Utilization of iPBS Markers for *Ambrosia artemisifolia* L. Ecotypes Evaluation

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Abstract

Ambrosia artemisifolia L. is a wild plant native to North America. It was introduced to Europe in the 19th century. It causes damage in agriculture by reducing the quantity and quality of production as well as respiratory problems and allergic reactions in humans. In Slovakia, the occurrence of ragweed was first recorded at the monitoring station in Žilina. It is widespread mainly in southern Slovakia. In this study, 37 samples of adult plants of ragweed collected from three localities in southern Slovakia, namely Balvany, Veľký Horeš and Malá nad Hronom were used. Up-to date only a few molecular data are available for *Ambrosia artemisifolia* L. genome, thus the aim of the study was the analysis of genetic variability and inter-retrotransposon based polymorphism in the set of studied ecotypes. Using the iPBS 1880 primer, the total number of amplified fragments was 136 with sizes ranging from 110 to 1315 bp. For the iPBS 2079 primer, the total number of amplified fragments was 108 with sizes ranging from 159 to 888 bp. Using the iPBS primer 2274, 114 fragments were produced with sizes ranging from 270 to 1467 bp. Based on constructed dendrograms, the marker 1880 provided the most distinctive profiles that separated the most of the analysed ragweed ecotypes.

Keywords: ragweed, iPBS polymorphism, variability

1. Introduction

The origin of *Ambrosia artemisiifolia* L. is in North America such as monoecious, wind-pollinated annual herb. It entered Europe in the 19th century with the import of seeds. During the Second World War, it spread to the regions of Europe through the Pacific climate, nowadays it occurs to a higher extent as an agricultural weed [1].

Since the first record of *A. artemisiifolia* in Slovakia, the number of its colonies and its spread rate has increased considerably, and the colonisation of this species has been successful mainly in the south-western part of the country. Highest airborne pollen counts were recorded in Nitra, Trnava and Bratislava Monitoring Stations situated in the areas most infested by *A. artemisiifolia* in Slovakia. [2]. This invasive plant destroys native ecosystems and reduces agricultural revenues. It causes problems with the respiratory system in humans, as it belongs to allergenic plants and is the main cause of hay fever and associated asthma [3]. Ambrosia is growing in Europe spreads as a neophyte, while climate changes can affect the growth of the plant as well as the allergenicity of its pollen. Global changes lead to changes in molecular mechanisms during pollen development and changes in allergenic proteins [4].

To investigate the population genetic structure in *A. artemisiifolia*, reliable and polymorphic molecular markers are needed. Up to now, only a limited studies that utilize DNA based markers for *A. artemisiifolia* are available. A set of genomic single sequence repeats (gSSR) markers have been reported tha were developed from

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French *A. artemisiifolia* populations [5,6] and used to assess the population genetic structure and patterns of colonization across continental and regional scales in Europe [7-12], North America [13] and China [14]. Thirteen genomic SSRs and thirteen expessed sequence tags – simple sequence repeats (EST-SSRs) were retained and used to characterize the genetic diversity and population genetic structure of *A. artemisiifolia* populations from the native (North America) and invasive (Europe) ranges of the species in another study [15].

Here, inter – Primer Binding Site (iPBS) markers were used, as they were reported as universal or semi-universal marker technique in plants [16]. The method of iPBS was succesfully used for analysis in many different plant species such *Linum ussitatisimum* [17], *Saussurea esthonica* [18], *Liparis loeselii* [19] or *Prunus armeniaca* [20] and was prooved as reliable DNA marker technique, but according to our knowledge, these markers were not utilized in the studies of genetic variability of common ragweed.

The aim of the performed analysis reported here was to analyse the possibility and effectivity of iPBS markers for the *Ambrosia artemisia*, L. in the variability studies of this specie.

2. Materials and methods

Biological material

A total of 37 biological accession of *Ambrosia artemisia* L. was collected in three localities of Southern parts of Slovak republic (figure 1). Collected ecotypes were transported into the laboratory and kept frozen until further analysis.

Figure 1. Localization of places where analysed accessions of common ragweed were collected for this study.

