Genetic Relationships Among Representatives of *Dasypyrum*, *Secale* and *Triticum* Species Revealed with RAPD and ISSR Markers

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Abstract

In this study the genetic similarity among *Dasypyrum*, *Secale* and *Triticum* species with RAPDs and ISSRs was analyzed. To show a level of similarity between the species, 12 populations of *Dasypyrum* (11 *D. villosum* and 1 *D. breviaristatum*), together with 12 accessions belonging to 3 *Secale* species and 12 accessions from 4 *Triticum* species were used. Genetic distances (GD) and bootstrap values were calculated and PCA analysis was conducted to present the relationships among the species. To estimate the genetic structure among and inside genera, as well as population differentiation, gene diversity (He), total genetic variation (Ht) and Wright’s fixation index (Fst) were computed. The highest values were found in *Triticum*, within which Ht was equal to 0.332±0.023 and Fst was 0.42. It confirmed that the material studied was highly differentiated. Both systems found *Dasypyrum* more related with *Triticum*, as compared to *Secale*. With RAPDs, genetic distance (GD) between *Triticum* and *Dasypyrum* was 0.435, respectively 0.460 for *Secale vs Dasypyrum*. In the case of ISSRs these values were 0.374 and 0.407, respectively. Despite the fact that the difference between the two GD indices was insignificant, one should not exclude the possibility of successful hybridization of *Dasypyrum* and *Secale*, especially when using bridge species.

Keywords: diversity, molecular markers, similarity, *Triticaceae*

Introduction

*Dasypyrum*, *Triticum* and *Secale* are three species from Triticeae tribe. The first taxon includes only two wild, allogamous species: *Dasypyrum villosum* L. ‘Candargy’ (diploid, haplome V) (Db) and *D. breviaristatum* (Lindb. f.) ‘Frederiksen’ (diploid and autotetraploid, haplome V³) (Db) (Baum et al., 2014; De Pace et al., 2011; Gradzielewska, 2006a). The other two genera include both wild and cultivated species.

*Dasypyrum* species, especially *Dasypyrum villosum*, are commonly known as relatives of bread wheat (*Triticum aestivum* L.) and they are widely used as donors due to their favorable traits to wheat (De Pace et al., 1990). *Dasypyrum villosum* (2n=2x=14, VV) possess many genes used in wheat breeding to improve the resistance; for example, resistance to powdery mildew, leaf and stem rusts, take-all, cereal eyespot, wheat streak mosaic virus (WSMV) and its vector wheat curl mite (De Pace et al., 2011; Gradzielewska, 2006b; Hyde, 1953; Liu et al., 1988; Murray et al., 1994; Yildirim et al., 1998; Zhang et al., 2005). Moreover, other desirable traits of *D. villosum* include salt and drought tolerance, winter hardiness and high protein content (Chen and Liu, 1982; Della Gatta et al., 1984; De Pace et al., 2001; Liu et al., 1988). Until now, a lot of hybrids and amphiploids between *D. villosum* and *Triticum* species, substitution and translocation lines, have been developed successfully using resistance genes *Pm21* (powdery mildew), *YrV3* (yellow rust) and *Wss1* (wheat spindle streak mosaic virus) which have been transferred into wheat cultivars (Blanco et al., 1987; Chen et al., 1995, 2002; De Pace et al., 2011; Hou et al., 2013; Liu et al., 1988; Minelli et al., 2005; Zhang et al., 2005).

*Secale cereale* L. (rye) is considered an important crop in the colder parts of Northern and Eastern Europe and Russia. It is a diploid (2n=2x=14) cross-pollinated, annual cereal with an effective gametophytic self-incompatibility system (Geiger and Miedaner, 2009; Haffke et al., 2014). Rye is also considered as a hardy crop, which can grow in sandy soils of low fertility and is more tolerant to drought, cold and other adverse growing conditions than other cereal crops. Although there are two rye biotypes (spring and winter), most of the world supply is obtained from winter varieties (Haffke et al., 2014). Among the rye cultivation
areas. Germany, Poland, Russia and Belarus have a significant contribution to rye production (FAOSTAT, 2013).

