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Habitat-related specifity of iPBS fingerprint in European populations of *Hedera helix* L.

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Abstract

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Inter primer binding site (iPBS) polymorphism was investigated for common ivy (*Hedera helix*, L.) to obtain the knowledge on genetic diversity in this species. Actually, a very limited information exists about application of DNA markers in *Hedera helix*. Natural and planted European populations of ivy were analysed using an iPBS marker 5´ACCTGGCGTGCCA3´ with a total number of 238 fragments generated. Of these, 86% were polymorphic. There were determined certain attributes of this marker such as the diversity index (DI) and polymorphism information content (PIC). The value of the diversity index was 0.79 and the polymorphic information index was 0.78. The proportion of polymorphisms of the individual amplified loci ranged from 0.32% to 6.98%. Cluster analysis was performed to determine the relationships among the European ivy populations where the distribution in the dendrogram under the habitat specifity was found for the used iPBS marker. We concluded that iPBS was very efficient in analysing the genetic diversity in *Hedera helix*, L. and that this marker can serve as a suitable tool to find genomically specific fingerprints relevant to the factors influencing the distribution of genetic variation.

Keywords

genetic diversity, *Hedera helix*, L., iPBS, population

Introduction

Genetic variability description specifies differencies among individuals or populations of the same species and serves as a very good tool for plant breeding and conservation programmes (Minn et al., 2015). Different types of DNA markers have been applied in evaluation of genetic diversity of different plants, considering also the effects of the plant growing environment and developmental stage (Nadeem et al., 2018). DNA markers are genes or intergenic sequences used to define and interpret the sequentional genetic variability in organisms (Govindaraj et al., 2015; Hasanova et al., 2017). Some of them are suitable for use without any background information about the species sequences, such as ivy. These are mainly RAPD, ISSR, AFLP or iPBS markers. All of them have already been

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applied successfuly in the genetic polymorphism analysis of genomically not appropriately described plant species (Mehmood et al. 2013; Alp and Gebologl; 2017). Inter primer binding sites markers (iPBS) were firstly defined by Kalendar et al. (2010). This method defines the length polymorphism among the individul insertions of retrotransposons when using the reverse transcriptase primer binding the sites as markers. Retrotransposons are well known as markers revealing insertion fingerprints based on different biotic or abiotic stress history of plant genomes (Kalendar et al., 2010). Up to date, this marker technique has been proved to be efficient in DNA polymorphism analysis of genomically poorly characterized plant species. Duan et al. (2015) used iPBS primers to analyse genetic diversity among ten wild and fifty-five cultivated varieties of peonies. Mehmood et al. (2013) used six iPBS primers in studies of genetic diversity of *Psidium guajava* Linn. Xu et al. (2018) studied genetic diversity in twenty-five collected genotypes of *Tetradium ruticarpum*. Alp and Gebologl (2017) examined the genetic variability of twelve coriander genotypes using 16 iPBS and 8 SSR primers. Borna et al. (2017) studied the applicability of iPBS markers to assess the molecular variation and genetic relationships between 89 genotypes of *Leonurus cardiaca* L. All of these studies have confirmed this marker system as applicable and efficient in genetic variability analyses and in population studies.

Common ivy (*Hedera helix*, L.) is one of the plant species documented with only a very limited information about its genomic variability assessed by DNA markers. Up to date, only a few data are available for markers based on the internal transcribed spacers (ITS), randomly amplified polymorphic DNA (RAPD) and polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP). Specific variable sites and polymorphism genetared by ITS in ivy were determined by Vargas et al. (1999) with regard to A/C substitution. Chloroplast, microsatellites and *trnK* PCR-RFLP analyses combined with haplotype sequencing define a total of 13 groups with natural occurence in ivy (Grivet and Petite, 2002). Haplotype analysis for characterization of the *Hedera* genus was later used also by some other authors (Ackerfield and Wen, 2003; Valcárcel et al., 2003; Green et al., 2011, 2013). Invasive populations of *Hedera* were mapped in Pacific Northwest native forests with using RAPD markers (Clarke et al., 2006). PBA markers were applied in analysis of European population of common ivy by Bošeľová and Žiarovská (2016). A poor knowledge of ivy's genome could be changed, having in mind the potential of DNA markers (Kumar et al., 2014). *Hedera helix*, L. is a multipurpose plant with possible many applications in future. Nowadays, about 500 different cultures of ivy are cultivated. Normaly, ivy is popular as a decorative plant and has many available cultivars including non-climbing cultivars used to cover the soil and to fix compact forms in plots. Owing to the evergreen and shade-loving qualities, the ivy is ideal for conservatories and can create attractive packaging for garden structures. Besides of the ornamental applications, ivy is a very promising plant for medicinal use (Lutsenko et al. 2010; Hooshyar et al., 2014), for plant protection (Pârvu et al., 2015) and technological use (nanoparticles) in the future (Lenanghan et al., 2013). Starting a breeding and selection of ivy, a variability and genomic characterization of natural variability of its population is a strategic knowledge as this species has been recognised as an example of a genomic plasticity occurring during the typical developmental changes from the juvenile to the adult phase (Obermayer, 2000).

