VARIABILITY OF *SESAMUM INDICUM* L. GERMPLASM IN THE REACTION TO *ALTERNARIA ALTERNATA* FUNGUS

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The article presents data on the reaction of 40 sesame genotypes in the treatment of the seeds with culture filtrate (CF) of the *Alternaria alternata* fungus. Cluster analysis (*k*-means) of the studied set of sesame lines and varieties, in the variant with FC *A. alternata* showed a cluster of 20 genotypes with higher indices of germination, root length and stem. Of these, genotypes L1, Cubaneț 57 and Liano have the highest indices for such important agronomic characteristics as the number of capsules per plant – 95–135 and the mass of 1,000 seeds – 3.01–3.91 g, which indicates the association of some valuable agronomic characters with resistance to pathogen, thus being quite attractive for breeding programs.

Keywords: sesam, *Alternaria alternata*, culture filtrate, reaction.

INTRODUCTION

Sesame (*Sesamum indicum* L.) belongs to the Pedaliaceae family and is one of the most important and old oleaginous crops (Bedigian *et al*., 2010). Species are commonly grown in tropical and subtropical regions of Asia, Africa and South America (Anilakumar, 2010). Although it is known as culture in Asia for more than 5000 years, the origin and evolution of the species are still uncertain (Ashri *et al*., 1998; Ara *et al*., 2017). It is assumed that the cultivation of sesame appeared in the Harappa region of the Indian subcontinent for more than 3000 years. (Ashri, 2007; Nayyar *et al*., 2017).

China, India, and Myanmar are the main susan producers, followed by Sudan, Nigeria, Pakistan, Bangladesh, Ethiopia, Thailand, Turkey and Mexico (FAO, 2004) (Nayyar *et al*., 2014).

Sesame seeds contain 20–30% proteins and about 50% high quality oil. The importance of sesame lies in the quality of the oil and the antioxidant content – sesamin, sesmolin, sesamol. Fried sesame oil has a higher concentration of sesamole – the heat-degradation product of sesamoline, considered to be a stronger antioxidant

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compared to its initial molecule, and at the same time a compound with high antimicrobial activity (Kumar, Singh, 2015). Sesame oil is used in the preparation of salads and margarine production, being also a raw ingredient in the paint, varnish, soap, perfume, insecticide and pharmaceutical products industry (Nayyar *et al*., 2017).

At a global level, diseases are major biological constraints in sesame production, bringing annual losses of about 7 million tonnes. Sesame seeds are susceptible to a wide variety of pathogens (Ara *et al*., 2017). Numerous microorganisms, especially fungi, represent a challenge both for the production of sesame and for the preservation of seeds (Nayyar *et al*., 2017).

It is worth mentioning that the occupied areas and the production of this crop decrease in the traditional areas due to severe biotic stress, such as bacterial staining (*Xanthomonas campestris* pv. Sesami), fusarium wilt (*Fusarium oxysporum* var. *erysiphoides*), alternariosis (*Alternaria sesami*) and cercosporosis (*Cercospora sesame*).

The predominant fungal genus associated with sesame seed diseases are Alternaria, Aspergillus, Fusarium, Cercospora, Penicillium and Rhizopus, Curvularia, Helminthosporium, Memnoliela, which causes the deterioration of the seeds in the soil before the germination and subsequently determine the mortality of plants and affect the leaves in the adult stage. The diseases caused by infected seeds are disastrous because they reduce seed vigor and weaken the plant at the start of growth. The quality and quantity of oil and protein are also negatively affected by various biological agents that significantly influence the product made from sesame seeds or their varieties (Altaf *et al*., 2004; Nayyar *et al*., 2014).

Among the most important susan diseases are the alternariosis that cause leaf damage in all the areas of sesame growing in the world: Kenya, Ethiopia, El Salvador, Nigeria, India, and the USA. The fungus *Alternaria sesami* produces small brown spots, irregular on the limb of the leaf which later form elongated lesions. The same symptoms may also appear on the stem and seeds. In the case of severe infection, the plants may become completely defoliated. It should be noted that the number of lesions on sesame plants produced by *A. sesami* depends on the growth stage of the host plant (Kolte, 1985). At the seedling stage, the disease is devastating. Alternariosis of the leaves was responsible for 20–40% loss of sesame culture in the Uttar Pradesh, India region (Marri *et al*., 2012).

