JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

APPLICATION OF RYE SSR MARKERS FOR DETECTION OF GENETIC DIVERSITY IN TRITICALE

Želmíra Balážová, Zdenka Gálová, Martin Vivodík*

Address(es): Mgr. Želmíra Balážová, PhD.,
Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology,
Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic, +421376414327.

*Corresponding author: zelmira.balazova@uniag.sk

doi: 10.15414/jmbfs.2016.5.6.623-626

ARTICLE INFO

Received 24. 2. 2016
Revised 6. 4. 2016
Accepted 14. 4. 2016
Published 1. 6. 2016

Regular article

OPEN ACCESS

ABSTRACT

Present study aims to testify usefulness of particular rye SSR markers for the detection of genetic diversity degree in the set of 20 triticale cultivars coming from different European countries. For this purpose, a set of six rye SSR markers were used. The set of six polymorphic markers provided 22 alleles with an average frequency of 3.67 alleles per locus. The number of alleles ranged between 2 (*SCM43*) and 5 (*SCM28*, *SCM86*). Resulting from the number and frequency of alleles diversity index (DI), polymorphic information content (PIC) and probability of identity (PI) were calculated. An average value of PIC for 6 SSR markers was 0.505, the highest value was calculated for rye SSR marker *SCM86* (0.706). Based on UPGMA algorithm, a dendrogram was constructed. In dendrogram cultivars were divided into two main clusters. The first cluster contained two cultivars, Russian cultivar Greneder and Slovak cultivar Largus, and second included 18 cultivars. Genetically the closest were two Greek cultivars (Niobi and Thisbi) and were close to other Greek cultivar Vrodi. It was possible to separate triticale cultivars of spring and winter form in dendrogram. Results showed the utility of rye microsatellite markers for estimation of genetic diversity of European triticale genotypes leading to genotype identification.

Keywords: Triticale, microsatellites, polymorphism, dendrogram

INTRODUCTION

Triticale (x *Triticosecale* Witt.) is the intergeneric hybrid between wheat and rye, has gained considerable importance in recent years in Europe **(Tams *et al.*, 2004)**. It combines desirable traits of both parents **(Salmanovicz *et al.*, 2013)**. It is a crop with broad genetic potential and is widely adapted to abiotic stress conditions such as aluminium toxicity, drought, salinity, acidic or waterlogged soils **(Kuleung *et al.*, 2004)**. Triticale is also resistant to different diseases of wheat **(Leonova *et al.*, 2005)**. Triticale constitutes a valuable genetic resource for transferring genes of interest from rye into wheat, particularly those related to biotic and abiotic stresses **(Vaillancourt *et al.*, 2007)**. Knowledge of germplasm diversity has a significant impact on the improvement of crop plants. Molecular markers can provide an effective tool for efficient selection of desired agronomic traits because they are based on the plant genotypes and thus, are independent of environmental variation. Nowadays, several molecular markers are developed, of which simple sequence repeats (SSRs) or microsatellites are the most widely used types **(Benor *et al.*, 2008)**. SSR markers were successfully used for many purposes, such as mapping (Tams *et al.*, 2004; Bickel *et al.*, 2011), testing the authenticity of genetic stocks **(Pestsova *et al.*, 2000)** and tagging resistance genes **(Peng *et al.*, 1999)**. Simple sequence repeat (SSR) markers show a relatively good transferability between closely related species **(Botes & Bitalo, 2013)** and they are one of the most promising molecular marker types to identify or differentiate genotypes within a species **(Salem *et al.*, 2008)**. They were sucessufully used in many plant species, e.g. wheat **(Röder *et al.*, 1995; Huang *et al.*, 2002, Gregáňová *et al.*, 2005, Hudcovicová *et al.*, 2013)**, rye **(Khlestkina *et al.*, 2004, Balážová *et al.*, 2016)**, triticale **(Kuleung *et al.* 2004; Tams *et al.*, 2004, da Costa *et al.*, 2007; Odrouškova & Vyhnanek, 2013, Balážová *et al.*, 2015)**, rice **(Jiang *et al*, 2010)**, maize **(Ignjatovic-Micic et al., 2015)**, and flax **(Bickel *et al.*,2011).**

The aim of this study was to testify usefulness and transferability of six rye SSR markers to triticale (x *Triticosecale* Witt.) and detect a genetic variability among a set of twenty European cultivars.