For a long time rye was found to be resistant to cereal diseases. Nevertheless, it has been increasingly infected by pathogens in recent times. There are several diseases which infect rye such as leaf and stem rust (Puccinia recondita, P. graminis f. sp. secalis), ergot (Claviceps purpurea), Fusarium diseases, leaf blotch (Rhynchosporium secalis), pink snow mould (Microdochium nivale) or soil-borne viruses (Geiger and Miedaner, 2009; Pociucha et al., 2013). Due to this fact there is a need to find new resistance donors for rye, which will be used in breeding programs. A good resistance source would be Dasypyrum villosum. Both species, Dasypyrum villosum and Secale cereal, are diploids with the same chromosome number in their genome, annual and cross-pollinated cereals. Therefore, it might be possible to transfer homologous genes between these two species, as it happened in the case of wheat, although interspecific hybridization reveals very little homology between wheat and D. villosum genomes (Hyde, 1953).

Recently, introduction of alien genetic variability to rye seems to be necessary, because diseases attack augmentation, especially rusts and powdery mildew. Hence looking for germplasm donors inside closely related species from Triticaceae seems to be essential. Dasypyrum villosum seems to be a valuable source of germplasm for rye, particularly as these species have been shown to be related, in some findings even more than Dasypyrum with Triticum (Baum, 1977, 1978a; b, 1983; De Pace et al., 2011; Linde-Laursen et al., 1992; Lucas and Jahier, 1988; Uslu et al., 1999; Vershinin and Heslop-Harrison, 1998). To conduct hybridization and gene transfer between species, information about their genetic similarity and relationship is often essential. In the light of many studies, the taxonomic position of Dasypyrum vis-à-vis Triticum and Secale is not clear. Many of the morphological, biochemical and cytological, as well as some molecular analyses, suggest a closer relationship of Dasypyrum with Secale (Baum, 1983; Lucas and Jahier, 1988; Uslu et al., 1999; Vershinin and Heslop-Harrison, 1998). On the other hand, Dasypyrum has been shown to be closely related to wheat as regards the level of the prolamin storage proteins (Montebove et al., 1987; Shewry et al., 1987), isozymes (Liu et al., 1995; Montebove et al., 1987) and RFLP analyses (Qi et al., 1998, 1999).

Molecular markers are useful in taxonomical troubleshoot, phylogenetic studies and evaluation of genetic diversity. From many techniques applied, random-type markers like RAPD and ISSR are still successfully used for these goals in plants (Bishoyi et al., 2014; Linos et al., 2014; Mucciarelli et al., 2014).

Random Amplified Polymorphic DNA (RAPD) is a technique using short (8-12 base pairs) arbitrary primers to amplify random segments of DNA along the whole genome. This method has been shown to have some disadvantages, like sensitivity to the changes of reaction conditions and dominant inheritance of markers. Nevertheless, it has a low cost, requires small amounts of DNA and does not need prior knowledge of the genome sequences. The data received in a short time and number of markers obtained is sufficient to discriminate species and genera. The source of RAPD polymorphism are mutations in sites complementary to the 3' end of primer, as well as deletions or insertions between primer binding sites and repetitive sequences (Kojima et al., 1998; Lynch and Milligan, 1994; MacPherson et al., 1993).

ISSR is also a simple technique using single arbitrary primers. However, reproducibility and informativeness of ISSRs are higher than in the case of RAPD. Moreover, ISSRs are inherited as dominant and rarely as codominant genetic markers. In this method, polymorphisms results from the differences in the length between inversely oriented and closely spaced microsatellites (Reddy et al., 2002; Ziętkiewicz et al., 1994). In rye (Bolibok et al., 2005; Matos et al., 2001) and in wheat (Nagaoka and Oghara, 1997) ISSRs have been applied for polymorphism detection.

The aim of this study is to present relations between an inside Dasypyrum, Secale and Triticum species on the genetic level with ISSR and RAPD markers. New findings on similarity of the wild Dasypyrum accessions to wheat and rye may shed light on genetic divergence and indicate further directions in intergeneric transfer.

Material and methods

Plant material and DNA extractions

12 populations of Dasypyrum, twelve species and subspecies of Secale and Triticum were analyzed (Tab.1). All genotypes were kindly supplied by Dr Harold Bockelman, National Small Grains Collection, U.S. Department of Agriculture, Agriculture Research Service, Aberdeen, Idaho, USA. Total genomic DNA was extracted from 15-30 coleoptiles of several days-old seedlings following the CTAB procedure (Doyle and Doyle, 1987).