In the present paper, we used the iPBS technique to characterize the length polymorphism of DNA in European populations of ivy and to determine the relationships among these populations.

Materials and methods

Materials

In the present investigation, twenty five populations of common ivy (*Hedera helix*, L.) were sampled across its natural geographic range in seven coutries. Healthy 2-year old ivy leaves were obtained from *in situ* habitats in Slovakia (5 populations, SVK 1-5), the Czech Republic (7 populations, CZE 11-18), Poland (POL 25), Croatia (6 populations, HRV 19-24), Scotland (2 populations, GBR 6-7), Germany (GER 8) and Austria (AUT 9-10). For each population, a total of ten leaves were sampled randomly from each of 5 random selected shrubs. Ethanol treatment was immediatelly performed in delivered samples for the purpose of surface sterilization. The delivered samples were treated immediately with ethanol – to ensure their surface sterilisation.

Molecular genotyping and data analysis

The iPBS primer 2270 (5′ACCTGGCGTGCCA3′) was used for the fingerprints amplification. Using a primer specific for a group type of retrotransposon primer binding site increases its specifity by reducing the number of amplicons to one class of retrotransposons as reported by Kalendar et al. (2010). Genomic DNA was extracted from 100 mg of frozen leaf tissue, using a GeneJet Plant Genomic DNA purification Kit (ThermoScientific) according to the instruction of the manufacturer. The following thermal and time profiles were used for the DreamTaq 2x MasterMix (ThermoScientific): 95 °C – 5 min; 45 cycles of: 95 °C – 1 minute; 55 °C – 2 minutes; 72 °C – 3 minutes with final 72 °C – 10 minutes. The description of the working regimens is not clear. Amplified fragments were analysed in 4% PAGE and scored for the binary matrices – presence of band was denoted as 1, its absence as 0. UPGMA analysis and dendrogram construction was performed in SYNTAX software using a Jaccard coefficient of similarity to define a relationships between different the iPBS profiles of the analysed populations.

Results and discussion

Lenght polymorphism variability was inspected among European populations of *Hedera helix*, L., using a PBS marker 2270. This is based on the results of previous screening analysis for different marker systems and their applicability to ivy polymorphism studies (Bošeľová et al., 2016). Evaluation of the obtained iPBS fingerprints was performed with a GelAnalyzer software. The total number of the obtained fragments was 238 distributed into 22 levels. The average number of fragments per population was 10.81. The fragment size ranged from 2,341 bp for the CZE population up to the 187 bp in some other populations (CZE, HRV, POL). The highest number of the obtained fragments per one population were 14 fragments (HRV-19). The lowest number – 4 fragments, were obtained in an AUT-10 population. There was no unique fragment generated by the used iPBS marker. The percentage of polymorphism reached 86%. The most similar iPBS fingerprints profile were shared by the populations SVK-5 and GBR-7 with the length of fragments shown in Table 1 and electrophoretic profiles evaluated by the GelAnalyzer software shown in Fig. 1.

Table 1. Characteristics of obtained fragment length in populations GBR and SVK

Accession	Length of amplified iPBS fragments in base pairs
GBR-7	1123, 923, 713, 328, 310, 287, 236
SVK-5	2049, 1112, 1012, 875, 685, 750, 324, 288, 240, 236

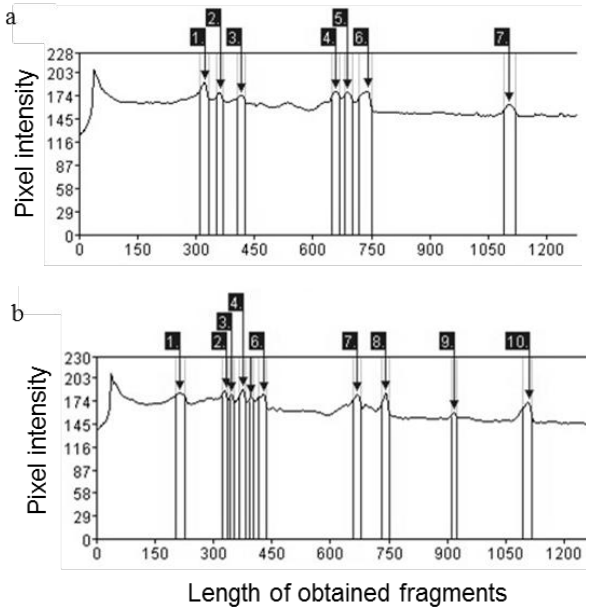

Fig. 1. Analysis of obtained fragments length for populations SVK-5 (A) and GBR-7 (B) evaluated with software GelAnalyzer.