MATERIAL AND METHODS

Plant material and DNA extraction

Twenty European triticale cultivars (x *Triticosecale* Witt.) were used for analysis (Table 1). Cultivars were provided by Gene Bank of Slovak Republic in Plant Production Research Center Piešťany, Bratislavská 122, 921 68 Piešťany, Slovakia (12 winter triticale and 8 spring triticale). Samples originated from 6 countries: i.e. Germany (6), Spain (4), Slovakia (4), Greece (4), Czech Republic (1), Russia (1).

DNA isolation

DNA was isolated from 100 mg freshly-collected leaf tissue according to GeneJET™ protocol (Fermentas, USA). The concentration and quality of DNA was checked up on 1.0 % agarose gel stained by ethidium bromide and detecting by comparing to λ-DNA with known concentration.

PCR conditions

For analysis, six microsatellite primer pairs were chosen according to the literature **(Saal and Wricke, 1999)**. Used primers belong to rye-derived primers (*SCM*) localised on 2R, 5R and 6R chromosomes (Table 2). PCR amplification was performed in 20 µl volume containing PCR water, 5 x Green *GoTaq*® Flexi Buffer, 100 µM dNTP Mix, 0.3 µM primers (Forward and Reverse primer), 1.5 mM $MgCl_2$, 0.4 U *GoTaq*® *polymerase* (Promega, USA). PCR reactions were performed in a thermocykler (Bio-Rad, USA) in 0.2 ml tubes. The PCR program consisted of these steps: an initial denaturation (1 cycle): 2 min. at 93 ^{0}C, (29 cycles) denaturation: 1 min. 93 ^{0}C, annealing 2 min. with different temperature for each primer pair and extension 2 min. at 72 ^{0}C.

Table 1 List of the used triticale cultivars

No.	cultivar	country of origin	form
1	Flavius	Slovakia	winter
2	Largus	Slovakia	winter
3	Pletomax	Slovakia	winter
4	Kandar	Slovakia	winter
5	Amarillo 105	Germany	winter
6	Trizeps	Germany	winter
7	Mungis	Germany	winter
8	Trimmer	Germany	winter
9	Trigold	Germany	winter
10	Cosinus	Germany	winter
11	Senatrit	Spain	spring
12	Sierra de Villuercas	Spain	spring
13	Sierra de Almaraz	Spain	spring
14	Tentudia	Spain	spring
15	Kinerit	Czech Republik	winter
16	Vrito	Greece	spring
17	Niobi	Greece	spring
18	Vrodi	Greece	spring
19	Thisbi	Greece	spring
20	Greneder	Russia	winter

Electrophoresis and staining conditions

The PCR amplicons (5µl) were resolved by electrophoresis in 6.0 % denaturating polyacrylamide gel and run with 1.0 x TBE buffer. Electric voltage and time were different for each marker. The electric voltage ranged from 1800 to 2000 V and time of electrophoretic separation differs for each marker (3-4 hours) influenced by the predicted size of fragments. After electrophoresis, gels were fixed and stained with silver according to [22]. Final PCR amplicons were scanned in UVP PhotoDoc-t® camera system. The size of alleles was determined by comparing with 10 bp standard lenght marker (Invitrogen: 100-330 bp). Each band was treated as a single allele.

Data analysis

For statistical evaluation, all gels were scored in a binary matice on the base of presence (1) or absence (0) of particular allele. Information about presence of alleles and their frequency were used for calculation of Jaccard coefficient of genetic similarity by SPSS modul (SPSS inc., USA). Final dendrogram was constructed by hierarchic cluster analysis using UPGMA (Unweighted Pair Group Method using arithmetic Averages) algorithm by SPSS statistic programme version 17.

