The gene Sr38 for bread wheat breeding in Western Siberia

E.S. Skolotneva1, V.N. Kelbin1, V.P. Shamanin2, N.I. Boyko3, V.A. Aparina3, E.A. Salina1, 4

1 Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
2 Omsk State Agrarian University named after P.A. Stolygin, Omsk, Russia
3 Siberian Research Institute of Plant Production and Breeding – Branch of the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
4 Kurchatov Genomic Center of ICG SB RAS, Novosibirsk, Russia

Abstract. Present-day wheat breeding for immunity exploits extensively closely related species from the family Triticeae as gene donors. The 2NS/2AS translocation has been introduced into the genome of the cultivated cereal Triticum aestivum from the wild relative T. ventricosum. It contains the Lr37, Yr17, and Sr38 genes, which support seedling resistance to the pathogens Puccinia triticina Eriks., P. striiformis West. f. sp. tritici, and P graminis Pers. f. sp. tritici Eriks. & E. Henn, which cause brown, yellow, and stem rust of wheat, respectively. This translocation is present in the varieties Trident, Madsen, and Rendezvous grown worldwide and in the Russian varieties Morozko, Svarog, Graf, Marquis, and Homer bred in southern regions. However, the Sr38 gene has not yet been introduced into commercial varieties in West Siberia; thus, it remains of practical importance for breeding in areas where populations of P. graminis f. sp. tritici are represented by avirulent clones. The main goal of this work was to analyze the frequency of clones (avirulent to the Sr38 gene in an extended West Siberian collection of stem rust agent isolates. In 2019–2020, 139 single pustule isolates of P. graminis f. sp. tritici were obtained on seedlings of the standard susceptible cultivar Khakasskaya in an environmentally controlled laboratory (Institute of Cytology and Genetics SB RAS) from samples of urediniospores collected on commercial and experimental bread wheat fields in the Novosibirsk, Omsk, Altai, and Krasnoyarsk regions. By inoculating test wheat genotypes carrying Sr38 (VPM1 and Trident), variations in the purity of (avirulent clones were detected in geographical samples of P. graminis f. sp. tritici were obtained on seedlings of the standard susceptible cultivar Khakasskaya in an environmentally controlled laboratory (Institute of Cytology and Genetics SB RAS) from samples of urediniospores collected on commercial and experimental bread wheat fields in the Novosibirsk, Omsk, Altai, and Krasnoyarsk regions. By inoculating test wheat genotypes carrying Sr38 (VPM1 and Trident), variations in the purity of (avirulent clones were detected in geographical samples of P. graminis f. sp. tritici were obtained on seedlings of the standard susceptible cultivar Khakasskaya in an environmentally controlled laboratory (Institute of Cytology and Genetics SB RAS) from samples of urediniospores collected on commercial and experimental bread wheat fields in the Novosibirsk, Omsk, Altai, and Krasnoyarsk regions.

Key words: Puccinia graminis f. sp. tritici; avirulent clones; resistance; Triticum aestivum; Sr38.

For citation: Skolotneva E.S., Kelbin V.N., Shamanin V.P., Boyko N.I., Aparina V.A., Salina E.A. The gene Sr38 for bread wheat breeding in Western Siberia. Vavilovski Zhurnal Genetiki i Selektcii = Vavilov Journal of Genetics and Breeding. 2021;25(7):740-745. DOI 10.18699/VJ21.084

Ген Sr38: значение для селекции мягкой пшеницы в условиях Западной Сибири

E.C. Сколотнева1, В.Н. Кельбин1, В.П. Шаманин2, Н.И. Бойко3, В.А. Апарина3, Е.А. Салина1, 4

1 Федеральный исследовательский центр Институт цитологии и генетики Сибирского отделения Российской академии наук, Новосибирск, Россия
2 Омский государственный аграрный университет им. П.А. Столыпина, Омск, Россия
3 Сибирский научно-исследовательский институт растениеводства и селекции – филиал Федерального исследовательского центра Институт цитологии и генетики Сибирского отделения Российской академии наук, Новосибирск, Россия
4 Курчатовский геномный центр ИЦиГ СО РАН, Новосибирск, Россия