Diseases of sesame leaves, such as those caused by *Alternaria* spp., lead to substantial loss of harvest, deterioration in seed quality and vigor. Of particular economic importance is the appearance of leaf spots caused by the *Alternaria sesami* and *A. alternata* species, which also cause the seed degradation, diminished germination and growth energy, as well as the infection of all aerial parts of the plant. The alternariosis of the sesame leaves has been recognized as a major biotic pressure (Alfat *et al*., 2004; Choi *et al*., 2014). Symptoms of leaf bleaching caused by causative agents include the formation of round spots up to irregular spots with a diameter of up to 10 mm that can confluence to form large necrotic patches resulting in premature abscesses.

Alternaria species are transmitted from region to region through various pathways, including aerial transmission of conidia, infestation of the planting material through soil, agricultural equipment and animals (Altaf *et al*., 2004; Nayar *et al*., 2017).

The toxicological consequences of fungal diseases are very important for sesame. *Alternaria* spp. produce non-host toxins (e.g., tenuazonic acid (TeA), alternaryol (AOH), alternariol monomethyl ether (AME), brefeldin A) contaminating the product. Consumption of food contaminated with Alternaria toxins has determined the incidence of esophageal carcinoma in humans (Nayar *et al*., 2017).

Disease control of sesame plants using agro-technical practices and resistant varieties are considered the safest management measures (Marri *et al*., 2012). It is important to respect the rotation scheme of sesame every three or four years (Belay, 2018).

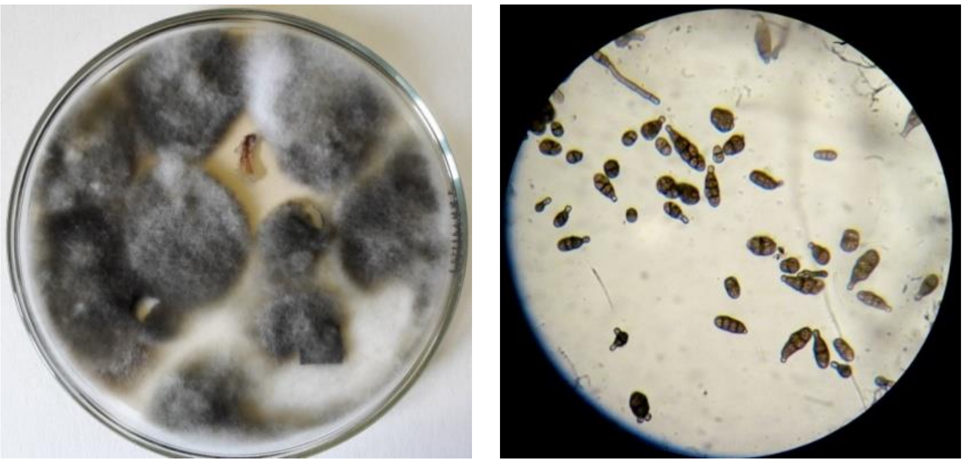
The appreciation of the growth and development of sesame on the experimental fields of the Institute of Genetics, Physiology and Plant Protection (IGFPP) in the Republic of Moldova showed that culture is useful and offers great opportunities for valorisation of the potential in our conditions, but the damage of some perspective genotypes by the fungal diseases may restrict or exclude some of them from improvement programs or the implementation process.

In connection with the above, the purpose of the work was the identification of the controlled reaction of a set of sesame genotypes of different ecological and geographical origin in the culture filtrate of one of the commonly encountered pathogens – *Alternaria alternata*.

MATERIAL AND METHODS

The elaboration strategy of the integrated disease management involves the knowledge of the epidemiology and the properties of the pathogenic population, and these studies have as a mandatory condition the correct identification of causative agents (Lupascu, Sasco, Gavzer *et al*., 2015).

In our researches, the fungi have been isolated on the PDA medium from sesame plants with signs of maladia – brown spots, necrotic roots, stem, leaves (Tuite, 1969). The identification of pathogens was performed on the basis of macro- and microscopic characters according to the mycological key (Barnett, Hunter, 1998) (Fig. 1).