For the assessment of the polymorphism between triticale genotypes and usability of SSR markers for differentiation we used diversity index (DI) **(Weir, 1990**), the probability of identity (PI) (**Paetkau *et al.*, 1995**) and polymorphic information content (PIC) (**Weber, 1990**).

They were calculated according to formulas:

Diversity index (DI)

$$DI = 1 - \sum p_i^2$$

Probability of identity (PI)

$$PI = \sum p_i^4 + \sum_{i=1}^{i=n-1} \sum_{j=i+1}^{n} \left(2 p_i p_j\right)^2$$

Polymorphic information content (PIC)

$$PIC = 1 - \left(\sum_{i=1}^{n} p_i^2\right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 p_i^2 \cdot p_j^2$$

where pi and pj are frequencies of ith and jth allele of given genotype.

Table 2 Sequences and melting temperature value (TM) of six SSR primers

SSR marker	*Locus on chromosome*	*Sequences of primers 5´ → 3´*	*SSR repetition*	*Annealing temperature*
SCM28	6RL	5' CTGGTCCTGGTCTGGTGGGTC 3' CGCATCGGGTGTGTCGCATAC	$(GT)_{26}$	60°C
SCM43	2RS	5' CTAGGGGATTACAGGGAGGGCA 3' GTTCCCTTGTCCTACTCGTTACCG	$(GT)_{11}$	60°C
SCM86	6RL	5' CAGATAGATGGGTGTTGTGCG 3' CTCTTCTCGACATCCACACTCC	$(GT)_{20}$	60°C
SCM120	5RL	5' CATTGTTGCGAGTGTTGAAGC 3' TGTGCTGTCGTCGATGTTGTC	$(AC)_{10}$	60°C
SCM180	6RL	5' GTTTCGTCCCCGTTGCCATC 3' ACGTGTCGCTTTCCATTGCCC	$(GT)_6 (GA)_7$	60°C
SCM268	5RS	5' GCGCACCCCACACAACACG 3' GCGGTGGCGGTTGAGGAC	$(CA)_9$	65°C

RESULTS AND DISCUSSION

Six SSR markers were used in this study with the aim to detect genetic diversity in triticale (x *Triticosecale* Witt.) cultivars. This work focused on two principal goals: i.e. a) to testify the usefulness and efficiency of 6 rye SSR markers in close-related triticale and b) to find out a genetic background among triticale cultivars (bred in different European countries). Six SSR markers provided a polymorphic spectrum.

A total of 22 alleles were detected (Table 3) with an average value of 3.67 alleles per locus. The number of alleles ranged from 2 (*SCM43*) to 5 (*SCM28, SCM86*). Very similar average number of detected alleles per locus was detected by authors **Vyhnánek *et al.* (2009)**, **Ondroušková & Vyhnánek (2013)** and **Kuleung *et al.* (2004). Ondroušková & Vyhnánek (2013)** used 25 SSR wheat and rye SSR markers for the study of genetic variability in 10 triticale cultivars. They detected 84 alleles (3.36 alleles per locus). **Vyhnánek *et al.* (2009)** studied 16 genotypes of triticale using 48 markers (27 wheat and 21 rye SSR markers) and detected 184 alleles with an average of 3.83 alleles per locus. **Kuleung *et al.* (2004)** detected on average 4.2 alleles per locus using 43 wheat and 14 rye SSR markers for the study of genetic diversity of 80 hexaploid accessions. Higher average number of alleles was detected by **Tams *et al*. (2004)** (6.8 alleles and per locus). This value is considerably higher compared to our results, but it could be caused by higher number of SSR markers. The highest number of alleles (5) was detected by SSR marker *SCM28* and *SCM86*. **Kuleung *et al*. (2006)** detected the same number of alleles by SSR marker *SCM268* (3) but lower number of alleles (3) compared to our results (4) by SSR marker *SCM120*.