The number of fragments obtained in the SVK-5 population was 10 and in the GBR-7 it was 8, but based on the GelAnalyzer software evaluation, there were differences in the total length of the amplified fragments. The number of the synthesized fragments obtained with the 2270 marker is shown in Fig. 2. The highest number of fragments was amplified in the populations HRV-19, HRV-22, CZE-18 and POL-25. The graph also clearly demonstrates that the least fragments were obtained in the samples AUT-10 and CZE-15. The same numbers of fragments were obtained in five groups of populations: the first group comprising SVK-4, AUT-9, CZE-12, CZE-13, CZE-14, HRV-20 and HRV-24; the second SVK-1, GBR-7, DEU-8 and CZE-16; the third SVK-2, SVK-3, GBR-6 and HRV-21; the fourth CZE-17 and HRV-23 and the last group comprising the populations with the same number of amplified fragments i.e. those of CZE-18 and POL-25.

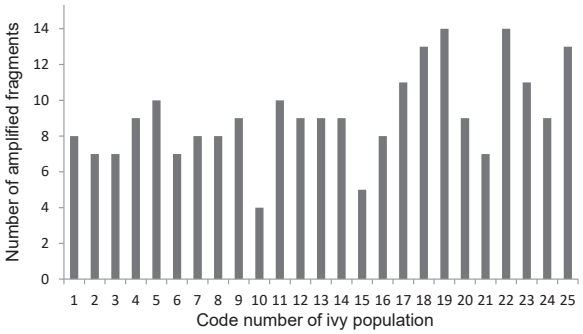

Fig. 2. Number of amplified iPBS loci in ivy populations revealed by iPBS marker 2270 used.

Based on the Jaccard coefficient of similarity, a dendrogram was constructed with the aid of the SYNTAX software (Fig. 3, Table 2). Using this marker, four clusters were generated after the populations from different countries were mixed and grouped together, but the habitat type is clearly visible in a grouping manner. This factor is present in the division between the clusters 1, 2 and 3 background grouping the populations from the urban environment, as well as in cluster 4 with all populations collected on borders of national parks.

Dendrogram clustering was done on the UPGMA levels, ranging from 0.083 to 0.585, corresponding to the obtained iPBS fingerprints profiles (Fig. 4). In the 1st cluster, the populations SVK-3 (High Tatras Mountains), CZE-11 and CZE-12 (Bohemian Karst) along with the population POL-25 (Ojców National Park) are present with the values of Jaccard coefficient of similarity ranging from 0.167 to 0.333. The second cluster included populations SVK-5, GBR-7 and DEU-8 with the values of Jaccard coefficient of similarity ranging from 0.083 to 0.167. The third cluster contained populations GBR-6, CZE-15 and CZE-16 with the values of Jaccard coefficient of similarity ranging from 0.333 to 0.585. The fourth cluster contained populations SVK-1, HRV-21, AUT-9 and CZE-17 with the values of Jaccard coefficient of similarity ranging from 0.471 to 0.154.