Isolation of Alternaria fungus on PDA medium from sesame leaf petioles

A. alternata conidia (300x)

Fig.1. Isolation and identification of *Alternaria* spp. fungi.

40 sesame genotypes were tested representing varieties and lines of different ecological and geographical origin. The seeds were obtained from genotypes grown on the IGFPP experimental sector.

For laboratory testing was used the culture filtrate (CF) of the *A. alternata* fungus prepared by inoculating the mycelium into the Czapek-Dox liquid medium containing the following components (g / l of distilled water): $NaNO_3$ – 3.0; K_2HPO4 – 1.0; $MgSO_4 \cdot 7H_2O$ – 0.5; KCl – 0.5; $FeSO_4 \cdot 7H_2O$ – 0.01; sucrose – 30.0 (Tuite, 1969). Sesame seeds were treated with CF for 18 hours.

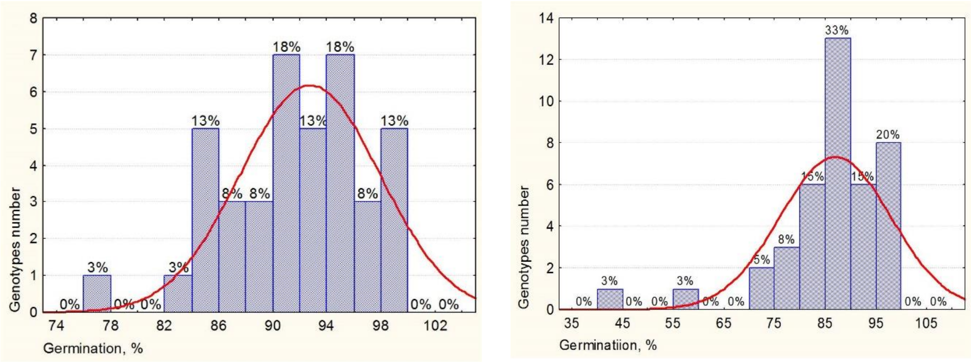
As a control served the seeds kept in the distilled water. Cultivation of the seedling took place in Petri dishes on filter paper wetted with distilled water, at a temperature of 22–24 °C for 6 days. As test indices of plant reaction served the important growth and developing characters at early stage of ontogenesis – germination, root length and stem length.

A considerable contribution to genetic diversity research of the plants has the classification method that would objectively identify the degree of similarity or difference between genotypes. For this purpose, was used the *k*-means clustering analysis, a centroid method based on the presentation of differences between objects / genotypes based on Euclidean distances. It is important to consider that incorrect choice of the numbers of clusters can cause erroneous results. That is why, when using the *k*-media method, the necessary number of clusters for classification should be analyzed. Thus, the basic features of the method are: 1) as the metric value is the Euclidean distance, 2) the number of clusters is selected by the researcher himself, 3) the clustering quality depends on the initial number of groups in which the lot is to be separated (Valliammalb, Geethalakshmi, 2012; Ravishanker *et al*., 2013; Nandini *et al*., 2017). In order to perform the *k*-means clusterian analysis we have programmed the distribution of the set of genotypes in 3 clusters, according to the possible values – large, medium and small of the characters (germination, root length and stem length of seedling) that served as cases.

The data were statistically processed in the STATISTICA 7 software package.

RESULTS AND DISCUSSIONS

Germination. In the control variant, seed germination at the 40 sesame studied genotypes ranged from 78 to 100% and the genotype rate of high germination (90–100%) constituted 70%, indicating the good suitability of the seeds for testing. Under the influence of *A. alternata* CF, the seeds rate with the germination level of 90–100% decreased to 35% (Fig. 2).

