

STUDYING AND UTILIZATION OF PLANT GENETIC RESOURCES

Original article

UDC 634.334

DOI: 10.30901/2227-8834-2022-2-149-158

**Symptomological identification of Citrus Psorosis Virus (CPsV) in citrus orchards of Northern Cyprus****Turgut Alas, Akın Akın, İbrahim Kahramanoğlu***European University of Lefke, Gemikonagi, Northern Cyprus***Corresponding author:** Turgut Alas, talas@eul.edu.tr

This study was conducted to perform symptomological identification of Citrus Psorosis Virus (CPsV) in Northern Cyprus. This is the oldest known citrus virus disease, which causes huge decrease in citrus fruits' quality. The first symptoms of the disease were found in Florida and California in the 1890s. The most important and well known symptoms of CPsV are dehiscence and spalling of the trunk and branches of old trees. Chlorotic spotting and ringspot may also be caused on the leaves of infected young trees.

There were reports not to kill the infected trees in the Mediterranean basin and America, but the disease leads to an important reduction in yield and fruit quality in time. Recent available verbal information among the farmers in Northern Cyprus claims that there is an increase in the symptoms of chlorotic spotting on leaves and spalling on tree trunks, which are thought to be caused by CPsV. Grafting is a common management technique on citrus trees in Northern Cyprus, where most of the varieties have been grafted on sour orange; and grafting is among the major ways of transmitting this virus, together with other mechanical practices.

The results of the present study showed that the virus disease in different citrus species and varieties of Northern Cyprus are mostly symptomatic. In the current work, citrus orchards which showed disease symptoms were all recorded and a detailed database was prepared for future molecular analyses. Thus, it is revealed that further molecular studies should be carried in order to perform a scientific investigation into the presence of this virus disease in Northern Cyprus and prove it molecularly.

Keywords: lemon, virus, observation, citrus, bark spalling**Acknowledgements:** the authors would like to extend their thanks to the farmers and citrus producers for their valuable help in sampling and observing the materials.

The authors thank the reviewers for their contribution to the peer review of this work.

For citation: Alas T., Akın A., Kahramanoğlu İ. Symptomological identification of Citrus Psorosis Virus (CPsV) in citrus orchards of Northern Cyprus. *Proceedings on Applied Botany, Genetics and Breeding*. 2022;183(2):149-158. DOI: 10.30901/2227-8834-2022-2-149-158

ИЗУЧЕНИЕ И ИСПОЛЬЗОВАНИЕ ГЕНЕТИЧЕСКИХ РЕСУРСОВ РАСТЕНИЙ

Научная статья

DOI: 10.30901/2227-8834-2022-2-149-158

Симптоматическая идентификация вируса псороза цитрусовых (CPsV) в цитрусовых садах Северного Кипра

Т. Алас, А. Акын, И. Кахраманоглу

Европейский университет Лефке, Гемиконаги, Северный Кипр

Автор, ответственный за переписку: Тургут Алас, talas@eul.edu.tr

Цель исследования – проведение симптоматической идентификации вируса псороза цитрусовых (CPsV) на Северном Кипре. Это старейшее известное вирусное заболевание цитрусовых, которое вызывает значительное снижение качества цитрусовых плодов. Симптомы впервые были обнаружены во Флориде и Калифорнии в 1890-х годах. Наиболее важными и хорошо известными симптомами CPsV являются расщепление и отслоение коры на стволах и ветвях старых деревьев. На листьях зараженных молодых деревьев также могут появляться хлоротические пятна и кольцевые пятна.

В отчетах сообщалось, что не следует уничтожать зараженные деревья в Средиземноморском бассейне и Америке, но со временем болезнь приводит к значительному снижению урожайности и качества плодов. По последним сведениям, полученным от фермеров Северного Кипра, наблюдается увеличение симптомов хлоротических пятен на листьях и сколов на стволах деревьев, которые, как полагают, вызваны CPsV. Прививка является обычным агроприемом для цитрусовых деревьев на Северном Кипре, где большинство сортов были привиты на кислый апельсин; и прививка является одним из основных способов передачи этого вируса, наряду с другими механическими методами.