DNA extraction

Total genomic DNA was extracted using the NucleoSpin®Plant II kit (Macherey-Nagel) following the instructions of manufacturer. Quantity and quality of extracted DNA was checked by NanoPhotometer® P360 (Implen).

iPBS fingerprinting

In iPBS analyses, a total of 15 primers (table 1) were firstly screened for their ability to generate amplicons. After the selection of the most suitable primers, PCRs were performed using the EliZyme™HS Robust MIX Red (Elisabeth Pharmacon), 10 ng of DNA and ^{8} pmol/µLiPBS primers in the final PCR mixture. The PCR amplification conditions were as follows: 95°C for 3 min [95°C for 1 minute, 55°C for 1 minute, 72°C for 2 minutes] 45 times and final extension 72°C for 10 minutes.

Table 1. List of iPBS primers used for the screening analyses

primer name	primer sequence
1830	5′ACTTCGGGTAAATTGTAAACTGTGTGGTTTGG3′
1838	5′TGTTAAATCGCGCCTCGGGTGGGAGCA3′
1845	5′AGCCTGAAAGTGTTGGGTTGTCG3′
1846	5′CTGGCAATTTCCATTGTCCTCGATGC3′
1854	5′GCAATCAGCCTGGACCAGTCCTCGTCC3′
1867	5′TCGACTTGATCCGCTGCTTGCCA3′
1880	5′AGAACTCCCTGGTGGCATCGTGAGC3′
1881	5′TCGAGGGTACCACCTCGACTCAG3′
1882	5′TCGACTTCTCATGCAATGGCAGCACC3′
1886	5′ATTCTCGTCCGCTGCCGCCCCTACA3′
1897	5′AGTTTGGCATAGAAAAATTCGAGCCAAC3′
2079	5′AGGTGGGCGCCA3′
2152	5′AGTGAGCATGGGAGTGGACAAGC3′
2270	5′ACCTGGCGTGCA3′
2274	5′ATGGTGGGCGCCA3′

Fragment separation and data analysis

Amplified IPBS fragments were separeted in 3% agarose electrophoresis and resulted fingerprints were transformed into binary matrices. Jaccard coefficient of genetic similarity was calculated for obtained matrices and dendrograms were prepared by UPGMA in Softwer GelAnalyzer 23.1 (http://www.gelanalyzer.com/).

3. Results and discussion

Firstly, all the primers were used for screening of their utilization for *A. artemisifolia* analysis (figure 2). A bulk DNA was used for analysis. A total of 43 fragments were amplified within the length range from 171 bp up to the 2605 bp (table 2).

For further analysis, iPBS primers 1880, 2079 and 2274 were selected as the number of amplified fragments among all of the tested iPBS markers was comparable.

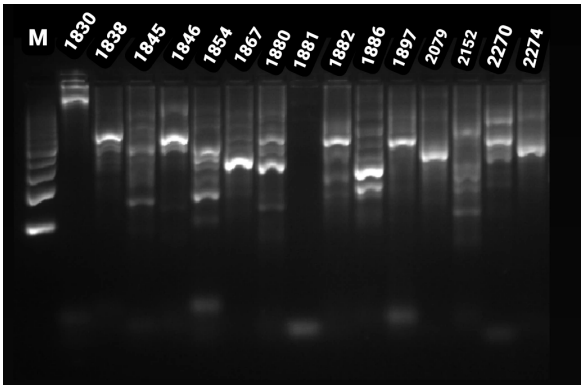

Figure 2. Fingerprints of individual tested iPBS primers in bulk DNA of *A. artemisifolia* L.

Table 2. Characteristics of iPBS amplicons for individual primers used in the study obtained for bulk DNA

primer name	no. of amplicons	length of amplicons in bp
1830	2	2605, 1857
1838	3	721, 581, 542
1845	4	1263, 911, 534, 182
1846	3	1538, 745, 618
1854	5	541, 461, 386, 293, 215
1867	3	744, 616, 428
1880	4	702, 532, 390, 177
1881	0	-
1882	3	692, 521, 307
1886	5	859, 602, 447, 324, 245
1897	1	668
2079	1	461
2152	5	863, 399, 323, 260, 171
2270	3	1180, 732, 519
2274	1	568

When using primer 1880, a total amplified fragments were 136. For samples from Balvany region (codes B1-B18), a total number of amplified fragment was 107 with the length from 110 up to the 1315 bp. For samples from Veľký Horeš region (codes VH19-VH23), a total number of amplified fragment was 23 with the length from 164 up to the 904 bp. For samples from Kicsina region (codes KZ24-KI37), a total number of amplified fragment was 62 with the length from 168 up to the 2 030 bp (table 3).

