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COMPARISON OF RAPD, ISSR AND SSR MARKERS IN ASSESSING GENETIC DIVERSITY AMONG RYE (*SECALE CEREALE* L.) INBRED LINES

ABSTRACT

Forty eight inbred lines of winter rye, of various origin and pedigree, were analysed using 19 RAPD (random amplified polymorphic DNA) primers, 8 ISSR (inter-simple sequence repeats) primers and 13 SSR (simple sequence repeats) primer pairs. On the basis of particular marker types, there were created three separate dendrograms and one combined similarity tree, prepared on account of the whole data. Correlation coefficients for individual technique based on genetic similarity matrices were not significant. By comparing the *GS* data obtained on the basis of singular methods with collective matrix, it was observed that the highest correlation rate was for ISSR method (r=0.68). The utility of each marker technique was compared by using marker index *MI*. Diversity detecting index (*DDI*) was suggested in the paper, which may prove helpful in planning and comparing researches on phenetic relationships.

Key-words: dendrogram, genetic diversity, molecular markers, rye.

INTRODUCTION

Recent progress in the development of new marker technologies allows for using them for various practical purposes. One of them is implementation of molecular markers in genetic identification in terms of cultivars, landraces, lines or clones.

Basing on DNA profiles of characterised objects, it is possible to determine their genetic similarity. The knowledge of genetic diversity on molecular level may be helpful with choosing the appropriate parents for breeding hybrids. The connection between the heterosis effect and genetic distance of parental forms has been demonstrated for example, for particular features of barley (Krystkowiak *et al.* 2009). Molecular analysis of genetic similarity was also used while assessing the level of genetic erosion of durum wheat cultivars

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(Martos *et al.* 2005).

Isoenzymes and markers based on PCR were used so far to establish phylogenetic relationship between species within genus *Secale* or among cultivars and landraces of species *S. cereale* (Matos *et al.* 2001, Persson and von Bothmer 2002, Ma *et al.* 2004, Chikmawati *et al.* 2005). Analysis of diversity of rye accessions have been carried out using single marker technique (Persson et al 2001, Myśków *et al.* 2001, Stojałowski *et al.* 2004), two (Loarce *et al.* 1996) or three of them (Bolibok *et al.* 2004, Lebiecka 2007).

The aim of this study was the assessment of genetic similarity of 48 inbred lines of winter rye representing various origin and pedigree and comparison of RAPD, ISSR and SSR markers as affective tools in diversity evaluation.

MATERIALS AND METHODS

Forty eight inbred lines of rye obtained from the collections of Institute of Plant Breeding and Acclimatization (IHAR) in Radzików, Danko Plant Breeding, Ltd. in Choryń and Department of Plant Genetics, Breeding and Biotechnology (ZGHBR) of West-Pomeranian University of Technology in Szczecin have been analysed in terms of genetic similarity (Table 1). Detailed pedigree of some of the objects was unknown.

DNA was sampled from the leaves of young plants, using ready to use sets for isolation (Qiagen). Primers selected during previous genetic mapping studies were used for molecular analysis, including 19 RAPD primers, 8 ISSR primers and 13 pairs of SSR primers. RAPD and ISSR primers sequences and symbols of SSR markers used in the study are available upon request. RAPD reaction conditions were the same as in Myśków *et al.* (2001). ISSR and SSR markers were analyzed as described by Stojałowski *et al.* (2004) and Milczarski *et al.* (2007), respectively.

Respective classes of markers were characterised using marker index *MI*, which is obtained by multiplying *PIC* (polymorphic information content) and *EMR* (effective multiplex ratio). *PIC* was calculated using the formula:

$$1-\sum p_i^2$$

where p is the frequency of i-th allel (Smith *et al.* 1997).

EMR is the average number of polymorphic loci in a single analysis for a particular set of objects (Powell *et al.* 1996, Milbourne *et al.* 1997).

DDI (diversity detecting index) was calculated from the proposed formula:

$$DDI=PIC\times n_m/n_{OTU}$$

where

n_m is the number of markers (polymorphic loci)

and n_{OTU} is the number of examined objects (operational taxonomic units - *OTU*).