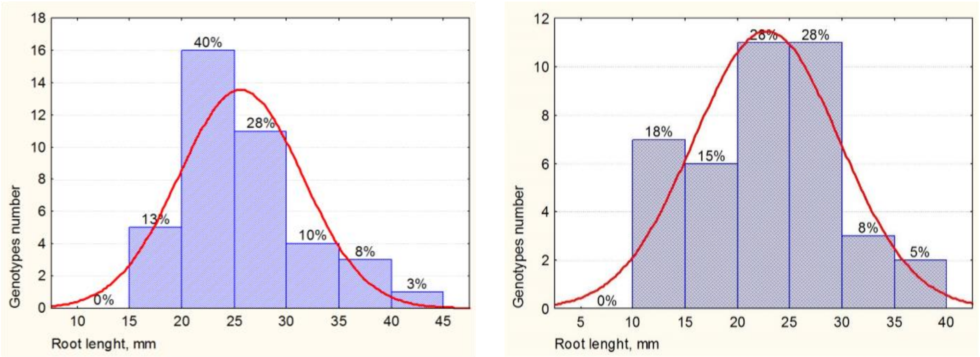


Control *A. alternata* CF

Fig. 2. The distribution of sesame genotypes based on seed germination.

Compared to the control, the most pronounced reduction of germination (-10 ... -51%) was recorded in 13 genotypes (32.5%). Among the most affected are Jerello, K-1621, VNIIMK 889, Iubileinii (-20 ... -22%) and K-1265 (-51%). Stimulation of germination with 8-10% in response to the action of FC was recorded in 3 varieties – Cubanet 57, N 162/0781 and Boiarin.

The length of the root. In the control variant, the root length of the studied genotypes ranged from 15–45 mm, and the rate of genotypes whose root length reached higher values (25–45 mm) than the average sample constituted 49%. In *A. alternata* CF variant, the character varied within 10–40 mm, and the frequency of genotypes whose rootstock was lower than the sample average decreased to 41% (Fig. 3).

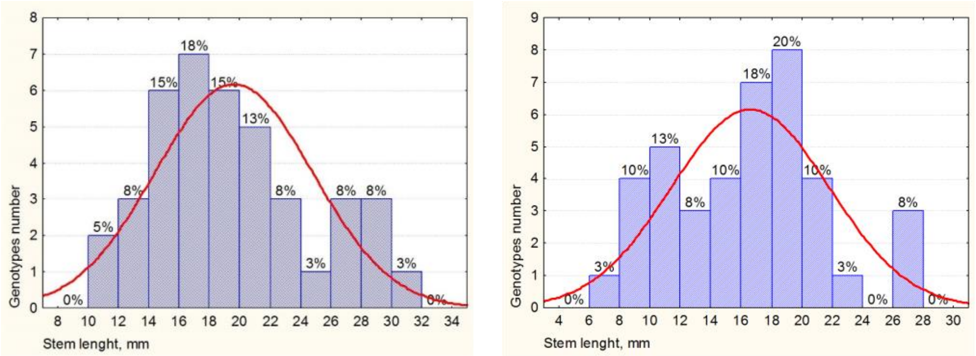


Control *A. alternata* CF

Fig. 3. The distribution of sesame genotypes based on the embryonic root length.

It is worth mentioning that under the influence of CF, in 21 genotypes (52.5%) the parameter diminished by 32–94% of the control. Among the most affected genotypes (32–52% of the control) can be mentioned Mancijurschi ulucisenii, Oro shot, k-1257, Iubileinii. In the 10 genotypes (25%) – Natasa, Cubanet 93, Cumhuriyet 99, K-1555, Lider, Belosemeanii 177, VNIIMK-1, Liano, Zaltsadovski, Biolsadovski – was established the increase in root growth with 6.2–59.3% in comparison with the control.

Length of stem. In the control variant, the length of the stem varied within 10–32 mm, and the frequency of genotypes with values higher than the sample average (20–32 mm) constituted 43%. Under the CF influence the strain length values decreased to 4–26 mm, and the genotype rate with stem length greater than the sample mean decreased to 21%, that is practically halved as compared to the control (Fig. 4).