Аннотация. Современная селекция пшеницы на иммунитет широко применяет генетический резерв близкородственных видов из семейства Triticeae. Транслокация 2NS/2AS привнесена в геном культурного злака Triticum aestivum от дикорастущего сородича T. ventricosum и содержит гены Lr37, Yr17 и Sr38, которые отвечают за устойчивость пшеницы на уровне проростков к буровой, желтой и стеблевой ржавчине с соответствующими возбудителями: Puccinia triticina Eriks., P. striiformis West. f. sp. tritici и P graminis Pers. f. sp. tritici Eriks. & E. Henn.
Introduction

Bread wheat *Triticum aestivum* has been cultivated in many countries for millennia. The exhaustion of the diversity of wheat genes potentially encoding commercially valuable traits, including pest resistance, is inevitable. Wild relatives in the Triticeae family are broadly used as genetic resources for traits, including pest resistance, is inevitable. Wild relatives in countries for millennia. The exhaustion of the diversity of *Triticum monococcum* L., *T. speltaoides* (Tausch) Gren., and *T. ventricosum* (McIntosh et al., 1996). A long chromosome stretch (25–38 cM) hosting by *Sr38* (McIntosh, 1993). The acquired genes and *Lr37* have been designed to facilitate the transfer of the *Lr37, Yr17*, and *Sr38* genes to commercial varieties. The first of the proposed markers was the dominant SCAR (Sequence Characterized Amplified Region) marker, located at 0.8 ± 0.7 cM apart from the *Yr17* gene (Robert et al., 1999). At present, two markers are widely used to identify the 2NS/2AS translocation in wheat genetic material (Helguera et al., 2003). The codominant CAPS (Cleavage Amplified Polymorphic Sequence) marker demands an additional step of digesting the diagnostic fragment with restriction endonucleases. The dominant PCR marker is targeted directly at a specific sequence of the typical allele inside the translocation. The amplification is done with the VENTRIUP-LN2 primer pair, and the products are resolved in agarose gel (https://maswheat.ucdavis.edu/protocols/Sr38), which is an obvious advantage of the marker.

Several molecular markers of the 2NS/2AS translocation have been designed to facilitate the transfer of the *Lr37, Yr17*, and *Sr38* genes to commercial varieties. The first of the proposed markers was the dominant SCAR (Sequence Characterized Amplified Region) marker, located at 0.8 ± 0.7 cM apart from the *Yr17* gene (Robert et al., 1999). At present, two markers are widely used to identify the 2NS/2AS translocation in wheat genetic material (Helguera et al., 2003). The codominant CAPS (Cleavage Amplified Polymorphic Sequence) marker demands an additional step of digesting the diagnostic fragment with restriction endonucleases. The dominant PCR marker is targeted directly at a specific sequence of the typical allele inside the translocation. The amplification is done with the VENTRIUP-LN2 primer pair, and the products are resolved in agarose gel (https://maswheat.ucdavis.edu/protocols/Sr38), which is an obvious advantage of the marker.

Here we analyze the frequencies of clones (a)virulent against *Sr38* in a West Siberian collection of stem rust agent isolates extended by adding samples from the Krasnoyarsk region. Another objective of this work is the DNA marker-assisted search for *Sr38*-carrying accessions. The study involved a collection of spring bread wheat lines and cultivars adapted for growing in West Siberia.

The *Sr38* gene became inefficient against stem rust in countries of Asia and Northern Africa when the aggressive southern race Ug99 started its expansion (Pretorius et al., 2000). However, this race has not yet been detected among wheat pathogens in Russia (Baranova et al., 2015; Skolotneva et al., 2020b). Moreover, it has been shown that low temperatures enhance *Sr38* expression (Helguera et al., 2003). Thus, it may be promising in wheat breeding in regions with temperate climate. As *Sr38* has not been widely introduced into commercial varieties grown in West Siberia (Sochalova, Lichenko, 2015), it is remains of practical significance for breeding for resistance in regions where pathogenic *P. graminis* f. sp. *tritici* populations are represented by avirulent clones.
Materials and methods
The extended West Siberian collection of the stem rust agent included samples from the Novosibirsk, Omsk, Altai, and Krasnoyarsk regions collected from commercial and experimental bread wheat fields in 2019–2020. A total of 139 *P. graminis* f. sp. *tritici* single pustule isolates were obtained from the collected urediniospores on seedlings of the standard susceptible cultivar Khakasskaya in an environmentally controlled laboratory (Institute of Cytology and Genetics, Novosibirsk) (Table 1).