Table 1

Origin and pedigree of 48 rye inbred lines used in the analysis of genetic similarity

Inbred line	Origin	Pedigree
541	ZGHBR	KaH9×[(MS69-8-1×Smolickie)F_2MS×KaH]F_1MP
620	ZGHBR	Dańkowskie Złote
153/79	ZGHBR	541-6-2-3×544-7-1-3
542-9-1-11	ZGHBR	(*S.montanum*×Smolickie)Bc_5×(MS69-8-1×KaH)F_1MP
544-7	ZGHBR	(*S.Kuprianowii*×Smolickie)Bc_5×[(MS69-8-1×Smolickie)F_2MS×KaH]F
585/92-1-2	ZGHBR	544×620-5
585/92-6-1	ZGHBR	544×620-5
585/92-6-3	ZGHBR	544×620-5
711/81K	ZGHBR	[(541×K221)F_1×541]F_1×541
723/81K	ZGHBR	{[(544-7-1-1×K221)F_1-7-1-5]F_1×544-7-1-5}F_1MP
C599/74	ZGHBR	unknown
DS2	ZGHBR	*S.dighoricum*×Smolickie
KaH6	ZGHBR	Kazimierskie-cultivar
L1	ZGHBR	Petkus Normal-cultivar (Germany)
Ot0-20	ZGHBR	Otello-cultivar (Sweden)
Ot1-3	ZGHBR	Otello-cultivar (Sweden)
ROP2	ZGHBR	Rogalińskie-cultivar
RXL10	ZGHBR	Zeelandzkie-cultivar
51527	IHAR	unknown
1304/LM-5	IHAR	unknown
1628/LM-1	IHAR	unknown
1792/LM-5	IHAR	unknown
2020/LM-3	IHAR	unknown
2035/LM-1	IHAR	unknown
2130/LM-1	IHAR	unknown
2143/LM-4	IHAR	unknown
2256/LM-2	IHAR	unknown
2298-1/LM-1	IHAR	unknown
2332-2/LM-1	IHAR	unknown
2374-1/LM-5	IHAR	unknown
2680/LM-2	IHAR	unknown
2683-1-7/LM-3	IHAR	unknown
2713/LM-3	IHAR	unknown
4475/LM-2	IHAR	unknown
5114/LM-1	IHAR	unknown
51334/LM-3	IHAR	unknown
53165/LM-4	IHAR	unknown
54206/LM-5	IHAR	unknown
5491/LM-1	IHAR	unknown
739/LM-3	IHAR	unknown
804/LM-1	IHAR	unknown
809/LM-1	IHAR	unknown
910/LM-2	IHAR	unknown
L304N/LM-1	IHAR	unknown
S19	DANKO	unknown
S76	DANKO	LG3×Amilo
S120	DANKO	LG3×Szk.10
S436N	DANKO	Mat.S×Dominator

NTSYSpc2.20 software (Rohlf, 1998) was applied for dendrogram construction, by *UPGMA* (unweighted pair-group method). During genetic similarity (*GS*) calculation simple matching function (*SM*) was used. The software permitted to assess the correlation coefficient of particular matrixes

of similarity values.

RESULTS

Primers giving polymorphic products among two inbred lines were chosen for molecular analysis. Genotypes of lines were compared in respect to 29 ISSR polymorphic loci revealed by 8 primers (1-6 loci per primer), 48 RAPD polymorphic loci (19 primers, 1-4 loci per primer) and 13 SSR loci giving 34 allelic bands (2-4 alleles per locus). Marker assortment within each marker system allowed for differentiating all inbred lines.

PIC was calculated for each polymorphic locus (Table 2). The lowest values were obtained in ISSR method, considering both particular loci values and average values. The highest possible *PIC* value in the case of dominant markers (0.5) was obtained for 5 out of 29 ISSR loci and for 5 out of 48 RAPD loci. SSR markers showed the highest *PIC* values. This marker system gave the lowest *MI*, since only one locus was presented in a single assay. ISSR and RAPD, as the markers allowing to discover polymorphism simultaneously in many loci, had higher *MI* values. The highest *MI* value was obtained with ISSR markers (Table 2).