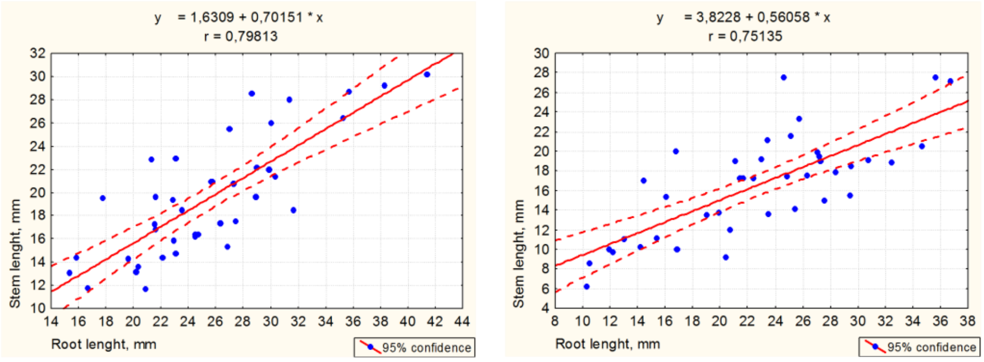


Control *A. alternata* CF

Fig. 4. The distribution of sesame genotypes based on strain length.

The decrease in growth under the influence of CF *A. alternata* was found in 22 genotypes (55%). Among the most affected varieties (22–52% of the control) can be mentioned Cadet, Manjurschi ulucisenii, Oro shot, Dulce, Oro 9/71. A number of 12 genotypes – Jerello, Zaltsadovski, Gusar, Biolsadovski, k-1265, Natasa, Cubant 93, K-1621, K-1555, Boiarin, VNIIMK 1, Zaltsadovzri – responded to treatment by growth stimulation (6–109%).

Correlation analysis revealed a pronounced dependence between root length and strain length in control variant (r = 0.80 *, p <0.05) and FC variant (r = 0.75 *, p <0.05) (Fig. 5).



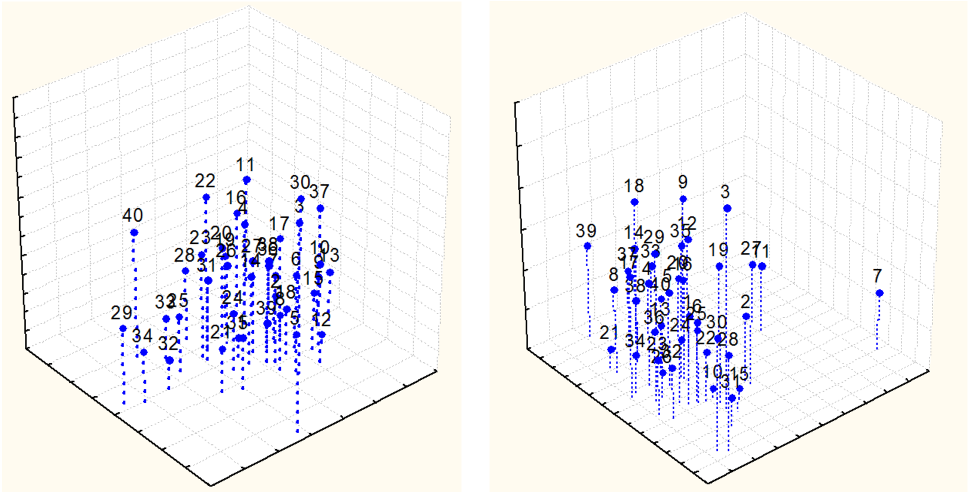
Control CF *A. alternata*

Fig. 5. Regression dependence between stem length and root length in plant reaction of *S. indicum* to the *A. alternata* culture filtrate.

However, the dependence slightly diminished under the influence of the pathogen, which denotes that the phenotypic plasticity of the sesame growth organs in the *A. alternata* reaction is relatively independent.

One of the modern statistical methods for classifying objects / genotypes is multidimensional scalling (Deschepper, Brys, Fortuna, 2017). In order to investigate the degree of similarity / distinction between sesame genotypes and the identification of the less susceptible to the treatment of seeds with *A. alternata* CF, the multidimensional scanning analysis was performed based on seeds germination (%), root length (mm) and length of the stem (mm) (Fig. 6).

The obtained data reveal the distribution of the sesame genotypes in the three-dimensional space on a rather extensive surface, which denotes the existence of their pronounced variability on the basis of the tested characters both in the control variant and in the variant with *A. alternata* culture filtrate.



Control *A. alternata* CF

Fig. 6. Multidimensional scalling of sesame genotypes based on the growth characters under controlled conditions.