RAPD and ISSR amplification

Amplification was performed according to RAPD method described by Williams et al. (1990) and the ISSR method described by Ziętkiewicz et al. (1994) with modifications. Analyses were conducted in a T1 Biometra thermal cycler. 15 µl of RAPD mixture contained: 1 × PCR Buffer (10 mM Tris pH 8.8, 50 mM KCl, 0.08% Nonidet P40), 160 µM of each dNTP, 5.3 pM of primer, 1.2 mM MgCl₂, 0.5 U Taq DNA Polymerase and 60 ng of template. 14 RAPD primers listed in Tab. 2 were used in amplification.

The applied program of thermal cycling was: initial denaturation 95 °C for 2 minutes and next 45 cycles: denaturation 94 °C for 45 s, annealing 37 °C for 45 s, extension 72 °C for 45 s and last cycle was followed by incubation at 72 °C for 10 minutes. Amplification was conducted in two repeats for each genotype.

ISSR analyses were conducted with 17 primers (Tab. 2). The reaction was run in 15 µl mixture, which contained: 1 × PCR Buffer (75 mM Tris pH 8.8, 20 mM (NH₄)₂SO₄, 0.01% Tween 20), 160 µM of each dNTP, 4.7 pM of primer, 1.5 mM MgCl₂, 0.4 mM of spermidine, 0.5 U Taq DNA Polymerase and 60 ng of template. Amplification was carried out in a T1 Biometra thermal cycler with the
program of thermal cycling as follows: initial denaturation 95 °C for 7 min, 38 cycles with denaturation step at 95 °C for 30 s, different annealing temperatures (the first three cycles 54 °C – 45 s, the next three cycles 53 °C – 45 s and 32 cycles 52 °C for 45 s) and extension 72 °C – 2 min for each step. The last cycle was followed by incubation at 72 °C for 7 minutes. Amplification was conducted in two repeats for each genotype.

RAPDs and ISSR products were separated on agarose gels, respectively in 1.5% and 2.5%, which contained 0.1% ethidium bromide in 1 x TBE buffer (89 mM Tris-borate, 2.5 mM EDTA). Separation was carried out for 1.5 h at 120 V. DNA marker GeneRulerTM 100 bp Plus DNA Ladder was used as a size ruler.

Data analysis

The amplified RAPD and ISSR fragments were scored as absent (0) or present (1), then formed into a binary matrix. Results of RAPD and ISSR allowed to calculate polymorphism information, which include PIC (polymorphism information content) according to Rick De et al. (2001) and Assay Efficiency Index (AEI, mean number of polymorphic fragments) (Pejic et al., 1998). Genetic Distance (GD) indexes between all the analyzed genotypes were calculated using Nei’s formula (Nei, 1972). GD and bootstrap values were calculated for the relationships between the species of Secale, Dasypyrum and Triticum using PHYLIP (Felsenstein, 1989) and visualised as dendrograms constructed using Treeview software (Page, 2004).
Results and discussion

In this study the genetic similarity among *Dasypyrum*, *Secale* and *Triticum* species with RAPDs and ISSRs was estimated. To show the level of *Dasypyrum* similarity vs. *Secale* and *Triticum*, 12 populations of *Dasypyrum* (11 *D. villosum* and 1 *D. breviaristatum*), together with 12 accessions belonging to 3 *Secale* species and 12 accessions from 4 *Triticum* species were analyzed (Tab. 1). 14 RAPD and 17 ISSR primers (Tab. 2) showing high level of polymorphism and reproducibility were selected, respectively from 80 and 60 previously screened.

As expected, ISSRs were more informative than RAPDs. In *Dasypyrum* 170 RAPD bands (from a total of 287) were amplified and only 4 fragments were monomorphic (polymorphic ones comprised 97.65%, AEIP11.9). Among *Secale* total RAPD bands were scored, out of them 130 were polymorphic (96.04%) and in the case of ISSRs from a total of 423 bands, 417 were polymorphic (98.58%). AEI were 12.14 for RAPD and 24.53 for ISSR. Summing up, ISSRs amplified more polymorphic bands, as was also obtained by other researchers (Kojma et al., 1998; Matos et al., 2001), but oddly, the percentage of polymorphic bands detected with both methods was nearly on the same level inside the tribes of *Secale* and *Triticum*.