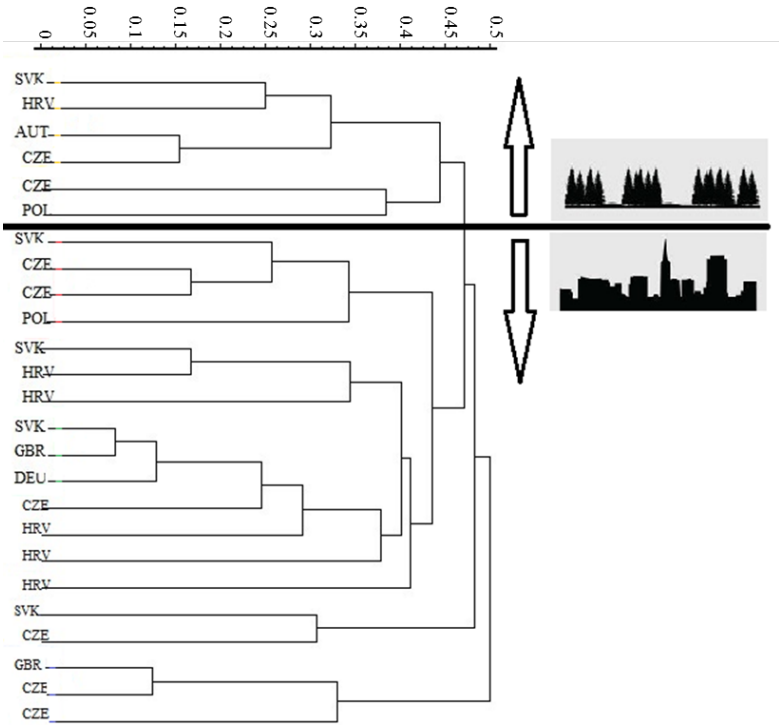

Fig. 3. Dendrogram of analysed ivy populations constructed from iPBS fingerprints of marker 2270.

Table 2. Comparison of amplicons number in the individual clusters of dendrogram constructed from iBPS data of ivy populations

Cluster	Minimum no. of amplicons	Maximum no. of amplicons
1	7	13
2	8	10
3	5	8
4	8	14

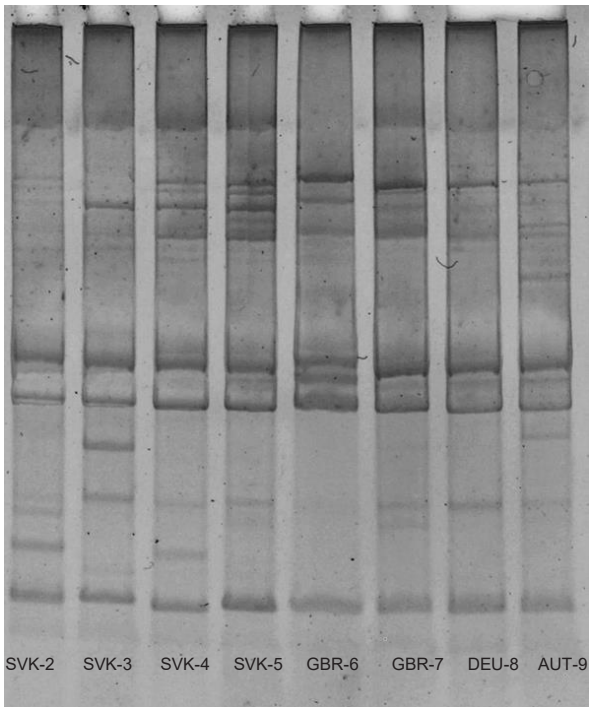

Fig. 4. The iPBS fingerprint profiles of 2270 loci for selected populations.

The existing genetic variability of the individual species within and among the populations is connected to this species ability to mirror the short- and long-term specific regimes of their living habitats (Davies et al., 2016). The analysis of the distribution of the genetic variability patterns specific for landscape nad ecological parameters is valuable for identification of the taxa most vulnerable to the anthropogenic impacts (Brandvain et al., 2014). The coupling of ecological and genetic data will provide the most suitable background for preserving the ability of the biota to respond the rapid environmental changes (Eckert, 2011; Gugger et al., 2011). The literature reports the following basic factors influencing the distribution of genetic variation: habitat specifity, plant-insect interactions, connectivity and disturbance, dispersal ability, species lifespan, reproductive rates and existing genetic diversity (Schierenbeck, 2017). Genetic diversity when analysed by neutral markers does not correspond to the adaptive ability of plant populations, but these types of markers are very useful for the interpretation of the past landscapes, refugia and gene flow (Holderegger et al., 2006). That is, why the selected genes or markers of active parts of plant genomes are used to interprete the plant genome response to the changes to the local climate and environment (Hoff-

Table 3. Amplified loci characteristics generated by 2270 marker in analysed ivy populations