Результаты настоящего исследования показали, что вирусное заболевание у различных видов и сортов цитрусовых Северного Кипра в основном носит симптоматический характер. В ходе текущей работы все цитрусовые сады, где были выявлены симптомы заболевания, регистрировали и подготовили подробную базу данных для будущих молекулярных анализов. Таким образом, выявлено, что необходимо провести дальнейшие молекулярные исследования для изучения наличия этого вирусного заболевания на Северном Кипре и доказать это молекулярными методами.

Ключевые слова: лимон, вирус, обследование, цитрусовые, откалывание коры

Благодарности: авторы хотели бы выразить свою благодарность фермерам и производителям продукции цитрусовых за их ценную помощь в процессе отбора и оценки материала.

Авторы благодарят рецензентов за их вклад в экспертную оценку этой работы.

Для цитирования: Алас Т., Акын А., Кахраманоглу И. Симптоматическая идентификация вируса псороза цитрусовых (CPsV) в цитрусовых садах Северного Кипра. *Труды по прикладной ботанике, генетике и селекции*. 2022;183(2):149-158. DOI: 10.30901/2227-8834-2022-1-149-158

Introduction

Citrus species are among the most widely produced and consumed fruit crops throughout the world. Citrus fruits are highly appreciated by consumers, especially due to their rich diversity of phytochemicals and high vitamin C (ascorbic acid) content. These are the important reasons which rapidly increased the demand for these fruits in the world markets. In this study, symptomological observation of Citrus Psorosis Virus (CPsV) was carried out. This pathogen was for the first time identified in the 1890s and was called psorosis. Bark spalling was the first characteristic symptom of this pathogen (Martín et al. 2002). There are differences in the degree of infection between countries, especially in the Mediterranean countries. For example, the spread of the disease in Italy is higher than in other countries (Alioto et al. 2000). In the 1930s, this pathogen was defined as Psorosis A and B. Afterwards it was reported that other races were related to this virus (Galipienso, 2000). CPsV contains single-stranded ribonucleic acid (RNA) and is present in all parts of the plant. The psorosis disease, which is commonly spread all over the world, does not cause quick decline and yield losses but leads to low productivity and gradual disappearance of the tree. Virus diseases can be detected and identified with serological, biological and molecular methods. Serological tests, such as the enzyme-linked immunosorbent assay (ELISA), agar-gel spreading reactions and precipitation reactions might be used for detection and identification of viruses. Moreover, molecular methods, such as hybridization, electron microscopy, dsRNA electrophoresis and polymerase chain reaction (PCR), can also be used (Clark, 1981; Luisoni, Boshia, 1994; Hull, 2002). When genetic sequences became available, molecular detection and characterization tests based on RT-PCR were also developed for Citrus Psorosis Virus (Barthe et al., 1998; Legarreta et al., 2000; Loconsole et al., 2009). Garcia et al. (1997) conducted a research on detecting the citrus psorosis-ringspot virus by RT-PCR and DAS-ELISA in 2003 in Italy. PCR and ELISA analysis showed similar results in three isolates that were separate-

ly isolated. In another study, Reyes et al. (2011) tested the resistance of transgenic sweet orange to CPsV in 2011 in France. Symptoms of psorosis on infected trees were examined separately as leaf, stem and branch symptoms. Results showed that this transgenic sweet orange was resistant.