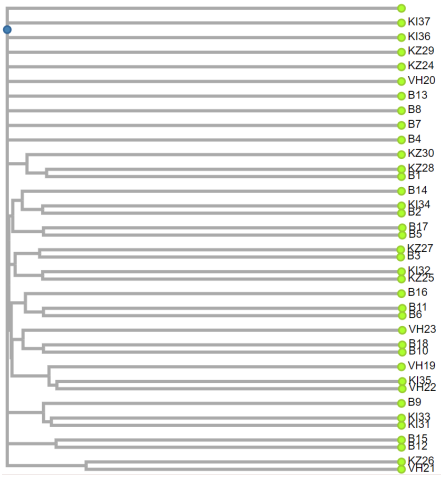

Figure 3. Dendrogram of analysed genotypes of *A. artemisifolia* L. using the iPBS primer 1880.

Table 3. Obtained lenght of iPBS amplicons for 1880 primer for analysed accessions

sample	no. of amplicons	length of amplicons in bp
B1	7	898, 804, 558, 466, 383, 299, 212
B2	7	1142, 1063, 785, 602, 496, 323, 131
B3	6	964, 820, 585, 490, 307, 217
B4	5	901, 645, 489, 347, 249
B5	7	990, 646. 455, 312, 239, 206, 146
B6	7	1225, 822, 584, 476, 314, 195, 167
B7	8	1127, 855, 549, 333, 297, 222, 182, 139
B8	5	1315, 809, 444, 211, 126
B9	7	1143, 1028, 687, 568, 462, 319, 142
B10	8	1138, 819, 625, 532, 394, 269, 193, 110
B11	5	796, 704, 472, 426, 314
B12	4	1042, 672, 574, 380
B13	6	1099, 616, 527, 387, 311, 145
B14	6	1308, 1175, 411, 330, 189, 131
B15	5	1042, 545, 396, 306, 125
B16	5	847, 406, 304, 183, 167
B17	5	990, 813, 532, 323, 194
B18	4	1270, 925, 298, 193
VH19	7	814, 589, 480, 427, 343, 265, 206
VH20	2	631, 362
VH21	4	904, 605, 409, 271
VH22	5	870, 665, 579, 476, 427
VH23	6	838, 670, 551, 476, 394, 164
KZ24	4	1041, 896, 372, 168
KZ25	6	1230, 688, 601, 487, 399, 213
KZ26	2	856, 409
KZ27	7	1038, 679, 529, 474, 415, 308, 217
KZ28	4	662, 575, 420, 212
KZ29	4	1046, 718, 288, 186
KZ30	4	659, 544, 383, 191
KI31	4	839, 643, 462, 228
KI32	6	1067, 870, 540, 487, 415, 252
KI33	6	851, 637, 577, 462, 292, 205
KI34	4	969, 697, 602, 459
KI35	4	929, 654, 427, 229
KI36	4	930, 638, 397, 218
KI37	3	588, 322, 245

Polymorphism at the level of 100 % was obtained for this primer in the analysed set of *A. artemisia* L. genotypes and all of them were separated in constructed dendrogram (figure 3).

The dendogram shows a total of 11 individual pairs of samples (KZ28-B1, KI34-B2, B17-B5, KZ27-B3, KI32-KZ25, B11-B6, B18-B10, KI35-VH22, KI33-KI31, B15-B12, KZ26-VH21), where the calculated similarity values based on the Jaccard coefficient ranged from 0.118 up to 0.350. The remaining branches of the constructed dendrogram are all the alone separated genotypes.

When using primer 2079, a total number of amplified fragments was 108. For samples from Balvany region (codes B1-B18), a total number of amplified fragment was 60 with the length from 159 up to the 888 bp. For samples from Veľký Horeš region (codes VH19-VH23), a total number of amplified fragment was 10 with the length from 199 up to the 599 bp. For samples from Kicsina region (codes KZ24-KI37), a total number of amplified fragment was 38 with the length from 178 up to the 566 bp. For this marker, 100 % polymorphism was obtained that allowed the separation of all of analysed *A. artemisia* L. genotypes in constructed dendrogram (figure 4).