1 – L_1, 2 – Djerelo, 3 – Zaltsadovski, 4 – Cubanet 54, 5 – Gusar, 6 – BiolSadovski, 7 – K – 1265, 8 – Bliscucii, 9 – Natasa, 10 – Cubanet 93, 11 – K 1550, 12 – K 1621, 13 – Cumhuriyet 99, 14 – K 1257, 15 – K 1555, 16 – Solnecinâi, 17 – Boiarin, 18 – Adaptovanii 2, 19 – Lider, 20 – Cubanet 55, 21 – Cadet, 22 – L_2, 23 – N162/0781, 24 – Taschentschi 25122, 25 – Conditerschi 2058, 26 – Belosemeannii177, 27 – VNIIMK-889, 28 – VNIIMK-1, 29 – Manjurschi ulucisenii, 30 – Margo, 31 – Liano, 32 – Delco, 33 – Dulce, 34 – Serebristii, 35 – Iubileinii, 36 – Zalt Sadovschi, 37 – UCR/82 n 209-SUAT, 38 – Margo Tall, 39 – Oro shot, 40 – Oro 9/71.

According to the classification of the sesame genotypes (Table 1) based on the model *k*-means for clusterian analysis, it was found that in the control variant the highest values of the studied characters – germination, root length and strain length were recorded in the genotypes of cluster 1, the averages of which accounted for 94.8%; 30.7 mm and 24.0% mm, respectively.

Table 1

Cluster analysis (*k*-means) of the distribution of sesame genotypes into classes based on the growth and development characteristics of the seedlings

Cluster	**Character**	**x**	**S**	**Genotype names**	**Cluster members**
Control					
1	Germination, %	94.8	17.8	12	1 – 3-3 s., p76, 21 – Kadet, 23 – N162/0781, 24 – Taşchentschii 122, 25 – Conditerschii 2058, 29 – Manjurschii ulucisennai, 31 – Liano, 32 – Delco, 33 – Dulce, 34 – Serebristai, 35 – Iubileinai, 40 – Oro 9/71.
	Root length, mm	30.7	16.8		
	Stem length, mm	24.7	3.5		

Table 1 (continued)

2	Germination, %	94.1	14.5	20	2 – Djerelo, 3 – Zaltsadovski, 5 – Gusar, 6 – BiolSadovski, 7 – K – 1265, 8 – Bliscucii, 9 – Natasa, 10 – Cubanet 93, 12 – K-1621, 13 – Cumhuriyet 99, 14 – K-1257, 15 – K-1555, 17 – Boiarin, 18 – Adaptovannai 2, 27 – VNIIMK-889, 30 – Margo, 36 – Zalt Sadovzri, 37 – UCR/82 n 209-SUAT, 38 – Margo Tall, 39 – Oro Shot.
	Rod length, mm	22.9	30.6		
	Stem length, mm	14.6	11.4		
3	Germination, %	85.8	13.1	8	4 – Cubanet 57, 11 – Donscoi belosemiannai, 16 – K-1748 Solnecinai, 19 – Lider, 20 – Cubanet 55, 22 – b., 3-3 p 96, 26 – Belosemiannai 177, 28 – VNIIMK-1.
	Root length, mm	24.4	17.1		
	Stem length, mm	20.7	5.9		
A. alternata CF					
1	Germination, %	87.6	41.2	16	8 – Bliscucii, 9 – Natasa, 12 – K-1621, 13 – Cumhuriyet 99, 14 – K-1257, 16 – K-1748 Solnecinâi, 18 – Adaptovanai 2, 21 – Kadet, 24 – Taschentschii 122, 29 – Manjurschii ulucisennai, 33 – Dulce, 35 – Iubileinai, 37 – UCR/82 n 209-SUAT, 38 – Margo Tall, 39 – Oro Shot, 40 – Oro 9/71.
	Root length, mm	16.3	17.3		
	Stem length, mm	12.3	13.7		
2	Germination, %	62.3	244.3	4	2 – Djerelo, 7 – K – 1265, 11 – Donscoi belosemiannai, 27 – VNIIMK-889.
	Root length, mm	21.2	17.5		
	Stem length, mm	17.2	18.6		
3	Germination, %	90.8	29.2	20	1 – L_1, 3 – Zaltsadovski,4 – Cubanet 57, 5 – Gusar, 6 – BiolSadovski, 10 – Cubanet 93, 15 – K-1555, 17 – Boiarin, 19 – Lider, 20 – Cubanet 55, 22 – b., 3-3 p 96, 23 – N162/0781, 25 – Conditerschii 2058, 26 – Belosemiannai 177, 28 – VNIIMK 1, 30 – Margo, 31 – Liano, 32 – Delco, 34 – Serebristai, 36 – Zalt Sadovzri.
	Root length, mm	28.1	18.1		
	Stem length, mm	19.8	15.4		