1996) by UPGMA (unweighted pair-group method with arithmetic averages). To estimate the genetic structure among and inside the tribe, gene diversity - He (Lynch and Milligan, 1994) was calculated with AFLPsurv (Vekemans et al., 2002). Variation between species within genus (Hw) and variation between genera (Hb) were established as components of total genetic variation (Ht). Wright's fixation index (Fst), which is a measure of population differentiation and genetic distance was computed based on genetic polymorphism data scored by measuring the genetic fixation among as opposed to within populations, Lynch and Milligan, 1994). PCoA based on matrix of Pearson correlation coefficients was performed in XLStat v.7.5.2.

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High informativeness of the chosen primers was also confirmed by the PIC index, which is the same as diversity index -DI (Botstein et al., 1980). PIC reflects informativeness of marker system employed and shows the distribution of identified polymorphisms. For dominant markers its values range from 0 (monomorphic) to 0.5 (highly discriminative) (Rick De et al., 2001). PIC has been widely used to compare diallelic and multiallelic molecular markers (Powell et al., 1996). In this study, the mean PIC indices of RAPD for the studied species were highly similar (Tab. 2), with values between 0.31±0.37 (Tab. 2). Powell et al. (1996) hypothesised that the numerical value of PIC index changes with the species analyzed, but the relative level of marker systems remains constant if the mechanisms generating DNA polymorphisms are similar across the species. The high PIC values found in the presented experiment indicates a high level of genetic diversity existing in the three species studied. Similarly, high values of PICs in the case of ISSR markers were found in rye (Bolibok et al., 2005). In rice, Sarla et al. (2005) also obtained high PICs for ISSR, as they examined highly diverse material containing varieties, landraces, ancestral landraces and wild accessions. High PICs were also detected for RAPD, for example in an orchid Dendrobium nobile Lindl. (Bhattacharyya and Kumaria, 2014) or an Indian medicinal plant Solanum trilobatum L. (Shilpha et al., 2013).

Tab. 3. Distribution of the genetic variation among and within Dasypyrum, Secale and Triticum (Lynch and Milligan, 1994)

| Genus            | Polymorphic loci | He ± S.E | Nei’s genetic distance |
|------------------|------------------|----------|------------------------|
|                  | Number           | Proportion [%] | Dasypyrum | Secale |
| Dasypyrum        | 554              | 61.1     | 0.207±0.007            | –       |
| Secale           | 377              | 41.6     | 0.146±0.006            | 0.228   |
| Triticum         | 523              | 57.7     | 0.223±0.007            | 0.150   | 0.188 |

Tab. 4. Number of specific RAPD and ISSR markers identified

| Accession        | Genome       | RAPD | ISSR |
|------------------|--------------|------|------|
| D. villosum      | V            | 0    | 3    |
| D. villosum + Secale | V+Vb+R     | 0    | 3    |
| D. villosum + Secale | V+R        | 0    | 1    |
| T. timopeevii    | AG           | 3    | 4    |
| T. monococcum    | Am           | 6    | 4    |
| T. turgidum      | AB           | 0    | 3    |
| T. aestivum      | ABD          | 4    | 3    |
| T. monococcum + T. turgidum | Am, AB | 0    | 1    |
| T. timopeevii + T. turgidum | AG, AB | 2    | 0    |
| T. aestivum + T. turgidum | ABD, AB | 2    | 1    |
| Total            | 47            | 122   |
Fig. 1. Cluster analysis dendrogram estimated among the *Dasypyrum*, *Secale* and *Triticum* species based on RAPD and ISSR markers using the unweighted pair group method of arithmetic average (UPGMA) and Nei’s distances. Bootstrap values are given for nodes.
In this experiment some fragments specific for genus, genome, species or genotype were found (Tab. 4). Many of these fragments were amplified with ISSR. Only three ISSR bands and none of RAPD were characteristic for Dasypyrum genus, while 3 other were shared with Secale. One RAPD and 4 ISSR markers of V genom of Dv were identified. For respective species of Secale, six RAPD fragments and 10 ISSRs were specific. The largest number of 20 RAPD and 16 of ISSR specific bands was identified for Triticum species. A significant number of bands, many more of ISSRs, specific to one of the analyzed accessions, were detected (Tab. 4). Some of these markers could be converted to SCARs suitable to trace introgression events, identification of genus, species or accessions.

The genetic relationships among Dasypyrum, Secale and Triticum were pictured as dendrograms constructed based on ISSRs and RAPDs (Fig. 1). On both dendrograms three genera formed 3 main clusters. Inside Dasypyrum cluster, Db was an outgroup, and accessions of Dv were clustered together. Turkish Dv accessions were demonstrated to be differential as they clustered into three different subclusters. One of them formed a group with accessions from USSR and Italy, two other on RAPD based dendrogram and one based on ISSR polymorphism clustered with one accession from Greece, and three formed their own subclaster (Fig. 1).