Table 2

Characteristics of three types of markers used for genetic similarity analysis of 48 inbred lines of rye

Characteristics	RAPD	ISSR	SSR
Number of assay (primers or primer pairs)	19	8	13
Number of products*	48	29	34
Number of polymorphic products	48	29	13
EMR number of polymorphic products per assay	2.53	3.63	1
PIC (DI, II(p)) – range of values	0.31-0.50	0.08-0.50	0.45-0.84
PIC (DI, Hav(p)) – mean	0.46	0.39	0.56
MI (EMR×PIC)	1.16	1.42	0.56
DDI =PIC×n_m/n_{OTU}	0.46	0.24	0.15

*for RAPD and ISSR only polymorphic products were considered

On the basis of particular marker types, 3 separate dendrograms were created. In addition a collective dendrogram was prepared using combined data (Fig. 1). Genetic similarity (*GS*) calculations gave values ranging from 0.41 to 0.82 for the common set of data, 0.29 to 0.94 for SSR, 0.37-0.93 for ISSR and 0.37-0.90 for RAPD markers.

Correlation coefficients for individual technique based on *GS* values matrixes were not significant. By comparing data obtained on the basis of individual marker systems with collective matrix, it was observed that the highest correlation coefficient was for ISSR method (r=0.68), lower for RAPD (r=0.25), and the lowest for SSR (r=0.06).

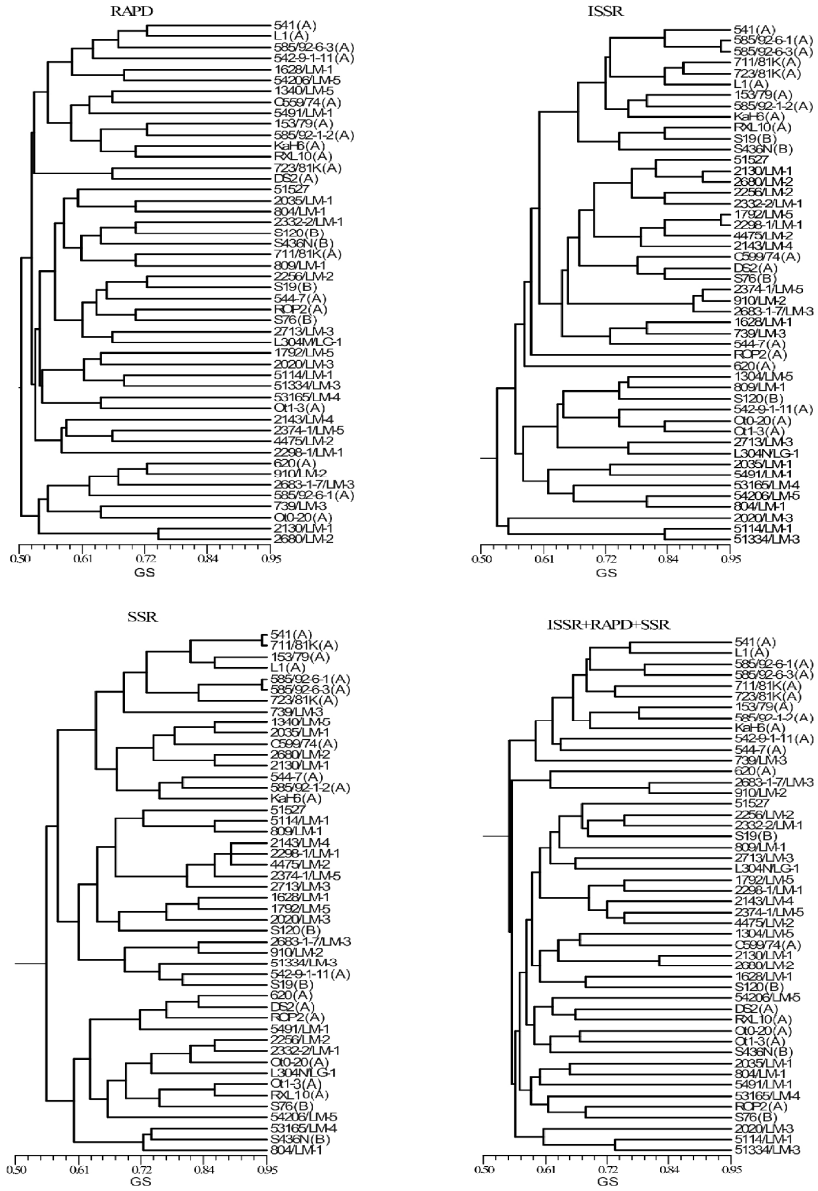

Fig. 1. Dendrograms of 48 rye inbred lines based on GS values calculated using RAPDs, ISSRs, SSRs and joined set of markers. Capital letters in brackets, following lines names, indicate their origin: (A) – from ZGHBR and (B) – from Danko Ltd. Lines from IHAR have common LM symbol in their names, except for line 51527