Typical damages of this disease were reported by farmers in Northern Cyprus to be common and widespread on various citrus species, where all of them were grafted on the sour orange rootstock. Sour orange rootstocks are highly susceptible to this virus. Since the sour orange rootstock is used in almost all old orchards in Northern Cyprus, the symptoms can be seen quite easily. As a solution to this problem, Troyer, Carrizo and Volkameriana rootstocks, tolerant or resistant to this disease, started to be used in newly established gardens. Also, previous studies showed that lemon species are more susceptible to this virus than other species (orange, grapefruit or mandarin). Moreover, mild stunting and yield reduction are common in lemon, orange, mandarin and grapefruit orchards of Northern Cyprus. Therefore, the present research was conducted to symptomatically analyze the presence of CPsV in different citrus species and varieties located in different places in Northern Cyprus. This study is the first one on this subject in Northern Cyprus, and it is important for the future in terms of preventing the damage caused by the presence of CPsV.

Material and methods

Orchard surveys and identification of symptoms

Orchard surveys in the present study were carried out in 2018 in various regions of Northern Cyprus over a total orchard area of 3,000 da. All of the citrus varieties surveyed in the present study were grafted on sour orange rootstocks. A total of 65 different citrus orchards (including 25 orange, 20 lemon, 15 grapefruit and 5 mandarin orchards) were selected for studying the symptoms of CPsV. Regions, varieties, tree ages and total numbers of trees are all presented in Table 1 together with the sample numbers.

Table 1. Orchards surveyed during the symptomological observations of Citrus Psorosis Virus

Таблица 1. Обследованные сады во время симптоматических наблюдений за вирусом псороза цитрусовых

Sample No.	Region	Species	Variety	Age	Number of trees
0 1	Yeşilırmak	Orange	Valencia	55	200
0 2	Güzelyurt			60	450
0 3	Lefke		Shamouti	65	250
0 4	Yayla		Valencia	55	350
0 5				60	400
0 6	Aydinköy		Shamouti	50	300
0 7	Bostancı		Valencia	55	150
0 8	Güzelyurt			65	100
0 9	Aydinköy		Shamouti	60	100
0 10				50	120
0 11	Yeşilırmak		Valencia	60	150
0 12				55	250
0 13	Lefke		Shamouti	55	100

Table 1. Continued
Таблица 1. Продолжение

Sample No.	Region	Species	Variety	Age	Number of trees	
O 14	Yayla	Orange	Valencia	60	200	
O 15				60	300	
O 16	Akçay			50	250	
O 17	Lefke			70	200	
O 18	Güzelyurt			65	250	
O 19	Bostancı			60	150	
O 20				60	150	
O 21	Gaziveren			45	150	
O 22	Aydıncöy			50	120	
O 23	Akçay			Shamouti	45	140
O 24	Lefke		55		220	
O 25	Güzelyurt		Valencia	50	300	
L 1	Lapta		Lemon	Cyprus Lemon	65	100
L 2					60	120
L 3					65	120
L 4	Güzelyurt	55			140	
L 5	Lefke	60			100	
L 6		50			150	
L 7	Güzelyurt	50			100	
L 8	Lefke	55			150	
L 9	Aydıncöy	65			80	
L 10	Bostancı	60			100	
L 11		60			120	
L 12	Yeşilirmak	55			150	
L 13		45			120	
L 14	Gemikonağı	40			100	
L 15	Lefke	60			80	
L 16	Yeşilirmak	65			100	
L 17		65			140	
L 18	Güneşköy	60			150	
L 19	Aydıncöy	55			100	
L 20	Güneşköy	50			140	
G 1	Kalkanlı	Grapefruit	Marsh Seedless	60	50	
G 2	Aydıncöy			60	80	
G 3	Gaziveren			50	80	
G 4	Lefke			55	100	
G 5				55	60	

Table 1. The end
Таблица 1. Окончание

Sample No.	Region	Species	Variety	Age	Number of trees		
G 6	Akçay	Grapefruit	Marsh Seedless	55	50		
G 7	Güneşköy			60	120		
G 8	Aydinköy			65	100		
G 9	Gaziveren			45	100		
G 10	Güzelyurt			50	120		
G 11				50	60		
G 12	Bostancı			55	60		
G 13				65	110		
G 14	Güzelyurt			60	100		
G 15	Yayla			60	100		
M 1	Kalkanlı			Mandarins	Local Variety	40	5
M 2	Aydinköy					50	3
M 3	Gaziveren					50	10
M 4	Bostancı					55	5
M 5	Güneşköy					55	5