As mentioned above, the usefulness of SSR markers is not influenced just by an overall number of detected alleles, but the most important factor is their distribution and frequencies. The way how to find out their effectiveness is to calculate Diversity index (DI), Polymorphic information content (PIC) and Probability of identity (PI) based on frequencies of alleles.

Diversity index (DI) for six rye SSR markers varied from 0.172 (*SCM43*) to 0.710 (*SCM28, SCM86*) with an average of 0.540. **Kuleung *et al*. (2006)** and **Tams *et al*. (2004)** calculated the same diversity index (0.54) for the study of genetic diversity of triticale cultivars using 57 SSR markers and 93 SSR markers, respectively. **Ondroušková & Vyhnánek (2013)** calculated similar average diversity index (0.55). Higher values of DI (0.65) were reported by **(Balážová *et al.*, 2015)** who used wheat genomic SSR markers for the study of genetic diversity of triticale cultivars.

Polymorphic information content (PIC) varied from 0.157 (*SCM43*) to 0.706 (*SCM86*) with an average of 0.505. An average value of PIC was similar compared to work **Ondroušková & Vyhnánek (2013)** who reported on average 0.5 and **Vyhnánek *et al.* (2009)** who calculated 0.48. Probabilities of identity (PI) ranged from 0.044 (*SCM86*) to 0.699 (*SCM43*) with an average of 0.264. **Korkovelos *et al.* (2008)** claimed that the more effective SSR in discriminating among genotypes is the one having the higher PIC and DI along with lower

PI values. According to this allegation, the most effective marker for the genetic diversity detection is *SCM86*. As appropriate markers, we also recommend *SCM28 and SCM180*, respectively. Only one marker (*SCM43*) reached considerably unfavourable results of DI, PIC, PI and number of alleles compared to average values of tested set.

Table 3 Statistical characteristics of used primers

SSR marker	Number of alleles	DI	PIC	PI
SCM28	5	0.710	0.689	0.074
SCM43	2	0.172	0.157	0.699
SCM86	5	0.710	0.706	0.044
SCM120	4	0.534	0.500	0.237
SCM180	3	0.607	0.532	0.222
SCM268	3	0.505	0.444	0.305
average	3.67	0.540	0.505	0.264

DI – Diversity index; PIC – Polymorphic information content; PI – Probabilities of identity

Resulting from number and frequencies of alleles, a UPGMA dendrogram based on Jaccard´s coefficient of similarity was constructed (Figure 1). Dendrogram revealed genetic relationships among analysed set of winter and spring triticale genotypes. Cultivars were divided into two main groups (1 and 2). First cluster (1) contained two cultivars, Slovak cultivar Largus and Russian cultivar Greneder. Second cluster of 18 cultivars was further subdivided into two subclusters (2a and 2b), subcluster 2a included 11 triticale cultivars and subcluster 2b contained 7 cultivars. All (4) Greek cultivars were included in subcluster 2b. Genetically the closest were two Greek cultivars, Niobi and Thisbi from subcluster 2b. We can consider their close genetic background. Another Greek cultivar Vrodi was also placed nearby Niobi and Thisbi. Most of cultivars from subcluster 2b are of spring form (85.7 %). Subclusters 1 and 2a comprised predominantly of winter triticale cultivars (84.6%).

Vyhnánek *et al.* (2009) tested set of 16 accessions using 48 SSR markers. One Russian genotype had been significantly differentiated from all set of accessions. **Da Costa *et al.* (2007)** divided 54 accessions of triticale based on wheat SSR markers into seven main groups using UPGMA dendrogram based on Jaccard's coefficient of similarity. Since most of the analyzed germplasm was derived from Mexican triticale, they detected a high similarity among triticale cultivars and moderate variability.

Kuleung *et al.* (2004) and **Tams *et al*. (2004)** reported that the diversity revealed by rye SSR markers in triticale was lower in comparison to wheat microsatellites. We can confirm this statement by our earlier study **(Balážová *et al.*, 2015)** where we have detected higher average values of PIC and DI of used wheat SSR markers to study genetic diversity of triticale cultivars compared to today´s study with rye SSR markers.