The frequencies of clones avirulent to the *Sr38* gene were determined on tester wheat genotypes: an isogenic line and Trident, respectively. Prior to the experiment, the seed material was verified with molecular markers to the gene, and plants *Sr38*-negative on the DNA array were rejected.

The protocols for seedling preparation and inoculation with fungus clones for the analysis of resistance are described in detail by Skolotneva et al. (2020a). The infection types on wheat tester lines were scored according to the Stackman four-point scale (Stackman et al., 1962).

The collection of 80 bread wheat lines and varieties adapted to the West Siberian conditions was kindly provided by Prof. V.P. Shamanin, Omsk SAU. DNA was isolated from seedling apices by the CTAB method (Rogers, Bendich, 1985). DNA was quantified with a Qubit 4 fluorometer (Invitrogen, United States).

The *Sr38* gene was identified in the material with the primers VENTRIUP (5'-AGGGCTACTGACCAAGGCT-3') and LN2 (5'-TGCAAGCTACAGCAGTTATGTCACACAAA-3') for the 2NS/2AS translocation. Amplification mixture: 1× SE-buffer AS (ammonium sulfate), 0.2 mM each dNTP, 0.2 μM each primer, 1.5 mM MgCl₂, 50 ng of genomic DNA, 1 U of Taq DNA polymerase (SibEnzyme, Russia), volume 25 μL. The reaction was carried out in a Bio-Rad T100 thermocycler (United States) according to the following program: pre-denaturation 7 min at 94°C followed by 30 cycles: 94°C, 30 s; 65°C, 30 s; 72°C, 30 s. Postextension was performed at 72°C for 10 min. The products were resolved in 2% agarose gel. Fragment sizes were assessed against the Step 50 plus DNA ladder (Biolabmix, Russia).

The final step of gene postulation was the phytopathological test of resistance with *P. graminis* f. sp. *tritici* isolates avirulent against *Sr38*. Plant resistance was assessed at the seedling stage as mentioned above. The Khakasskaya variety was chosen as the susceptible control. The experiment was carried out on ten plants of each genotype in two replications.

Results and discussion
While assessing stem rust agent isolates from various localities in West Siberia, we detected a variation in the frequencies of fungus clones not attacking tester genotypes with *Sr38*, that is, avirulent against them (see Table 1). The variation showed a longitudinal cline from the minimum frequency in the Omsk region to the nearly 100% avirulence in the population of the Krasnoyarsk region. The polymorphism of the detected infection types in response to the inoculation with single pustule *P. graminis* f. sp. *tritici* isolates from different samples is illustrated in Figure 1. All types scoring 1, 2, 3, and 3+ were detected, but those corresponding to resistance and medium resistance were predominant in isolates from the Altai and Krasnoyarsk regions. Noteworthy is the occurrence of avirulent clones in the Novosibirsk and Altai samples, not observed in the analysis of the races of the West Siberian population in 2017 (Skolotneva et al., 2020b). This fact may be due to importation of *P. graminis* f. sp. *tritici* inoculum from southern regions. It is known that the *Sr38* gene is efficient in northern Kazakhstan and China (Kozyshbaev, 2018; Li et al., 2018).

In general, clones avirulent against *Sr38* constitute 60% of the West Siberian population. If we reject the collection from the Omsk region, where the gene has been considered inefficient against the local agent for several years (Shamanin et al., 2020), the frequency of fungus clones not injuring genotypes with *Sr38* increases to 78%. Therefore, the gene can be useful in gene pyramiding for eastern West Siberia. The efficiency of the genotypes *Sr25+Sr38* and *Sr31+Sr38* has been demonstrated in the Urals, where *Sr38* alone cannot sufficiently protect plants from stem rust (Druzhin et al., 2018). An additional valuable feature of the 2NS/2AS translocation is that it bears the resistance genes *Lr37* and *Yr17*, which remain efficient against West Siberian isolates of brown and yellow rust agents (Skolotneva et al., 2018; Gultyaeva, Shadayuk, 2020).

