is characterised by significant genetic diversity, which is widely utilised in breeding for productivity, adaptability, and resistance to diseases and other stress factors [1]. The Bean Breeding and Genetics programme at Michigan State University, in particular, focuses on developing high-yielding bean varieties of various commercial classes that combine resistance to diseases and stresses with good agronomic traits (Michigan State University, n.d.).

Among bean diseases, anthracnose, caused by the fungus *Colletotrichum lindemuthianum*, is one of the most significant. According to Michigan State University Extension, anthracnose is among the most economically significant bean diseases and can significantly reduce crop yield [16]. The disease affects leaves, stems, petioles, pods, and seeds, and in severe cases leads to a deterioration in seed qual-

ity and significant yield losses [11, 26]. Anthracnose poses a particular threat to bean collection samples, as seed infection and high plant susceptibility hinder the maintenance of populations and the long-term conservation of valuable genotypes [11].

One of the key characteristics of *C. lindemuthianum* is its high pathogenic variability. A large number of physiological races of the pathogen have been described for beans, and their differentiation is based on a standard set of differential varieties [9, 23, 26]. Studies conducted in various regions have shown significant diversity among pathogen populations in terms of virulence and molecular characteristics, which substantially complicates breeding for resistance and requires a multi-race evaluation of the breeding material [3, 14, 18, 20].

The search for bean resistance genes against anthracnose has been ongoing for many years and remains one of the key areas of breeding and phytopathological research. During this period, numerous resistance genes and loci have been identified and mapped, their inheritance patterns studied, and links established between individual genes, resistance clusters and the racial composition of the pathogen [5, 7, 10, 17]. Alongside classical genetic studies, QTL analysis, genome-wide association studies (GWAS) and meta-QTL approaches have been actively employed in recent years, enabling a deeper understanding of the genetic architecture of resistance and the identification of new promising genomic regions [8, 13, 19, 21, 25]. The multifaceted

nature of these studies indicates that bean resistance to anthracnose has a complex genetic basis and requires a comprehensive approach when evaluating breeding material [12, 17, 15, 19].

In this regard, evaluating the resistance of samples to only one pathogen race may be insufficient and lead to an overestimation of the breeding material. The use of several physiological races allows for a more accurate identification of sources of resistance, promising donors and genotypes of interest for practical breeding [2, 4, 22, 26].

Against the backdrop of increasing demands for the environmental safety of agriculture and the need to preserve the crop gene pool, anthracnose-resistant varieties acquire dual value: on the one hand, as source material for breeding; on the other, as more suitable for preservation in collections and further use in sustainable crop production [1, 15, 24].

In this context, investigating the response of bean samples to several races of *C. lindemuthianum* is of considerable interest both for plant pathology and for the breeding and conservation of agrobiodiversity [3, 12, 22]. The aim of the study was to assess the resistance of 10 bean samples and 2 control variants to races 2 and 7 of *Colletotrichum lindemuthianum* under greenhouse conditions and to identify forms with potential for further breeding work and the conservation of agrobiodiversity.

Despite extensive research on common bean anthracnose, the response of the present Michigan State University-derived accession set to races 2 and 7 of *C. lindemuthianum* requires targeted evaluation. The object of this study is common bean accessions and control samples grown under greenhouse conditions. The subject of the study is their phytopathological response to artificial inoculation with *C. lindemuthianum* races 2 and 7. The research hypothesis is that individual accessions will maintain resistance to both races and can therefore be considered promising material for breeding and agrobiodiversity conservation. The main tasks were to grow seedlings, conduct separate inoculation with each pathogen race, assess disease symptoms according to the CIAT 1-9 scale, compare the reactions of all samples, and identify resistant genotypes. The study used greenhouse experimentation, artificial inoculation, visual phytopathological assessment and comparative analysis. The scientific novelty of the work lies in the multi-race screening of this specific set of accessions and

in linking the results with the practical preservation and use of bean agrobiodiversity.

Materials and methods

The study involved 10 samples of common bean (*Phaseolus vulgaris* L.) provided by Michigan State University, including breeding lines, varieties, and control samples: B24218, K22604, N22616, CHARRO, B24219, BELUGA, ADAMS, KONA, BLACK PEARL, EIGER, as well as two control samples – MDRK (control), MICH (control) (Michigan State University, n.d.).