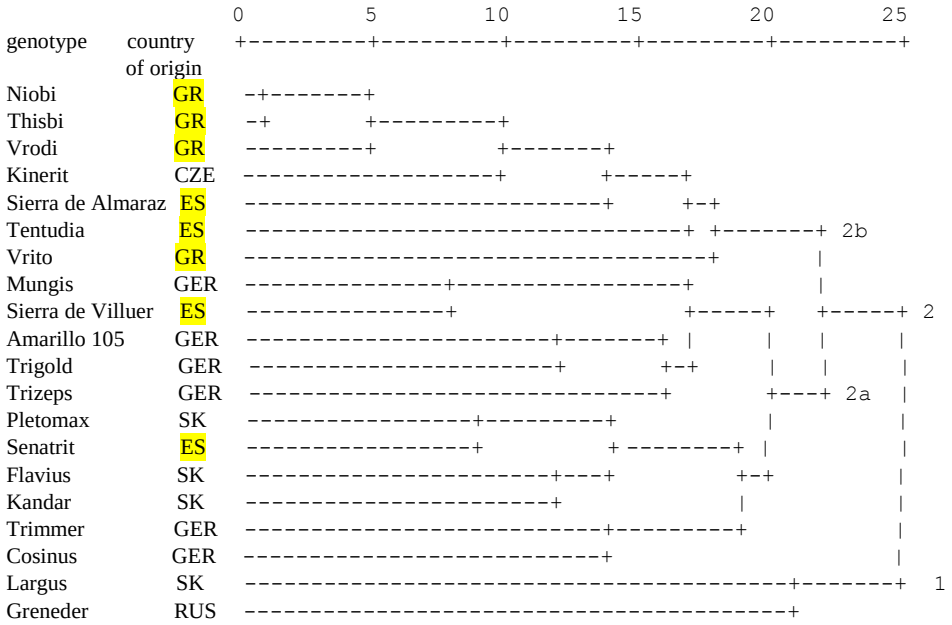

Figure 1 Dendrogram of 20 triticale genotypes based on data from six rye SSR markers. Yellow labelled cultivars are of spring form

CONCLUSION

A tested set of six rye SSR markers allowed significantly distinguish 18 out 20 cultivars. Only two Greek cultivars, Niobi and Thisbi, had not been separated from each other. In dendrogram triticale of spring form significantly separated from winter form triticale cultivars. An average value of PIC and DI indicate that used SSR markers are moderately polymorphic. We can recommend all rye SSR markers used for diversity analysis accept one marker (*SCM43*) which reached considerably unfavourable results of PIC and DI values. Anyway, used rye SSR markers represent an efficient and useful marker system for detection of genetic variability in closed-related triticale.

Acknowlegments: This work was funded by European Community under project No. 26220220180: Building Research Centre „AgroBioTech" (50%) and KEGA project No 021SPU-4/2015 (50%). This work originated also thanks to the support within Operational Programme Research and Development for the project: "Transfer, use and dissemination of research results of plant genetic resources for food and agriculture" (ITMS: 26220220058), cofinanced from the resources of the European Union Fund for Regional Development.

REFERENCES

Balážová, Ž., Trebichalský, A., Gálová, Z., Hornyák Gregáňová, R. (2015). Application of wheat SSR markers for detection of genetic diversity in triticale (x Triticosecale witt). *Genetika*, 47, 983 -992. http://dx.doi.org/10.2298/gensr1503983b

Balážová, Ž., Petrovičová, A., Gálová, Z., Vivodík, M. (2016). Molecular characterization of rye cultivars. *Potravinárstvo*, 10 (1), 54-58. http://dx.doi.org/10.5219/522

Bassam, B.J., Caetano-Anolles, G., P.M. Gresshoff. (1991). Fast and sensitive silver staining of DNA in polyacrylamide gels. *Analytical Biochemistry*, 196, 80-83. http://dx.doi.org/10.1016/0003-2697(91)90120-i