Donors of the *Sr38* gene were sought in the Russian breeding material with a specific molecular marker for the 2NS/2AS translocation. As the breeding programs should be targeted at West Siberia, the Omsk SAU collection of spring bread wheat lines and varieties adapted to the region was screened. The gene presence was postulated by genotyping with specific primers (VENTRIUP-LN2) and phytopathological tests with avirulent fungus clones.

### Table 1. Percentages of avirulent *P. graminis* f. sp. *tritici* clones on *Sr38*-bearing tester wheat varieties

| Sampling locality | Year       | Number of single pustule isolates | Percentage of avirulent clones on testers with *Sr38* |
|-------------------|------------|----------------------------------|-----------------------------------------------------|
| Omsk region       | 2020       | 33                               | 9                                                   |
| Novosibirsk region| 2019, 2020 | 57                               | 65                                                  |
| Altai region      | 2019       | 21                               | 71                                                  |
| Krasnoyarsk region| 2020       | 28                               | 100                                                 |
| Total             |            | 139                              | 60                                                   |
Fig. 1. Infection types of *P. graminis* f. sp. *tritici* from various regions tested on genotypes with *Sr38*.

Reaction type scores with fungus isolates: from the Novosibirsk region: (1) 3+, (2) 3–, (3) 1; from the Altai region: (4) 1; from the Krasnoyarsk region: (5) 2; from the Omsk region: (6) 3, (7) 3+.

Table 2. Pedigrees of some wheat lines from the West Siberian collection (Omsk SAU) resistant to stem rust against the natural infectious background of the Omsk region, 2019

| Breeding line | Pedigree | Field scores |
|---------------|----------|--------------|
| Lutescens 12-18 | MN6616M/3/ NL456/VEE#5/ DUCULA/4/ KARAGANDINSKAYA 70 | 20MR |
| Lutescens 34-16 | OMSKAYA 36/ BAVIS/ TERTSIYA | 10MR |
| Lutescens 81-17 | ERITROSPERMUM 55-94-01-20/5/ PYN/ BAU/3/ MON/ IMU// ALD// PYN// VEE#5/ SARA// DUCULA/6/ FITON 42 | 10MR |
| Lutescens 66-16 | 27.90.98.3/3/ KA/ NAC// TRCH/4/ ALTAYSKAYA 530 | 25MR |
| 9-31 | UKR-OD 1530.94/ AE.SQUARROSA(1027)/ Pamyati Azieva | 20MR |
| 8-26 | AISBERG/ AE. SQUARROSA(369)/ Omgau 90 | 20MR |

Positive signals corresponding to the diagnostic 259 bp long amplicon were obtained from DNA templates of seven experimental wheat lines: Lutescens 12-18, Lutescens 34-16, Lutescens 81-17, Lutescens 66-16, Erythrospermum 79/07, 9-31, and 8-26 (Fig. 2). The pedigrees of these varieties and hybrid lines are shown in Table 2. The dramatic variation in the origins of the supposed *Sr38* carriers deserves special attention, as it augments the value of the accessions as diverse resistance donors.

*Puccinia graminis* f. sp. *tritici* isolates eliciting stable responses on *Sr38*-bearing tester wheat genotypes were picked from infection samples of the Krasnoyarsk region for phytopathological tests of the West Siberian collection of bread wheat cultivars and hybrids. Infection types 0 and 1, indicative of resistance, were observed on inoculated plants of Lutescens 12-18, Lutescens 34-16, Lutescens 81-17, Lutescens 66-16, Erythrospermum 79-07, 9-31, and 8-26 (Fig. 3). In addition to the susceptible control (cv. Khakasskaya), we added for reference genotype 2, which lacks *Sr38* according to genotyping with molecular markers. They showed the maximum development of stem rust signs, scored 3 and 4. Part of the tested Lutescens 34-16 plants were susceptible to fungal isolates avirulent against *Sr38* (45S and 45R in Fig. 3). This observation indicates that the breeding material contained biotypes differing in stem rust resistance. The molecular marker is dominant; therefore, it cannot rule out heterozygosity for the character, as found in phytopathological tests. The presence of resistant *Sr38* alleles, expressing in response to the infection by avirulent clones of the fungus in accordance with Flor’s gene-for-
The gene Sr38 for bread wheat breeding in Western Siberia

gene relationship, describing the interaction between a host and a pathogen, was proven in the remaining West Siberian bread wheat accessions: Lutescens 12-18, Lutescens 81-17, Sr38, 77, Erythrospermum 79/07, 8-26. The results of immunological screening of these lines in field tests of breeding material against the natural infectious background point to medium stem rust resistance in Sr38 carriers (see Table 2). This fact is consistent with phytopathological tests on seedlings with isolates from the Omsk SAU West Siberian collection inoculated with P. graminis f. sp. tritici population.