Cluster analysis gives no clear answer as to the position of Triticum species vs. Secale and Dasypyrum. Results of RAPD analysis suggest closer relation between Triticum and Dasypyrum, which is further not confirmed by ISSR analysis where Secale and Triticum are better clustered (Fig. 2). This problem may be further resolved with PCA analysis (Fig. 2). Distribution of genotypes on the first two principal components accounting for 37.3% of overall genetic variation indicates that Triticum is located between Dasypyrum and Secale. Moreover, the accession of D. breviaristatum is grouped with Triticum. This finally supports that Dasypyrum is more closely related to wheat when compared with rye. Additionally, taking into account genetic distances, gene transfer from Dasypyrum to rye may be more difficult than to wheat. These observations are further supported by values on Nei’s genetic distance, which is the lowest for combination of Triticum and Dasypyrum (0.150) and the highest for Secale vs Dasypyrum (0.228).

The high level of polymorphism found inside Dasypyrum and topography of Dasypyrum clade was expected, as the situation within the genus is yet to be untangled. The V genome of D. breviaristatum was repeatedly shown to be highly different from V of diploid D. villosum, due to deficiency of chromosomes pairing, their different morphology and composition (Blanco et al., 1996; Linde-Laursen and Frederiksen, 1991; Pignone et al., 2000; Sakamoto, 1986; Uslu et al., 1999; Vershinin et al., 1996). Moreover, Uslu et al. (1999) suggested even higher distance of Db to Dv than of the latter to Th. bessarabicum and S. cereale. Yang et al. (2006) also showed by RAPD a closer relationship of Db with Th. intermedium and T. aestivum than with Dv. The results presented here with RAPDs as well as with ISSRs showed that in cluster analysis Db constitute an outgroup, but belonging to Dasypyrum (Fig. 1). Similarly, Fan et al. (2013), basing on molecular evolution of a single-copy gene encoding plastid acetyl-CoA carboxylase (Acl1), showed Dv in the same clade as Db; however, Dv was placed closer to one of Elymus repens accessions than to Db. On the other hand, Baum et al. (2014) through analyses of 5S nrRNA gene sequences found that the Db 4x cytotype is an allotetraploid containing haploids of Dv and diploid cytotype of Dv (VvVv). Close relationship between Dv and Db was also supported earlier with Southern-blot with repeated genome sequences and plastid DNA probes, RFLPs, isosymes and in situ with gladin (Blanco et al., 1996), rDNA and repetitive probes (Galasso et al., 1997). However, the results of PCA analysis confirm previous reports that Db may be grouped together with Triticum and Nei’s distances to Secale, Triticum and Dasypyrum villosum are respectively 0.424, 0.458 and 0.344 for RAPDs and 0.399, 0.379 and 0.343 for ISSRs. In Triticeae a high level of synteny exists at the genes or single-copy markers, but in repetitive DNA the significant differences, crucial for evolution and speciation, was found (Galasso et al., 1997). These explain similarities as well as differences found among species and genera.

Inside Triticum clade the phylogenetic relationships showed identical arrangement on both dendrograms. The accessions studied were separated into species according to present classification and genomic composition. The first outgroup consisted of three diploid species of T. monococcum (Tab. 1, 29P31). Next, five tetraploid wheat species clustered together and a group consisting of T. aestivum hexaploid was separated. These results do not reveal new taxonomic relationships, but may serve as a good background for establishing relationships with more distinctly related taxa, such as Dasypyrum and Secale.

The evolutionary events in Secale showed on dendrograms were generally the same as those presented earlier (Bolibok-Brągowska et al., 2014; Couadrado and Jouve, 2002; De Bustos and Jouve, 2002). In the case of Secale the RAPD and
ISSR clades were very similar, with two exceptions (Fig. 1). On the RAPD dendrogram one accession of \textit{S. cereale} ssp. \textit{cereale} was not grouped with the two other, but in the case of ISSRs, all three accessions were put together. On the other hand, \textit{S. cereale} ssp. \textit{segetale} were clustered with other \textit{Secale} subspecies with RAPDs, but based on ISSRs they were shown to be closer to \textit{S. vavilovii}. The first was in agreement with Bolbok-Bragoszewska \textit{et al}. (2014) and the last with the dendrogram of Chikamawtai \textit{et al}. (2005) constructed basing on AFLPs.