Population	No of presented loci	No of missing loci	Frequency of presented loci	Frequency of missing loci
SVK-1	36	0	1	0
SVK-2	13	23	0.3611	0.6389
SVK-3	11	25	0.3056	0.6944
SVK-4	9	27	0.25	0.75
SVK-5	11	25	0.3056	0.6944
GBR-6	12	24	0.3333	0.6667
GBR-7	7	29	0.1944	0.8056
DEU-8	11	25	0.3056	0.6944
AUT-9	10	26	0.2778	0.7222
AUT-10	12	24	0.3333	0.6667
CZE-11	4	32	0.1111	0.8889
CZE-12	11	25	0.3056	0.6944
CZE-13	11	25	0.3056	0.6944
CZE-14	11	25	0.3056	0.6944
CZE-15	9	27	0.25	0.75
CZE-16	5	31	0.1389	0.8611
CZE-17	8	28	0.2222	0.7778
CZE-18	10	26	0.2778	0.7222
HVR-19	12	24	0.3333	0.6667
HVR-20	15	21	0.4167	0.5833
HVR-21	11	25	0.3056	0.6944
HVR-22	8	28	0.2222	0.7778
HVR-23	15	21	0.4167	0.5833
HVR-24	11	25	0.3056	0.6944
POL-25	8	28	0.2222	0.7778

man and Willi, 2008). Molecular-based population genetic data are very useful for determining the ecological and habitat events in the past and for detection of patterns of the recent genetic divergence. This can be achieved using different types od DNA markers (Davey and Blaxter, 2010). Here, the habitat based specifity was obtained with the aid of the iPBS fingerprints, too.

The coefficient of cophenetic correlation reached the value 0.65 for marker 2270. The diversity index value was 0.79 and the polymorphic information index was 0.78, with the distribution of the allele in the genotypes given in Table 3. The proportion of polymorphisms of the individual amplified loci ranged from 0.32% to 6.98%.

In the past, the DNA markers applied in the studies of habitat specifity in plant species were barcode markers (Fehrmann et al., 2012; Katayama et al., 2016) or universal markers based on microsatellite sequences (Kothera et al., 2007). DNA markers based on retrotransposons, especially iPBS markers are well applicaple for the diversity studies in plants. Andeden et al. (2012) analyzed the genetic diversity within the species of wild growing chickpeas using iPBS retrotransposons and ISSR markers. With using 10 ISSR primers, these authors detected in total, 136 bands among 71 entries belonging to class 6, out of which 135 were polymorphic (99.3%), with an average of 13.5 polymorphic fragment per a primer, whereas with iPBS there were detected 130 bands of 100% polymorphism, with an average of 13.0 bands per a primer. The average polymorphic information content value was 0.91 for both marker systems. Baloch et al. (2015) used iPBS primers to determine the molecular characteristic of six wild and one cultivated lentils – 10 iPBS primers were used to amplify 151 fragments. Mehmood et al. (2013) used 6 iPBS primers in studies of genetic diversity in *Psidium guajava* Linn, where 113 fragments were amplified in the range of 150 bp to 3,000 bp. Guo et al. (2014) evaluated the molecular diversity of 35 grape varieties, using fifteen selected iPBS primers, and recorded 99 polymorphic DNA fragments. Xu et al. (2018) studied the genetic diversity of 25 collected genotypes of *Tetradium ruticarpum,* using 30 primers of ISSR and 10 iPBS primers. With ISSR primers, 151 fragments were amplified and 165 amplicons synthesized on iPBS. This study has shown that iPBS displayed a higher proportion of polymorphic loci than ISSR markers, and that the markers investigated were reliable and effective for analyzing the genetic diversity of *Tetradium ruticarpum*.

Demirel et al. (2018) used iPBS markers to identify genetic similarity and relationships in *Solanum tuberosum* genotypes. These authors used 17 primers of iPBS to amplify 290 fragments, of which 224 were polymorphic. Alp and Gebologl (2017) examined the genetic variability of twelve coriander genotypes (*Coriandrum sativum*), using 16 iPBS and 8 SSR primers. Their mean PIC values ranged

from 0.1975 to 0.4911 for iPBS markers and from 0.2778 to 0.4266 for SSRs, which makes the two marker systems comparable. In this study, in total sixteen different iPBS markers were used and all of the analysed coriander genotypes were differentiated sucessfully. Borna et al. (2017) studied the applicability of iPBS markers for assessment of molecular variations and genetic relationships between 89 genotypes of *Leonurus cardiaca* L. In this study, 7 iPBS primers were used to amplify a total of 191 fragments ranging from 180 bp to 4,000 bp. Záhumenická et al. (2018) used iPBS markers for investigation of genomic stability in *Amenome sylvestris* diploid and tetraploid plants. All these studies demonstrate that iPBS analysis is quick, reliable and that this approach produces sufficient polymorphisms for large-scale DNA fingerprinting purposes.

Conclusion

Using DNA markers in the studies of habitat specifity in plant species is a very good choice, because the basic variability of DNA sequences mirrors the plant rariry that is a combination of the species' abundance and the habitat occupied by this species. What is more, the retrotransposons belong to genomic elements conserving specific biotic and abiotic features of the individual ranges which the plant species live in.

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