The most informative dendrogram is that based on the combined data of ISSR, RAPD and SSR markers (Fig.1). It consist of the two major groups of lines. The first group is formed by 11 lines originating from the collection

developed in Szczecin (A) and one line developed in IHAR (739/LM-3). The second group is formed mainly by lines from IHAR with few lines from Danko Ltd. and from Szczecin, scattered among them. The third minor group consist of three lines i.e. 2683-1-7/LM3, 910/LM-2 and 620. Clustering of lines from rye collection kept in Szczecin can be attributed to their pedigree; majority of them were derived from Kazimierskie and/or Smolickie cultivars used as components in early crosses.

The correctness of grouping may be traced also on the basis of location in one group of sublines derived from the same parental line (lines 585/92-1-2, 585/92-6-1 and 585/92-6-3 on ISSR, SSR and the combined dendrogram.

A pair of lines Ot0-20 and Ot1-3 derived from Otello cultivar was found in direct vicinity on both the ISSR and collective dendrogram and in one group on SSR dendrogram, which is the additional prove for the dendrogram correctness.

DISCUSSION

The selection of the optimal molecular system for identification and analysis of relations between plant accessions is difficult. Attempts to obtain authoritative comparison between marker systems resulted in development of certain parameters, describing properties of each technique. Marker index (*MI*) is commonly used to describe general marker ability to detect polymorphism. The highest values of *MI*, (5-10) are characteristic for AFLP markers (Powell *et al.* 1996, Archak *et al.* 2003, Varshney *et al.* 2007) and SAMPLs (Bolibok *et al.* 2005), what is connected with multiplex banding patterns developed with these methods.

Average *MI* values for SSRs are usually equal to *PIC* values and do not exceed 1.0. *MI* for SSR markers presented in this study is 0.56, which exceed the *MI* level (0.38) for SSRs found in other rye materials (Bolibok *et al.* 2005). SSRs of soybean (Powell *et al.* 1996) and barley (Varshney *et al.* 2007) represent the same *MI* levels as that found in our study. Higher *MI* values (0.73 and 0.71) were obtained respectively for potato (Milbourn *et al.* 1997) and wheat (Prasad *et al.* 2000). High *MI* values result from polyallelic character of SSR loci in these species. Average number of alleles per locus is 5.76 for potato and 1-13 for wheat. Mean number of alleles per locus in rye is 2.62 (this study) and 2.74 (Bolibok *et al.* 2005).

PIC value calculated for RAPDs analysed in other sets of inbred rye lines was 0.374 (Myśków *et al.* 2001) and 0.32 (Lebiecka 2007). *PIC* value for RAPDs used in genetic similarity analysis of potato reached the level of 0.362 (Milbourne *et al.* 1997), and that for rice bean (*Vigna umbellata*) - 0.243 (Muthusamy *et al.* 2008). *PIC* value obtained in this study (0.46) is higher than those reported earlier.

In the case of ISSR markers, *PIC*=0.39 is comparable to the result obtained

for other rye accessions – 0.35 (Lebiecka 2007) and higher than *PIC*=0.23 obtained for rice bean (Muthusamy *et al.* 2008). Marker index for ISSRs used by Bolibok *et al.* (2005) in the analysis of different set of rye inbred lines is equal to 2.32 (calculated according to adequate formula), which is higher than our result (*MI*=1.42). Extremely high *MI* value (3.15) was obtained for cashew (Archak *et al.* 2003), what confirms some authors suggestions that ISSRs are very efficient tool in detecting DNA polymorphism (Nagaoka and Ogihara 1997).

Out of all presented marker systems, ISSR seems to be the most favourable and informative, taking into account both high marker index and structure of dendrogram, comparable to that built on the basis of joined data. It was not observed by Lebiecka (2007) for the other set of inbred rye lines analysed with the same three markers techniques. Correlations between *GS* values calculated for different marker systems were very low in our study. Analogous comparison presented in other investigations showed *r*=0.113 for barley (Hou *et al.* 2005), 0.32 for *Vigna mungo* (Souframanien and Gopalakrishna 2004) and as much as 0.63 for cashew (Archak *et al.* 2003).