Symptomological observations

Observations were carried out by professionals who studied each and every single tree independently in the above given orchards. During those observations, the most important symptomological characteristics of CPsV were searched for and observed. The most distinguishing symptoms of CPsV are bark spalling (Figure 1, a, b), splitting and bark spalling on the trunk (Figure 1, c), and gumming on the tree trunk (Figure 1, d, e).

Results and discussion

In this research, a total of 25 orange (19 Valencia and 6 Shamouti), 20 Cyprus lemon, 15 Marsh Seedless grapefruit and 5 local mandarin orchards were observed for the identification and quantification of the symptoms of the Citrus Psorosis Virus (CPsV) disease in Northern Cyprus. Each and every tree was individually analyzed for CPsV and photographs were taken from the orchards that showed symptoms. Thus, the percentage of trees showing CPsV symptoms in orchards was calculated.

Infected tree ratio

Totally, 9,028 citrus trees (from 65 citrus orchards) were surveyed within the current work in Northern Cyprus. The calculated percentages of trees with CPsV symptoms are given in Table 2, Table 3, Table 4 and Table 5 for oranges, lemons, grapefruits and mandarins, respectively. According to the results obtained, the percentage infection of CPsV was 0.72% in all trees including orange, mandarin, lemon and grapefruit orchards.

During the studies, a total of 5,350 different orange trees were analyzed for CPsV and 29 of them were identified

with CPsV infections symptomatically. The ratio of infected trees was then calculated as 0.54% in orange orchards. As mentioned above, two different orange varieties, namely: Valencia and Shamouti, were surveyed and observed in this research. The vast majority of the virus symptoms were recorded for Valencia trees, with 27 trees in total, and only 2 Shamouti trees were observed to be infected with the virus. Detailed information about the infected trees is given in Table 2.

Totally, 2,360 lemon trees were analyzed for CPsV infection in the present work and 28 of them were identified for the CPsV symptoms. The ratio of infected trees was then calculated as 1.17% in lemon orchards. This ratio was found to be about twofold of the infection in orange trees. Detailed information about the infected trees is given in Table 3.

A total of 1,290 different grapefruit trees were surveyed and analyzed for the CPsV symptoms in the present study, and 6 grapefruit trees were found to be infected with CPsV symptomologically. The results showed that the ratio of infected trees with CPsV (symptomologically) was 0.47% in grapefruit orchards. This infection ratio was slightly below the infection rate in orange trees. Six infected grapefruit trees were noted from 5 different regions of Northern Cyprus, namely: Kalkanlı, Lefke, Akçay, Güneşköy and Aydıncık (Table 4).

In the current study, a total of 28 different mandarin trees were observed from 5 different orchards and, according to the results obtained, only 2 mandarin trees had CPsV symptoms. The ratio of infected trees with CPsV was calculated as 7.14% in mandarin orchards. This is the highest ratio in the present study, but mostly it can be explained by a smaller number of surveyed trees. The 2 infected mandarin trees were noted in the Aydıncık and Güneşköy regions of Northern Cyprus (Table 5).