The relationships of \textit{Triticum}, \textit{Secale} and \textit{Dasypyrum} were estimated several times. Generally, it can be noticed that morphologically \textit{Dasypyrum} resembles \textit{Secale} species and was put as a sister group of \textit{Secale} within the same clade (Baum, 1978a, 1983; Frederiksen and Seberg, 1992; Kellogg, 1989; Seberg and Frederiksen, 2001). Similar relationships were found on the level of non-coding sequences as in heterochromatic regions (Linde-Laursen \textit{et al}. 1992; Vershinin and Haslop-Harrison, 1998) and species specific repetitive sequences- pHs62 and pSc119.2 (Schubert \textit{et al}. 1990; Uslu \textit{et al}. 1999). On the other hand, at the level of coding sequences as storage proteins (Montebove \textit{et al}. 1987; Shewry \textit{et al}. 1987) and isozymes (Liu \textit{et al}. 1995; Montebove \textit{et al}. 1987), \textit{Dasypyrum} was grouped with \textit{Triticum}.

In the presented paper, the phylogenetic relationships among the three species were shown to be slightly different, as was visualised on the dendrograms. RAPDs assembled \textit{Triticum} and \textit{Dasypyrum} together, and \textit{Nei}'s average distance between these the two genera (0.435) was lower for \textit{Triticum} vs \textit{Secale} (0.447) and \textit{Secale} vs \textit{Dasypyrum} (0.461). ISSRs put \textit{Triticum} together with \textit{Secale}, indicating them to be more related (0.370) than each of them with \textit{Dasypyrum} (0.374 and 0.407, respectively). Both marker systems analyzed together showed \textit{Dasypyrum} to be more similar to \textit{Triticum} than to \textit{Secale}, as was calculated with GD indices (Tab. 3). Nevertheless, differential between the two GD indices was slight (0.078), so it could not exclude the possibility of successful hybridization among \textit{Dasypyrum} and \textit{Secale}, especially using bridge species, which was conducted earlier. The first fertile hybrid of \textit{Secale} × \textit{Dv} (\textit{S. fragile} × \textit{Dv}) was produced by Sando (1935). Kostoff (1937) obtained trigenic hybrid with \textit{T. dicoccum} as a bridge species. The hybrid of \textit{S. cereale} with \textit{Dv} reported by Nakajima (1951), had more success in crossing \textit{Secale} with \textit{Dasypyrum} by using tetraploid wheats as bridge species (Nakajima, 1969, 1970). Other trigenic hybrids were produced by Jiang \textit{et al}. (1989), Fu \textit{et al}. (1997) and Knobloch (1968) and trigeneric ones by Jahier \textit{et al}. (1988) and Yuan \textit{et al}. (1997). Tetraploid amphidiploid \textit{S. cereale} × \textit{H. villosa} was produced by Łapiński and Gruszecza (1997). As was presented, hybridization of \textit{Secale} and \textit{Dasypyrum} was performed only several times, but little is known about useful \textit{Dv} genes' influence on hybrids.

Conclusions

The current study demonstrates usefulness of RAPD and ISSR markers for estimation of genetic relationships among \textit{Triticaceae}, between representatives of three analyzed species: \textit{Dasypyrum}, \textit{Secale} and \textit{Triticum}. The findings showed that the choice of random markers techniques is still appropriate and attractive due to a low cost of analysis and quick acquisition of data. The results obtained in the study enrich the earlier data achieved by morphological, storage, proteins, isozymes and cytological analyses. Polymorphisms resulting from mutations in sites complementary to the 3’ end of primer and from deletions or insertions between primer binding sites and repetitive sequences (RAPD), just as those found in the length between inversely oriented and closely spaced microsatellites (ISSR) showed \textit{Dasypyrum} to be more related to \textit{Triticum} species, comparing to \textit{Secale}. Nevertheless, the slight differential between the two GD indices could not exclude the potential use of \textit{Dasypyrum} germplasm to enrich a genetic pool of rye, which is a very important bread crop in north-eastern Europe and Russia.

Acknowledgments

We would like to thank Prof. Grzegorz Maziarczyk (Catholic University of Lublin, Lublin, Poland) for taking his time to read and comment on the language of the text.

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