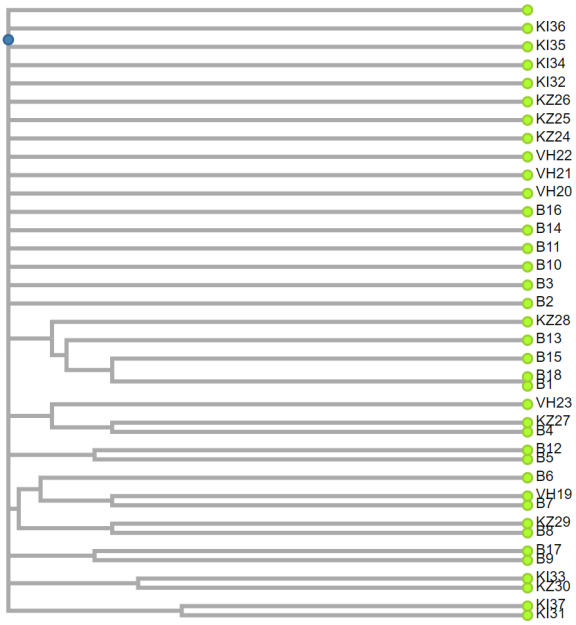

Figure 4. Dendrogram of analysed genotypes of *A. artemisifolia* L. using the iPBS primer 2079.

Here, the dendogram show only eight pairs of analysed accessions, that shared similar fingerprints obtained by iPBS 2079 (B1-B18, KZ27-B4, B12-B5, VH19-B7, KZ29-B8, B17-B9, KI33-KZ30 and KI37-KI31), with the calculated values of Jaccard similarity indices within the range from 0.125 up to the 0.333. All the other accessions were alone standing with specific fingerprints obtained by primer 2079 (table 4).

Table 4. Obtained lenght of iPBS amplicons for 2079 primer for analysed accessions.

sample	no. of amplicons	length of amplicons in bp
B1	3	473, 296, 195
B2	4	737, 448, 360, 283
B3	4	425, 369, 247, 159
B4	3	877, 453, 353
B5	5	888, 780, 518, 416, 193
B6	5	604, 481, 422, 334, 191
B7	4	604, 516, 432, 307
B8	2	496, 262
B9	3	532, 439, 215
B10	3	499, 409, 201
B11	2	484, 397
B12	2	468, 416
B13	4	465, 401, 245, 195
B14	3	617, 504, 210
B15	3	467, 296, 244
B16	3	505, 413, 350
B17	4	538, 421. 359, 215
B18	3	473, 296, 195
VH19	2	521, 432
VH20	1	575
VH21	2	542, 423
VH22	1	599
VH23	4	528, 450, 258, 199
KZ24	2	513, 313
KZ25	2	547, 309
KZ26	2	530, 454
KZ27	3	528, 453, 205
KZ28	4	566, 473, 355, 223
KZ29	4	506, 422, 262, 214
KZ30	3	552, 447, 216
KI31	1	492
KI32	3	526, 466, 287
KI33	2	552, 265
KI34	3	550, 455, 367
KI35	3	490, 419, 320
KI36	3	474, 394, 271
KI37	3	492, 382, 178

When using primer 2274, a total number of amplified fragments was 114. For samples from Balvany region (codes B1-B18), a total number of amplified fragment was 59 with the length from 337 up to the 1467 bp. For samples from Veľký Horeš region (codes VH19-VH23), a total number of amplified fragment was 17 with the length from 270 up to the 940 bp. For samples from Kicsina

region (codes KZ24-KI37), a total number of amplified fragment was 38 with the length from 302 up to the 1006 bp. In this case, 100% polymorphism was obtained again and all of analyzed genotypes were separated in constructed dendrogram (figure 5).