Conclusion
The analysis of West Siberian P. graminis f. sp. tritici isolates shows that the Sr38 gene is promising for breeding wheat in the Krasnoyarsk region and for gene pyramiding in the Novosibirsk and Altai regions. The following bread wheat cultivars and experimental lines from the Omsk SAU collection (see Table 2). This fact is consistent with phytopathological tests on seedlings with isolates from the Omsk SAU West Siberian collection inoculated with P. graminis f. sp. tritici population.

Fig. 3. Reaction type scores of bread wheat cultivars and hybrids from the Omsk SAU West Siberian collection inoculated with P. graminis f. sp. tritici isolates avirulent against Sr38.

Seedlings: Kh, cv. Khakasskaya (score 4); 2, genotype 2 from the Omsk SAU collection (score 3); 34, Lutescens 12-18 (score 1); 45R and 45S, Lutescens 81-17 (score 0); 57, Lutescens 66-16 (score 0), 77, Erythrospermum 79/07 (score 0); 89, line 9-31 (score 0); 90, line 8-26 (score 1).

References
Baranova O.A., Lapochkina I.F., Anisimova A.V., Gajunnill N.R., Iordanskaia I.V., Makarova I.Yu. Identification of Sr genes in new common wheat sources of resistance to stem rust race Ug99 using molecular markers. Russ. J. Genet. Appl. Res. 2016;6(3):344-350. DOI 10.1134/S2079059716030011.
Barianna H.S., McIntosh R.A. Cytogenetic studies in wheat. XV. Location of rust resistance genes in VPM1 and their genetic linkage with other disease resistance genes in chromosome 2A. Genome. 1993; 36(3):476-482. DOI 10.1139/g93-065.
Bespalova L.A., Ablova I.B., Khudokormova Zh.N., Puzynraya O.Yu., Nabokov G.D., Agaeva E.V., Tarhov A.S. Genetic determination of resistance of winter wheat varieties to Puccinia spp. In: Proceedings of the Int. Conf. “State and Prospects for the Development of Agricultural Science in a Changing Climate”. Krasnodar: EDVI Publ., 2019a:11-18. (in Russian)
Bespalova L.A., Ablova I.B., Khudokormova Zh.N., Puzynraya O.Yu., Nabokov G.D., Agaeva E.V., Tarhov A.S. Genetic protection of winter wheat varieties against rust diseases. Risovodstvo = Rice Growing. 2019b;4(45):30-37. (in Russian)
Druzhin A.Ye., Sibikeev S.N., Vlasovets L.T., Golubeva T.D., Kalinueva T.V. The study of agronomic valuable and adaptive traits in a new cultivar of spring bread wheat Alexandrite produced by introgression breeding. Uspekhi Sovremennogo Yestestvoznanii = Advances in Current Natural Sciences. 2018;9-12-17. DOI 10.17513/ use.36859. (in Russian)
Dubcovsky J., Luo M.C., Zhong G.Y., Bransteitner R., Desai A., Klian A., Kleinohns A., Dvojak J. Genetic map of diploid wheat, Triticum monococcum L., and its comparison with maps of Hordeum vulgare L. Genetics. 1996;143(2):983-999. DOI 10.1093/genetics/ 143.2.983.
Dyck P.L., Lukow O.M. The genetic analysis of two interspecific sources of leaf rust resistance and their effect on the quality of common wheat. Can. J. Plant Sci. 1988;68(3):633-639. DOI 10.4141/ cjp88-076.
Friese B., Jiang J., Raupp W.J., McIntosh R.A., Gill B.S. Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. Euphytica. 1996;91(1):59-87. DOI 10.1007/BF00035277.
Gultyaeva E.I., Shaydayuk Ye.L. Virulence of Russian populations of stripe rust causal agent. Mikologiya i Fitopatologiya = Mycology and Phytopathology. 2020;54(4):299-304. DOI 10.31857/S0026364820004042. (in Russian)