Seeds were sown in growing containers using the standard substrate SyperMix Peat Moss and Perlite Mix from Michigan Grower Products Inc. Once the plants had reached the seedling stage suitable for artificial infection (10-12 days), inoculation was carried out by spraying, followed by incubation in a specially equipped mist chamber conducive to disease development. The experimental design for assessing the resistance of bean samples to races 2 and 7 of *Colletotrichum lindemuthianum* is shown in Figure 1. Plants were inoculated by spraying a conidial suspension onto leaves and stems, then maintained under high humidity (>80%) in a mist chamber for a minimum of three days. Resistance was assessed in the seedling and young plant stages following artificial inoculation with the pathogen.

Two physiological races of the bean anthracnose pathogen – races 2 and 7 of *Colletotrichum lindemuthianum* – were used to assess resistance. Each race was studied separately. Inoculation was carried out separately for each pathogen variant, which allowed the reaction of each sample to a specific race to be assessed and forms with broader resistance to be identified [23].

Artificial infection of plants was carried out by applying an inoculum of the pathogen to the above-ground parts of the plants. The approach involving artificial inoculation followed by exposure of plants to high humidity conditions is widely used in screening beans for resistance to anthracnose [16, 22, 26]. Following inoculation, the plants were placed in high-humidity conditions necessary for the successful development of the infection and the manifestation of disease symptoms. The use of a mist chamber/high-humidity chamber is in line with modern approaches to the phytopathological screening of beans [16, 22].

Figure 1

Scheme of the experimental study of resistance of *Phaseolus vulgaris* L. samples to races 2 and 7 of *Colletotrichum lindemuthianum*



The resistance of bean samples to anthracnose caused by *Colletotrichum lindemuthianum* was assessed under greenhouse conditions at the early stages of plant development using the standard CIAT 1-9 scale developed by A. van Schoonhoven and M.A. Pastor-Corrales, 1987. Two physiological races of the pathogen, race 2 and race 7, were used as inoculum, and inoculation was carried out separately for each race to evaluate the reaction of each sample to a specific pathotype. Plant response was assessed visually after the appearance of disease symptoms on the hypocotyl, cotyledon leaves, and other above-ground parts. When interpreting the results, the severity of infection, the location of symptoms, and the general condition of the plant were considered. According to the CIAT scale, scores of 1–3 indicated resistant samples, 4–6 moderately resistant samples, and 7–9 susceptible samples [24]. Samples showing slight or no damage upon inoculation with both races were considered resistant and promising for further selection. Samples with pronounced symptoms of damage were classified as

susceptible. Particular attention was paid to forms capable of maintaining resistance to both races of the pathogen, as it is precisely such genotypes that are of greatest interest for hybridisation and subsequent use in agrobiodiversity conservation programmes [12, 22].

The results were evaluated on the basis of visual phytopathological analysis, comparing the response of all samples studied to each pathogen race. The main objective was to identify genotypes with a resistant response and to detect samples exhibiting reduced resistance under greenhouse conditions. Comparisons were carried out separately for the reaction to race 2, the reaction to race 7, and overall resistance to both races. This approach enabled the identification of samples showing promise for further breeding, hybridization, and the conservation of agrobiodiversity.

The methodological scheme of the study included the following elements: the research question was whether the tested bean accessions differ in their resistance to races 2 and 7 of *C. lindemuthianum*;

the hypothesis was that several accessions would remain resistant to both races; the main stages were seed sowing, seedling growth, preparation of inoculum, separate inoculation with each race, incubation under high humidity, symptom assessment, and comparative interpretation of results. The methods consisted of greenhouse cultivation, artificial infection, visual scoring according to the CIAT scale and comparative phytopathological analysis. The main research result expected from this methodology was the identification of resistant and susceptible samples for further breeding use.

Results and discussion

Studies were conducted under greenhouse conditions on the cultivation of bean seedlings and the subsequent assessment of their resistance to anthracnose. In the initial stage, seeds of the genotypes under study were selected and sown, after which the plants were grown to the seedling stage. Next, an inoculum of the anthracnose pathogen was prepared and the plants were artificially infected. The experimental design comprised the following main stages: sowing of genotypes, seedling growth, preparation of the inoculum, inoculation of plants, incubation

under high-humidity conditions, and subsequent assessment of symptoms on the CIAT scale (1–9) with photographic documentation of lesions.