Benor, S., Zhang, M., Wang, Z., Zhang, H. (2008). Assessment of genetic variation in tomato (*Solanum lycopersicum* L.) inbred lines using SSR molecular markers. *Journal of Genetics and Genomics*, 35, 373− 379. http://dx.doi.org/10.1016/s1673-8527(08)60054-5

Bickel, C.L., Gadani, S., Lukacs, M., C.A. Cullis. (2011). SSR markers developed for genetic mapping in flax (*Linum usitatissimum* L.). *Research and Reports in Biology*, 2, 23-29. http://dx.doi.org/10.2147/rrb.s16091

Botes, W.C., Bitalo, D. (2013). Identification, evaluation and optimalization of a minimum simple sequence repeat marker set for triticale breeding. *Journal of Apllied Biology and Biotechnology*, 1, 16-23.

Da Costa, C.T., Albuquerque, A.Ch.S., Nascimento, A., Marcelino, F.C., Pereira, J.F. (2007). Genetic diversity of Brazilian triticales evaluated with genomic wheat microsatellites. *Pesquisa Agropecuária Brasileira*, 42, 1577-1586. http://dx.doi.org/10.1590/s0100-204x2007001100009

Gregáňová, Ž., Kraic, J., Gálová, Z. (2005). Effectiveness of microsatellites in differentiation of elite wheat cultivars. *Biologia* , 60, 665-670.

Huang, X.Q., Börner, A., Röder, M.S., Ganal, M.W. (2002). Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite

markers. *Theoretical Applied Genetics*, 105, 699-707. http://dx.doi.org/10.1007/s00122-002-0959-4

Hudcovicová, M., Ondreičková, K., Hauptvogel, P., Kraic, J. (2013). Genetic diversity in domestic and introduced wheats. *Agriculture (Poľnohospodárstvo)*, 59, 101–110. http://dx.doi.org/10.2478/agri-2013-0009

Ignjatovic-Micic, D., Ristic, D., Babic, V., Andjelkovic, V., Vancetovic, J. (2015). A simple SSR analysis for genetic diversity estimation of maize landraces. *Genetika*, 47, 53-62. http://dx.doi.org/10.2298/gensr1501053i

Jiang, S.K., Huang, C., Zhang, X.J., Wang, J.Y., Chen, W.F., Xu, Z.J. (2010). Development of Highly Informative Microsatellite (SSR) Marker Framework for Rice (*Oryza sativa* L.) Genotyping. *Agricultural Sciences in China*, *9*, 1697-1704. http://dx.doi.org/10.1016/s1671-2927(09)60268-6

Khlestkina, E.K., Than, M.H.M., Pestsova, E.G., Röder, M.S., Malyshev, S.V., Korzun, V., Börner, A. (2004). Mapping of 99 new microsatellite-derived loci in rye (*Secale cereale* L.) including 39 expressed sequence tags. *Theoretical Applied Genetics*, 109, 725-732. http://dx.doi.org/10.1007/s00122-004-1659-z

Korkovelos, A.E., Mavromatis, A.G., Huang, W.G., Hagidimitriou, M., Giakoundis, A., Goulas, C.K. (2008). Effectiveness of SSR molecular markers in evaluating the phylogenetic relationships among eight *Actinidia* species. *Scientia Horticulturae*, 116, 305–310. http://dx.doi.org/10.1016/j.scienta.2008.01.011

Kuleung, C., Baenziger, P.S., Dweikat, I. (2004). Transferability of SSR markers among wheat, rye and triticale. *Theoretical and Applied Genetics*, 108, 1147-1150. http://dx.doi.org/10.1007/s00122-003-1532-5

Kuleung, C., Baenzinger, P.S., Kachman, S.D., Dweiket, I. (2006). Evaluating the Genetic Diversity of Triticale with Wheat and Rye SSR Markers. *Crop Science*, 46, 1692-1700. ISSN 1435-0653. http://dx.doi.org/10.2135/cropsci2005.09-0338