Helguera M., Khan I.A., Kolmer J., Lijavetzky D., Zhong-Qi L., Dubcovsky J. PCR assays for the Lr37-Yr17-Sr38 cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. Crop. Sci. 2003;43(5):1839-1847. DOI 10.2135/cropsci2003.1839.
Koshybayev M. Wheat Diseases. Ankara: Food and Agriculture Organization of the United Nations (FAO), 2018. (in Russian)
Li T.Y., Ma Y.C., Wu X.X., Chen S., Xu X.F., Wang H., Cao Y.Y., Xuan Y.X. Race and virulence characterization of Puccinia graminis f. sp. tritici in China. PLoS One. 2018;13(5):e0197579. DOI 10.1371/journal.pone.0197579.
Maia N. Obtention des bles tendres résistants au pietin-verse par croisement interspecifiques bles × Aegilops caryophyllae. C.R. Sciences Acad. Agric. Fr. 1967;53:149-154.
McIntosh R.A., Wellings C.R., Park R.F. Wheat Rusts an Atlas of Resistance Genes. Australia: CSIRO Publ., 1995. DOI 10.1007/BF03214019.
Pretorius Z.A., Singh R.P., Wagoire W.W., Payne T.S. Detection of virulence to wheat stem rust resistance gene Sr31 in Puccinia graminis f. sp. tritici in Uganda. Plant Dis. 2000;84(2):203. DOI 10.1094/ pdis.2000.84.2.203b.
Robert O., Abelard C., Dedryver F. Identification of molecular markers for the detection of the yellow rust resistance gene Yr17 in wheat. Mol. Breed. 1999;5(2):167-175. DOI 10.1023/A:1009672014114.
Rogers S.O., Bendich A.J. Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. Plant Mol. Biol. 1985;5(2):69-76. DOI 10.1007/BF0020088.
Seal S., Spielberg W., jabir J., Sivasithamparam K., Lagudah E.S. Resistance gene analogs within an introgressed chromosomal segment derived from Triticum ventricosum in Puccinia graminis f. sp. tritici. Planta. 2000;210(5):577-584. DOI 10.1007/ s004250000063.
Shamanin V.P., Pototskaya I.V., Skolotneva E.S., Skolotneva E.S., Hodorson D., Hovmoller M., Morgunov A.I. Stem rust in Western Siberia – race composition and effective resistance genes. Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Jour-
nal of Genetics and Breeding. 2020;24(2):131-138. DOI 10.18699/ 
VJ20.60823.
Skolotneva E.S., Kelbin V.N., Morgunov A.I., Boyko N.I., Shama- 
nin V.P., Salina E.A. Races composition of the Novosibirsk popula-
tion of Puccinia graminis f. sp. tritici. Mikologiya i Fitopatologiya =
Mycology and Phytopathology. 2020a;54(1):49-58. DOI 10.31857/
S0026364820010092. (in Russian)
Skolotneva E.S., Kosman E., Patpour M., Kelbin V.N., Morgounov A.,
Shamanin V.P., Salina E.A. Virulence phenotypes of Siberian wheat 
stem rust population in 2017–2018. Front. Agron. 2020b;2:6. DOI 
10.3389/fagro.2020.00006.

ORCID ID
E.S. Skolotneva orcid.org/0000-0001-8047-5695
V.N. Kelbin orcid.org/0000-0002-3455-5704
V.P. Shamanin orcid.org/0000-0003-4767-9957
N.I. Boyko orcid.org/0000-0002-5026-4907
V.A. Aparina orcid.org/0000-0003-2714-7216
E.A. Salina orcid.org/0000-0001-8590-847X

Acknowledgements. This work was supported by the Russian Foundation for Basic Research, project 19-316-90051, and State Budgeted Project 0259- 
2019-0001 for the Institute of Cytology and Genetics, Novosibirsk.
Conflict of interest. The authors declare no conflict of interest.
Received April 15, 2021. Revised August 23, 2021. Accepted August 23, 2021.