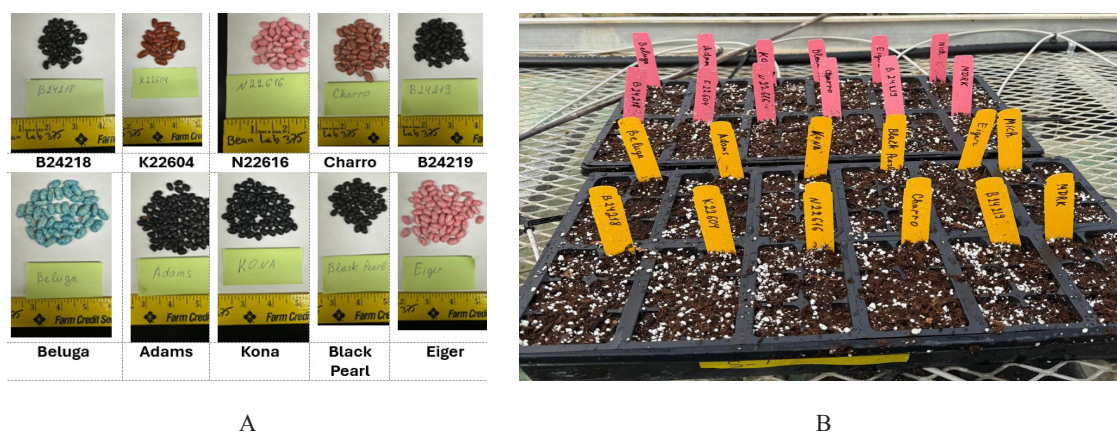
The greenhouse inoculation experiments showed that the bean samples under investigation differed in the degree of damage when infected with races 2 and 7 of *Colletotrichum lindemuthianum*.

Overall, the majority of the genotypes studied demonstrated high resistance to the anthracnose pathogen. When infected by both race 2 and race 7 of *C. lindemuthianum*, the majority of samples showed no symptoms or only minimal symptoms, suggesting that these forms are promising candidates for further selection.

An assessment of the resistance of 12 bean samples to races 2 and 7 of *C. lindemuthianum*, using the CIAT 1–9 scale, revealed that 9 genotypes were resistant to both races of the pathogen. These include B24218, K22604, N22616, CHARRO, B24219, ADAMS, KONA, BLACK PEARL and EIGER. These samples were rated 1 on the CIAT scale, which corresponds to the absence of visible disease symptoms on all parts of the plant. This indicates strong resistance to races 2 and 7 of *C. lindemuthianum* and confirms the high breeding potential of these varieties as source material for further work.

Figure 2

Seeds of common bean (Phaseolus vulgaris L.) genotypes provided by Michigan State University and used in the anthracnose resistance evaluation: A – seeds, B – sowing seeds



When infected with race 2, the majority of the material studied showed weak symptom development, indicating a favourable response to the pathogen and allowing these samples to be considered promising for further selection. A similar pattern was observed with race 7: most samples maintained good resistance.

The lowest resistance was found in the control samples MDRK and MICH, which recorded a damage score of 9 on the CIAT scale. These variants were characterised by pronounced tissue necrosis and damage to leaves, petioles, stems, and branches, corresponding to high susceptibility to both races of the pathogen. The BELUGA sample occupied an

intermediate position: it exhibited mild symptoms corresponding to 2 points on the CIAT scale, which may indicate partial resistance.

The obtained results are consistent with the literature data on the high pathogenic variability of *Colletotrichum lindemuthianum* and the race-specific nature of bean resistance to anthracnose. To reliably assess the bean gene pool, it is important to use several physiological races of the pathogen, as this approach allows the identification of samples with a broader spectrum of resistance.

From a practical point of view, samples resistant to two races are of increased breeding value. They can be used as resistance donors in hybridisation programmes, as well as source material for developing varieties less reliant on chemical control methods. Thus, such forms are significant not only for breeding but also for the greening of bean cultivation technologies.

There is particular interest in such research within the North American context. According to American phytopathological and university sources, bean anthracnose in the US remains a significant disease of dry beans, particularly due to seed-borne infection and the disease's dependence on cool, damp weather. For the state of Michigan, one of the leading centres for dry bean production in the US, even local outbreaks of anthracnose have not only phytopathological but also economic significance.