Leonova, I.N., Dobrovolskaya, O.B., Kaminskaya, L.N., Adonina, I.G., Koren, L.V., Khotyljova, L.V., Salina, E.A. (2005). Molecular analysis of the triticale lines with different *vrn* genes systems using microsatellite markers and hybridization in situ. *Russian journal of Genetics*, 41, 1014-1020. http://dx.doi.org/10.1007/s11177-005-0193-7

Ondroušková, J., Vyhnánek, T. (2013). Study of genetic variability of triticale varieties by SSR markers. *Journal of Microbiology, Biotechnology and Food Sciences*, 2, 2366-2368. http://www.jmbfs.org/wp-content/uploads/2013/04/jmbfs_0369_ondrouskova.pdf

Paetkau, D., Calvert, W., Stirling, I., Strobeck, C. (1995). Microsatellite analysis of population structure in Canadian polar bears. *Mol. Ecol*, 4, 347-354. http://dx.doi.org/10.1111/j.1365-294x.1995.tb00227.x

Peng, J.H., Fahima, T., Röder, M.S,, Li, Y.C., Dahan, A., Grama, A., Ronin, Y.I., Korol, A.B., Nevo, E. (1999). Microsatellite tagging of the stripe-rust resistance gene YrH52 derivedfrom wild emmer wheat, Triticum dicoccoides, and suggestive negative crossover interference on chromosome 1B. *Theoretical Applied Genetics*, 98, 862-872. http://dx.doi.org/10.1007/s001220051145

Pestsova, E., Ganal, M.W., Röder, M.S. (2000). Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. *Genome*, 43, 89–697. http://dx.doi.org/10.1139/gen-43-4-689

Röder, M.S., Plaschke, J., König, S.U., Börner, A., Sorrels, M.E., Tanksley, S.D., Ganal, M. (1995). Abundance, variability and chromosomal location of mocrosatellites in wheat. *Molecular Genetics and Genomics*, 246, 327-333. http://dx.doi.org/10.1007/bf00288605

Saal, B., Wricke, G. (1999). Development of simple sequence repeat markers in rye (*Secale cereale* L.). *Genome*, 42, 964–972. http://dx.doi.org/10.1139/gen-42-5-964

Salem, K.F.M., El-Zanaty, A.M., Esmail, R.M. (2008). Assessing wheat (*Triticum aestivum L.*) genetic diversity using morphological characters and microsatellite markers. *World Journal of Agricultural Sciences*, 4, 538-544. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.415.1802&rep=rep1&type=pdf

Salmanovicz, B.P., Langner, M., Wiśniewska, H., Apolinarska, B., Kwiatek, M., Błaszczyk, L. (2013). Molecular, Physicochemical and Rheological Characteristics of Introgressive Triticale/*Triticum monococcum* ssp. *Monococcum* Lines with Wheat 1D/1A Chromosome Substitution. *International Journal of Molecular Sciences*, 14, 15595-15614. http://dx.doi.org/10.3390/ijms140815595

Tams, S.H., Bauer, E., Oettler, G., Melchinger, A.É. (2004). Genetic diversity in European winter triticale determined with SSR markers and coancestry coefficient. *Theoretical and Applied Genetics*, 108, 1385-1391. http://dx.doi.org/10.1007/s00122-003-1552-1

Vaillancourt, A., Nkongolo, K.K., Michael, P., Mehes, M. (2007). Identification, characterisation, and chromosome locations of rye and wheat specific ISSR and SCAR markers useful for breeding purposes. *Euphytica*, 159, 297–306. http://dx.doi.org/10.1007/s10681-007-9492-5

Vyhnánek, T., Nevrtalová, E., Slezáková, K. (2009). Detection of the genetic variability of triticale using wheat and rye SSR markers. *Cereal Research Communications*, 37, 23-29. http://dx.doi.org/10.1556/crc.37.2009.1.3

Weber, J. L. (1990). Informativeness of human (dC-dA)n x (dG-dT)n polymorphism. *Genomics*, 7, 524-530. http://dx.doi.org/10.1016/0888-7543(90)90195-z

Weir, B.S. (1990). *Genetic data analysis.* Sinauer Associated, Sunderland, Mass.