Correlation coefficient between *GS* values depends not only on the kind of molecular technique and analysed species but also on the range of discovered diversity. Tams *et al.* (2005) obtained high *r* values (0.71) between AFLP- and SSR-based data for triticale varieties and breeding lines. Graner *et al.* (2004) reported even higher values (r=0.87-0.93) for barley lines analysed with three different techniques. Nagaoka and Ogihara (1997) used three molecular techniques (ISSR, RAPD, RFLP) to study genetic similarities among six wheat species. Each marker system resulted in the same grouping of *OTU*s within dendrograms. High *r* values and identical topologies of dendrograms suggest that each method of molecular marker development used independently could be a reliable source of information about relationships between analysed accessions. It was shown (Matos *et al.* 2001) that phylogenetic relationships of rye were better illustrated by joining data based on ISSRs and RAPDs than by analysis of simple sets of data. Loarce *et al.* (1996) revealed the best coincidence of genetic relationship and pedigree data of eight closely related rye cultivars for common set of RFLP and RAPD markers. Visual evaluation of the four dendrograms examined here support the results of other authors.

This study points out on the importance of a sufficient number of marker loci. The calculation of the number of loci required for accurate elucidation of genetic relationship among accessions is difficult. We have proposed a *DDI* parameter which could be helpful in preliminary estimation of the necessary number of marker loci. Diversity detecting indexes in our study were: DDI_{RAPD}=0.46, DDI_{ISSR}=0.24, DDI_{SSR}=0.15 and *DDI*=0.85 for combined data. Other papers showing low correlation coefficients between markers had the following *DDI*: DDI_{RAPD}=1 and DDI_{ISSR}=1.4 for barley (Hou *et al.* 2005); DDI_{SSR}=2.82 and

DDI_{AFLP}=0.67 for 128 accessions of triticale (Tams *et al.* 2005). Since in both examples, the dendrograms had different topologies, calculated *DDI* values were too low for a proper assessment of genetic diversity. The identical trees of similarities developed on the base of different molecular markers, were reported by Nagaoka and Ogihara (1997) for six wheat species. Values of *DDI* calculated using results presented therein were approximately: DDI_{RFLP}=14.5, DDI_{RAPD}=12.5 and DDI_{ISSR}=8.3. They were apparently sufficient in the assessment of genetic diversity in the case of wheat. Similar or even higher *DDI* values (DDI_{RAPD}=17.5 and DDI_{ISSR}=6.0) were not sufficient to obtain identical dendrograms (r=0.673) of ten landraces of *Vigna umbellata* (Muthusamy *et al.* 2008). Differences between species, their genome size, ploidy level, range of genetic variation are probably additional factors affecting dendrogram construction.

Neverthless, the *DDI* index may prove helpful in planning research on phenetic relationships and may be used for estimation of approximate number of marker loci necessary for accurate determination of relationship between examined accessions.

CONCLUSIONS

The most reliable clustering of rye lines was obtained on the combined set of PCR markers, including RAPD, ISSR and SSR loci.

Among three presented marker systems, ISSR seems to be the most informative, according to both high marker index and topology of dendrogram, comparable to tree constructed on the basis of joined data.

REFERENCES

Archak S., Gaikwad A.B., Gautam D., Rao E.V.V.B., Swamy K.R.M., Karihaloo J.L., 2003. Comparative assessment of DNA fingerprinting techniques (RAPD, ISSR and AFLP) for genetic analysis of cashew (*Anacardium occidentale* L.) accessions of India. Genome, 46, 362-369.

Bolibok H., Rakoczy-Trojanowska M., Hromada A., Pietrzykowski, R., 2005. Efficiency of different PCR-based marker systems in assessing genetic diversity among winter rye (*Secale cereale* L.) inbred lines. Euphytica, 146, 109-116.

Chikmawati T., Skovmand B. Gustafson J.P., 2005. Phylogenetic relationship among *Secale* species revealed by amplified length polymorphisms. Genome, 48, 792-801.

Graner A., Dehmer K.J., Thiel T., Borner A., 2004. Plant genetic resources: benefits and implications of using molecular markers. in: M.C. de Vicente (Ed.), Issues in Genetic Resources, IPGRI, Rome, Italy, 11, 26-32.