We mention that among these 12 genotypes that formed a separate cluster, 9 have a vegetation duration of 120 .... 131 days, that is, they are early and medium early. Three genotypes Conditerschii 2058, Manjurschii ulucisennai, Liano have a greater physiological maturation period – 136–155 days. Regarding other important characters – the size of the plants, the number of capsules *per* plant and the mass of 1,000 seeds, it was found that they were quite different in the genotypes of cluster 1. Thus, the size of the plants in 8 samples varied within the limits of 119.5 ... 155.25 cm, at other 4 – 107 ... 115 cm (Tashchenschii 122, Manjurschii ulucișennai, Dulce, Iubileinai), and the number of capsules *per* plant recorded values within wide limits – 43.4 ... 134. The mass of 1,000 seeds in 10 samples was between 2.56 and 3.94 g, and only in 2 samples – Serebristai, Oro 9/71 – between 2.32 and 2.35 g.

So within the cluster 1 sesame genotypes were found with valuable agronomic traits that also showed important physiological characteristics at the early stage of ontogenesis – high germination, well-developed growth organs can ensure a good development of plants right from the beginning of the vegetation.

In the case of the distribution of sesame genotypes in classes based on the growth and development characteristics of the seedlings when treating the grains with *A. alternata* culture filtrate, the samples with the highest germination values (x = 90.8%), the root length (x = 28.1 mm) and the strain (x = 19.8 mm) formed cluster 3 with a number of 20 genotypes. In the case of the variant with CF, the high values of the growth and development characters mean not only morphological particularities, but also of sensitivity to the pathogen, thus deducing to cluster 3 the most resistant sesame genotypes.

Based on the field assessments, it was found that in 15 genotypes in the mentioned cluster the period of physiological maturation constituted 120 ... 133 days, in 5 samples being 136 ... 155 days. The size of the plants in 10 samples was within the limits of 131.5 ... 162.5 cm, and in the other 10 – 100 ... 128 cm. In the case of the number of capsules *per* plant, 16 genotypes were recorded at 62.6 ... 134.9, and at 4 – 40.25 ... 58.9. The mass of 1,000 seeds for 13 genotypes out of the total of 20 was within the limits of 2.64 ... 4.15 g, and for the other 7 it ranged between 2.34 and 2.59 g. L1, Cubanet 57 and Liano have the highest rates for the number of capsules *per* plant – 95–135 and the mass of 1000 seeds – 3.01–3.91 g.

So, based on the cluster analysis of the distribution of 40 sesame genotypes in sensitivity classes based on the reaction of the seedlings to culture filtrate of *A. alternata*, 20 pathogens were identified, many of which also possess important morphological and agronomic traits, which makes them particularly prospective for implementation in production or involvement in breeding programs as potential donors of valuable complex characters.

CONCLUSIONS

1. Sesame genotypes (*Sesamum indicum* L.) show a wide variability regarding the sensitivity of the growth and development organs of the seedlings to the action of the culture filtrate of *A. alternata*, which offers opportunities for selection of the most resistant to the pathogen.
2. Cluster analysis (*k*-means) of the set of lines and varieties of sesame being studied, in the variant with culture filtrate of *A. alternata* showed a cluster of 20 genotypes with higher indices of germination, root and stem length. Of these, genotypes L1, Cubanet 57 and Liano have the highest indices for such important agronomic characteristics as the number of capsules per plant – 95–135 and the mass of 1,000 seeds – 3.01–3.91 g.

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