The use of samples obtained from Michigan State University makes the results of this study particularly relevant for the practical selection of resistant breeding material. The identification of resistant genotypes in this experimental design can serve as a basis for further work on hybridisation and the development of varieties that combine resistance to anthracnose with other economically valuable traits.

Based on the results of the assessment of the resistance of 12 bean samples to races 2 and 7 of *Colletotrichum lindemuthianum* on the CIAT 1–9 scale, presented in Table 1, it was established that the majority of the genotypes studied exhibited high resistance to the anthracnose pathogen.

Of the 12 samples studied, 9 genotypes were found to be resistant to both races of the pathogen. These include: B24218, K22604, N22616, CHARRO, B24219, ADAMS, KONA, BLACK PEARL and EIGER. These samples were rated 1 on the CIAT scale, corresponding to the complete absence of visible disease symptoms on all parts of the plant. This indicates a marked resistance to races 2 and 7 of *C. lindemuthianum* and confirms the high breeding potential of these varieties as source material for further work.

At the same time, two control samples – MDRK and MICH – showed susceptibility to both races of the pathogen. They were recorded as having a disease score of 9, characterised by severe necrosis affecting more than 25% of the plant's tissues, with damage to leaves, petioles, stems, and branches, which in some cases leads to the death of the plant. The data obtained confirm the high susceptibility of these control varieties and demonstrate the pronounced pathogenicity of the strains used.

The BELUGA sample occupied a special position. Despite the absence of severe damage, it exhibited minor symptoms of the disease, corresponding to 2 points on the CIAT scale. This manifested as the presence of small, sunken reddish spots on the main vein on the underside of the leaf. Consequently, according to the CIAT scale, this sample can be classified as resistant; however, the presence of mild symptoms indicates partial resistance and the need for further study.

Table 1

Results of the assessment of bean samples' resistance to races 2 and 7 of Colletotrichum lindemuthianum on the CIAT 1–9 scale (van Schoonhoven & Pastor-Corrales, 1987)

No.	Sample of specimen	Race 2	Race 7	Disease symptoms
1	B24218	R	R	Grade 1. No visible disease symptoms on any part of the plant.
2	K22604	R	R	Grade 1. No visible disease symptoms on any part of the plant
3	N22616	R	R	Grade 1. No visible disease symptoms on any part of the plant
4	CHARRO	R	R	Grade 1. No visible disease symptoms on any part of the plant
5	B24219	R	R	Grade 1. No visible disease symptoms on any part of the plant
6	MDRK control	S	S	Grade 9. Severe necrosis in over 25% of plant tissue; lesions on leaves, petioles, stem and branches; possible plant death.

Continuation of the table

No.	Sample of specimen	Race 2	Race 7	Disease symptoms
7	BELUGA	R	R	Grade 2. Small (1–2 mm), concave reddish lesion on the primary vein on the underside of leaves; resistant with mild symptoms.
8	ADAMS	R	R	Grade 1. No visible disease symptoms on any part of the plant
9	KONA	R	R	Grade 1. No visible disease symptoms on any part of the plant
10	BLACK PEARL	R	R	Grade 1. No visible disease symptoms on any part of the plant
11	EIGER	R	R	Grade 1. No visible disease symptoms on any part of the plant
12	MICH control	S	S	Grade 9. Severe necrosis in over 25% of plant tissue; lesions on leaves, petioles, stem and branches; possible plant death.

The assessment thus carried out showed that the material under investigation is generally characterised by a high level of resistance to anthracnose. The most valuable are the nine samples that showed no visible signs of the disease when infected with both races of the pathogen, while BELUGA may be considered resistant with mild symptoms. These sam-

ples are of practical interest for further selection, hybridisation and artificial pollination with the aim of creating new resistant bean varieties. The control samples MDRK and MICH confirmed their susceptibility, whilst the BELUGA sample stood out as a form with mild symptoms of the disease, requiring further study.