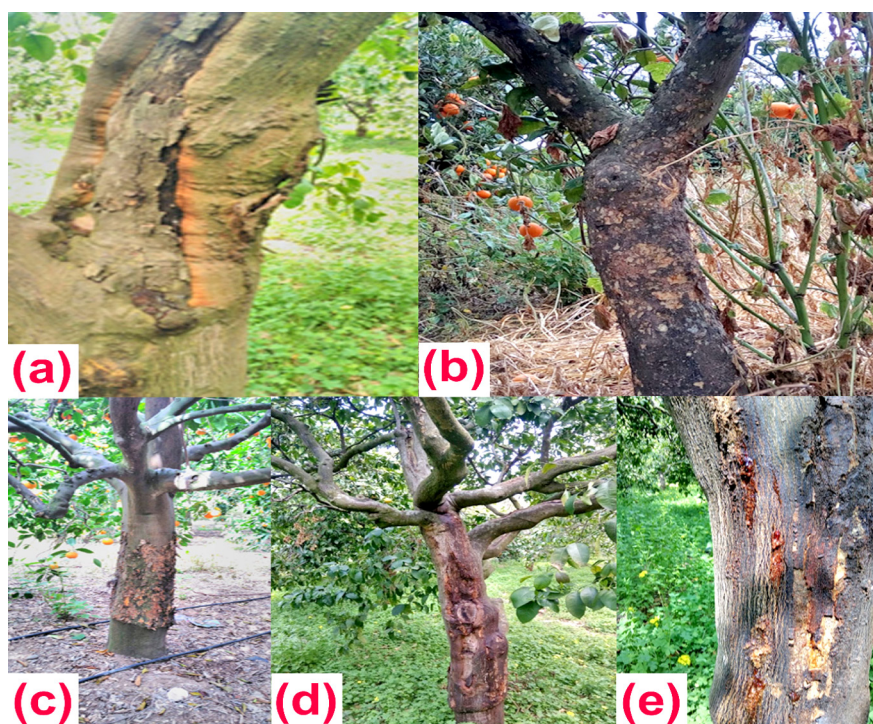


Figure. Most distinguishing symptoms of CPsV; (a) and (b): bark spalling; (c): splitting and bark spalling on the trunk, and (d) and (e): gumming on the tree trunk

Рисунок. Наиболее выраженные симптомы CPsV; (a) и (b): откалывание коры; (c): расщепление и отслоение коры на стволе; (d) и (e): гуммирование на стволе дерева

Table 2. Details on the orange trees infected with Citrus Psorosis Virus

Таблица 2. Подробная информация о зараженных деревьях апельсина вирусом цитрусового псороза

Region	Variety	Age	Number of trees	Number of infected trees	Ratio (%)
Yeşilirmak	Valencia	55	200	3	1.50
Güzelyurt		60	450	1	0.22
Lefke	Shamouti	65	250	0	0.00
Yayla	Valencia	55	350	1	0.29
		60	400	6	1.50
Aydinköy	Shamouti	50	300	0	0.00
Bostancı	Valencia	55	150	2	1.33
Güzelyurt		65	100	1	1.00
Aydinköy	Shamouti	60	100	0	0.00
	Valencia	50	120	0	0.00
Yeşilirmak		60	150	3	2.00
		55	250	4	1.60
Lefke	Shamouti	55	100	0	0.00
Yayla	Valencia	60	200	0	0.00
		60	300	1	0.33
Akçay	Valencia	50	250	2	0.80
Lefke		70	200	0	0.00

Table 2. The end
Таблица 2. Окончание

Region	Variety	Age	Number of trees	Number of infected trees	Ratio (%)
Güzelyurt	Valencia	65	250	0	0.00
Bostancı		60	150	0	0.00
		60	150	1	0.67
Gaziveren		45	150	0	0.00
Aydinköy		50	120	0	0.00
Akçay	Shamouti	45	140	1	0.71
Lefke		55	220	1	0.45
Güzelyurt	Valencia	50	300	2	0.67
Total			5350	29	0.54

Table 3. Details on the lemon trees infected with Citrus Psorosis Virus

Таблица 3. Подробная информация о зараженных деревьях лимона вирусом цитрусового псороза