Hou Y.C., Yan Z.H., Wei Y.M., Zheng Y.L., 2005. Genetic diversity in barley from west China. Barley Genetics Newsletter, 35, 9-22.

Krystkowiak K., Adamski T., Surma M., Kaczmarek Z., 2009. Relationship between phenotypic and genetic diversity of parental genotypes and the specific combining ability and heterosis effects in wheat (*Triticum aestivum* L.). Euphytica, 165, 419-434.

Lebiecka K., 2007. Przydatność markerów molekularnych SSR oraz ISSR do konstrukcji map genetycznych i analizy zmienności genotypów żyta. West-Pomeranian University of Technology in Szczecin, Doctoral thesis.

Loarce Y., Gallego R., Ferrer E., 1996. A comparative analysis of the genetic relationships between rye cultivars using RFLP and RAPD markers. Euphytica, 88,107-115.

Ma R., Yli-Matilla T., Pulli S. 2004. Phylogenetic relationship among genotypes of worldwide collection of

spring and winter ryes (*Secale cereale* L.) determined by RAPD-PCR markers. Hereditas, 140, 210-221.
Martos V., Royo C., Rharrabti Y, Garcia del Moral L.F. 2005. Using AFLPs to determine phylogenetic relationships and genetic erosion in durum wheat cultivars released in Italy and Spain throught the 20th century. Field Crops Research, 91, 107-116.
Matos M., Pinto-Carnide O., Benito C., 2001. Phylogenetic relationship among Portuguese rye based on isozyme, RAPD and ISSR markers. Hereditas, 134, 229-236.
Milbourne D., Meyer R., Bradshaw J.E., Baird E., Bonar N., Provan J., Powell W., Waugh R, 1997. Comparison of PCR-based marker systems for analysis of genetic relationship in cultivated potato. Mol Breed, 3, 127-136.
Milczarski P., Banek-Tabor A., Lebiecka K., Stojałowski S., Myśków B., Masojć P., 2007. A New genetic map of rye based on RAPD, ISSR and STS markers and its alignment with the reference DS2xRXL10 map. J Appl Genet, 48, 11-24
Myśków B., Masojć P., Banek-Tabor A., Szołkowski A, 2001. Genetic diversity of inbred rye lines evaluated by RAPD analysis. J Appl Genet 42, 1-14.
Nagaoka T., Ogihara Y., 1997. Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor Appl Genet, 94, 597-602.
Persson K., Von Bothmer R., 2002. Genetic diversity amongst landraces of rye (*Secale cereale* L.) from northern Europe. Hereditas, 136, 29-38.
Persson K., Diaz O., Von Bothmer R., 2001. Extent and patterns of RAPD variation in landraces and cultivars of rye (*Secale cereale* L.) from Northern Europe. Hereditas, 134, 237-243.
Powell W., Morgante M., Andre C., Hanafey M., Vogel J., Tingey S., Rafalski A., 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breed, 2, 225-238.
Prasad M., Varshney R.K., Roy J.K., Balyan H.S., Gupta P.K., 2000. The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theor Appl Genet, 100, 584-592.
Rohlf F.J., 2005. NTSYSpc: Numerical Taxonomy system, ver. 2.20. Exeter Publishing, Ltd.: Setauket, NY.
Smith J.S.C., Chin E.C.L., Shu H., Smith O.S., Wall S.J., Senior M.L., Mitchell S.E., Kresovich S., Ziegle J., 1997. An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparison with data from RFLPS and pedigree. Theor Appl Genet, 95, 163-173
Souframanien J., Gopalakrishna T., 2004. A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. Theor Appl Genet, 109, 1687-1693
Stojałowski S., Milczarski P., Masojć P., 2004. Usefulness of ISSR markers for identification of inbred lines and mapping of rye genome. Biul. IHAR, 231, 237-246.
Tams S.H., Melchinger A.E., Bauer E., 2005. Genetic similarity among European winter triticale elite germplasm assessed with AFLP and comparison with SSR and pedigree data. Plant Breeding, 124, 154-160.
Varshney R.K., Chabane K., Hendre P.S., Aggarwal R.K., Graner A., 2007. Comparative assessment of EST-SSR, EST-SNP and AFLP markers for evaluation of genetic diversity and conservation of genetic resources using wild, cultivated and elite barleys. Plant Science, 173, 638-649.