Figure 3

Development of anthracnose symptoms on young bean plants 12 and 15 days after inoculation with *Colletotrichum lindemuthianum*

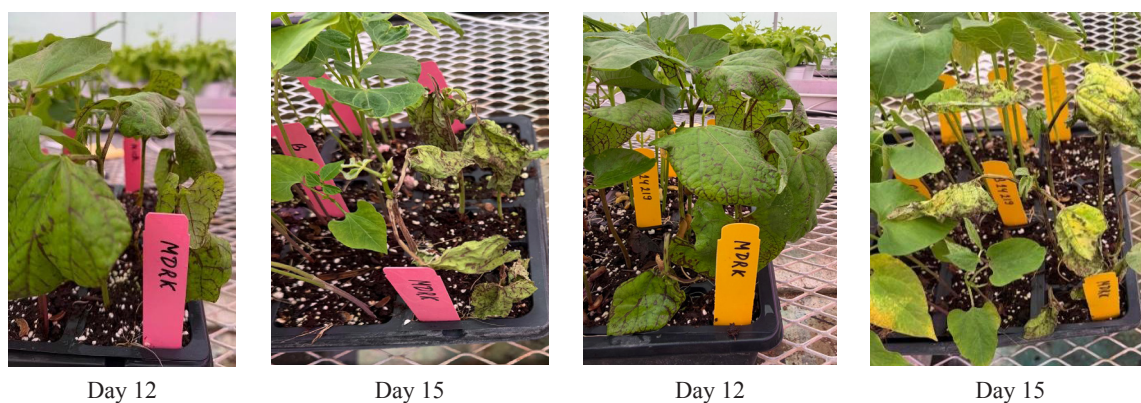


Figure 3 clearly illustrates the progression of anthracnose in young bean plants following inoculation with *Colletotrichum lindemuthianum*. It was found that on the 12th day after infection, the symptoms of the disease were mild and manifested primarily as small dark spots on isolated areas of the leaf surface. By the 15th day, the severity of the infection had increased: the spots became more noticeable, grew in size and took on a more distinct necrotic character. The observed dynamics confirm the gradual development of the pathological process and indicate the progression of the infection

over time. Such visual changes are consistent with the general pattern of anthracnose development and may serve as further confirmation of differences in the samples' response to infection.

Thus, greenhouse assessment of resistance to races 2 and 7 of *C. lindemuthianum* can be considered an important step in the scientifically grounded management of bean agrobiodiversity. It contributes simultaneously to phytopathological monitoring, primary selection, and the more reliable conservation of the crop's genetic resources. From a practical breeding perspective, samples showing resis-

tance to both races are of particular value, as they may serve as donors of resistance in hybridisation programmes and as source material for the development of varieties less dependent on chemical protection. Therefore, such forms are important not only for breeding purposes, but also for the advancement of more environmentally sustainable bean cultivation technologies.

This type of research is especially relevant in the North American context. In the state of Michigan, one of the major centres of dry bean production in the USA, even local outbreaks of anthracnose are important not only from a phytopathological standpoint, but also in economic terms. The inclusion of samples obtained from Michigan State University further increases the practical significance of this study for the identification and selection of resistant source material.

Conclusion

As a result of greenhouse assessments of the resistance of 12 bean (*Phaseolus vulgaris* L.) samples to races 2 and 7 of *Colletotrichum lindemuthianum*, it was established that the material studied is generally characterised by a high level of resistance to the anthracnose pathogen. It was found that 9 samples exhibited resistance to both races of the pathogen and showed no visible symptoms of infection, indicating their high breeding value. The control samples MDRK and MICH proved susceptible to infection, whereas the BELUGA sample demonstrated mild symptoms and may be considered resistant according to the CIAT scale, although it requires further study.

The use of two physiological races of the pathogen allowed for a more objective assessment of the response of the genotypes under study and confirmed the necessity of multi-race testing in the phytopathological characterisation of bean breeding material. This approach is of fundamental importance for identifying forms with a broader spectrum of resistance and for enhancing the reliability of selection in the breeding process.

The identified resistant accessions are of practical interest as resistance donors for further hybridisation, artificial pollination, and the creation of new bean varieties adapted to conditions where anthracnose is prevalent. The results obtained highlight the importance of a comprehensive assessment of the bean gene pool for the conservation, rational use, and scientifically sound management of the crop's agrobiodiversity. The practical implementation of these results may include the use of the identified resistant accessions as donor material in breeding programmes aimed at developing bean varieties with stable resistance to anthracnose. Further research should include testing these accessions against additional physiological races and evaluating their agronomic traits under field conditions.

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Author Contributions

A. D. Dukenbayeva: study design, experimental work, data analysis and manuscript preparation. H. Awale: methodological training, consultations and practical recommendations related to the experimental procedure.

Conflict of Interest

The author declares no conflict of interest.

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