Region	Variety	Age	Number of trees	Number of infected trees	Ratio (%)
Lapta	Cyprus Lemon	65	100	3	3.00
		60	120	4	3.33
		65	120	0	0.00
Güzelyurt		55	140	0	0.00
Lefke		60	100	2	2.00
		50	150	1	0.67
Güzelyurt		50	100	0	0.00
Lefke		55	150	0	0.00
Aydinköy		65	80	0	0.00
Bostancı		60	100	6	6.00
		60	120	4	3.33
Yeşilirmak		55	150	2	1.33
		45	120	1	0.83
Gemikonağı		40	100	0	0.00
Lefke		60	80	0	0.00
Yeşilirmak	65	100	0	0.00	
	65	140	0	0.00	
Güneşköy	60	150	3	2.00	
Aydinköy	55	100	1	1.00	
Güneşköy	50	140	1	0.71	
Total			2360	28	1.17

Table 4. Details on the grapefruit trees infected with Citrus Psorosis Virus**Таблица 4.** Подробная информация о зараженных деревьях грейпфрута вирусом цитрусового псороза

Region	Variety	Age	Number of trees	Number of infected trees	Ratio (%)
Kalkanlı	Marsh Seedless	60	50	1	2.00
Aydinköy		60	80	0	0.00
Gaziveren		50	80	0	0.00
Lefke		55	100	0	0.00
		55	60	2	3.33
Akçay		55	50	1	2.00
Güneşköy		60	120	1	0.83
Aydinköy		65	100	1	1.00
Gaziveren		45	100	0	0.00
Güzelyurt		50	120	0	0.00
		50	60	0	0.00
Bostancı		55	60	0	0.00
		65	110	0	0.00
Güzelyurt		60	100	0	0.00
Yayla		60	100	0	0.00
Total			1290	6	0.47

Table 5. Details of the mandarin trees infected with Citrus Psorosis Virus**Таблица 5.** Подробная информация о зараженных деревьях мандарина вирусом цитрусового псороза

Region	Variety	Age	Number of trees	Number of infected trees	Ratio (%)
Kalkanlı	Local Variety	40	5	0	0.00
Aydinköy		50	3	1	33.33
Gaziveren		50	10	0	0.00
Bostancı		55	5	0	0.00
Güneşköy		55	5	1	20.00
Total			28	2	7.14

Discussions and conclusion

Citrus Psorosis Virus is a worldwide disease of citrus orchards. It is an infectious filamentous ophiovirus which causes annual losses of about 5%. Moreover, it is a progressive decline of trees through affecting the conductive tissues (Achachi et al., 2014). The most characteristic symptoms of Citrus Psorosis Virus disease on trees are bark spalling on the trunk and branches (Rosa et al. 2007). In this study, the citrus trees chosen as samples mostly showed the bark spalling symptom in Northern Cyprus.

Citrus species most severely affected are orange, grapefruit and mandarin (Milne et al. 2003). The results of this study showed that the most affected species was mandarin (7.4%). In addition to that, lemon orchards showed the se-

cond largest percentage with the infection ratio of 1.17%. Observations carried in 65 different citrus orchards in Northern Cyprus also showed that the symptomological presence of CPsV mostly occurred as leaf wrinkles and chlorosis on leaves, bark spalling and gumming on trunks and branches.

The results of the present study made it possible to obtain a database on the quantification of CPsV in Northern Cyprus. Information about the symptomologically infected trees was all recorded for further molecular analysis. Besides, it is well known that citrus farmers in Northern Cyprus generally import rootstocks and scions from other countries. Under the absence of a quarantine and monitoring system, it seemed so difficult to prevent the emergence of such problems in Northern Cyprus, which may cause different diseases and pests to be carried to the island. Current results made it very impor-

tant to perform similar studies on different crops for the identification and quantification of different pests and diseases in the forthcoming period. Moreover, molecular investigations should also be performed.

Detection and identification of viruses infecting different crops constitute the basic steps for the development of effective crop disease management systems. Methods used to detect Citrus Psorosis Virus in citrus trees are based on using indicator plants. Moreover, laboratory tests, like ELISA and RT-PCR, for molecular detection and characterization of various CPsV forms are so common and important (Garcia et al., 1997; Martín et al., 2004; Zaneck et al., 2006). Furthermore, in order to make molecular detection of the virus easier, the periods when the virus is at its maximum concentration in plants under the conditions of Northern Cyprus should be determined.

Northern Cyprus, as part of an island, has an advantage in the context of preventing the onset of such disease, which is its natural biological isolation. However, this advantage can only be supported with the development of quarantine measures and audits at the entrance points of the island. It is of utmost importance to prevent the transfer of other virus diseases to the island.

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Information about the authors

Turgut Alas, Department of Horticulture, Faculty of Agricultural Sciences and Technologies, European University of Lefke, 10 via Mersin, Gemikonagi 99010, Northern Cyprus, talas@eul.edu.tr, <https://orcid.org/0000-0001-9565-4506>

Akın Akın, Department of Horticulture, Faculty of Agricultural Sciences and Technologies, European University of Lefke, 10 via Mersin, Gemikonagi 99010, Northern Cyprus, akinakin393@gmail.com, <https://orcid.org/0000-0002-3081-5070>

İbrahim Kahramanoğlu, Department of Horticulture, Faculty of Agricultural Sciences and Technologies, European University of Lefke, 10 via Mersin, Gemikonagi 99010, Northern Cyprus, ibrahimcy84@yahoo.com, <https://orcid.org/0000-0002-6074-6395>

Информация об авторах

Тургут Алас, кафедра садоводства, факультет сельскохозяйственных наук и технологий, Европейский университет Лefке, 99010 Северный Кипр, Гемиконаги, ул. Мерсин, 10, talas@eul.edu.tr, <https://orcid.org/0000-0001-9565-4506>

Акын Акын, кафедра садоводства, факультет сельскохозяйственных наук и технологий, Европейский университет Лefке, 99010 Северный Кипр, Гемиконаги, ул. Мерсин, 10, akinakin393@gmail.com, <https://orcid.org/0000-0002-3081-5070>

Ибрагим Кахраманоглу, кафедра садоводства, факультет сельскохозяйственных наук и технологий, Европейский университет Лefке, 99010 Северный Кипр, Гемиконаги, ул. Мерсин, 10, ibrahimcy84@yahoo.com, <https://orcid.org/0000-0002-6074-6395>

Ethical statement: this research was developed from the data obtained during the MSc studies of Akin Akin, guided by Dr. Turgut Alas, and was carried out under the umbrella of European University of Lefke.

Заявление о соблюдении этических норм: эта статья написана на основе данных, полученных в ходе магистерских исследований Акына Акына под руководством доктора Тургута Аласа и проводившихся под эгидой Европейского университета Лefке

Contribution of the authors: T. Alas and A. Akin – conceptualization and methodology; T. Alas and İ. Kahramanoğlu – validation; A. Akin – investigation; A. Akin and İ. Kahramanoğlu – data management; T. Alas – writing the original draft preparation; İ. Kahramanoğlu – writing the review and editing; T. Alas and İ. Kahramanoğlu – visualization. All authors have read and agreed to the published version of the manuscript.

Вклад авторов: Алас Т., Акын А. – концептуализация и методология; Алас Т., Кахраманоглу И. – валидация; Акын А. – исследование; Акын А., Кахраманоглу И. – работа с данными; Алас Т. – подготовка оригинального чернового варианта; Кахраманоглу И. – рецензирование и редакция; Алас Т., Кахраманоглу И. – визуализация. Все авторы прочитали и согласились с опубликованной версией рукописи.

Conflict of interests: the authors declare no conflicts of interests.

Конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

The article was submitted on 15.03.2022; approved after reviewing on 22.04.2022; accepted for publication on 03.06.2022. Статья поступила в редакцию 15.03.2022; одобрена после рецензирования 22.04.2022; принята к публикации 03.06.2022.