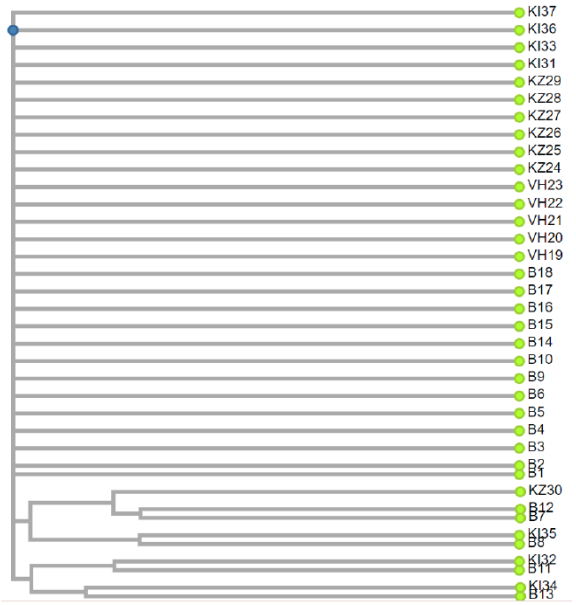

Figure 5. Dendrogram of analysed genotypes of *A. artemisifolia* L. using the iPBS primer 2274.

Here, the dendogram show only four pairs of analysed accessions, that shared similar fingerprints obtained by iPBS 2079 (B12-B7, KI35-B8, KI32-B11 and KI34-B13), with the calculated values of Jaccard similarity indices within the range from 0.143 up to the 0.250. All the other accessions were alone standing with specific fingerprints obtained by primer 2274 (table 5).

The iPBS is a retrotransposon marker system based on the amplification of the region covered by binding sites of the reverse transcriptase primer for two contiguous retrotransposons that are in opposite orientations [16]. Besides being used for both, plant and animal kingdoms, iPBS is an advantageous DNA fingerprinting technique in many ways as it does not require prior sequence knowledge [21, 22] (Demirel et al., 2018; Karık et al., 2019). The iPBS technique became a widely applied in plant variability studies. iPBS primers were used to assess the molecular variation and genetic relationships of 89 genotypes of *Leonurus cardiaca* L.), native to the Mediterranean regions of Europe and Asia. Fingerprinting with seven iPBS primers produced a total of 191 fragments ranging from 180 to 4000 bp. The samples originating from Iran were reported to be highly divergent and have rich genetic diversity and clearly provide a basis for selection and breeding [23].

Table 5. Obtained lenght of iPBS amplicons for 2274 primer for analysed accessions.

sample	no. of amplicons	length of amplicons in bp
B1	3	1019, 557, 433
B2	6	1351, 1115, 919, 667, 620, 481
B3	3	1058, 615, 496
B4	3	1026, 876, 625
B5	4	1390, 1063, 824, 682, 544
B6	3	1104, 635, 495
B7	1	684
B8	3	1136, 664, 324
B9	4	1016, 732, 542, 427
B10	3	1050, 651, 533
B11	3	955, 654, 530
B12	4	1393, 1163, 684, 558
B13	5	1107, 956, 678, 545, 337
B14	3	1166, 844, 365
B15	4	1467, 1296, 837, 398
B16	1	819
B17	3	1118, 810, 348
B18	3	1183, 794, 470
VH19	3	859, 517, 424
VH20	4	873, 584, 480, 326
VH21	4	940, 762, 551, 441
VH22	3	925, 766, 531
VH23	3	881, 532, 270
KZ24	2	838, 586
KZ25	3	827, 536, 325
KZ26	3	805, 593, 487
KZ27	3	849, 552, 460
KZ28	2	920, 613
KZ29	3	567, 502, 302
KZ30	4	981, 684, 645, 541
KI31	3	983, 661, 559
KI32	3	956, 608, 531
KI33	2	1075, 702
KI34	3	1006, 623, 545
KI35	2	981, 664
KI36	3	939, 650, 538
KI37	2	916, 685

Genetic analysis of cultivated grapevines, where each cultivar consisting of a number of clones obtained by vegetative propagation from selected vines grown from a single seedling were performed by iPBS molecular markers to assess the genetic variability of 33 grapevine genotypes collected in Russia [24]. Based on amplification, 4

from 30 iPBS primers were selected, that produced a total of 1412 fragments, ranging from 300 to 6000 bp. The results of the molecular variation analysis indicate high divergence of grapevine genotypes and rich genetic diversity. IPBS primers proved to be a useful tool for clonal selection. In this study, the iPBS marker system was successful in distinguishing of ecotypes of *Ambrosia artemisifolia* L., what suggests that the iPBS marker technique is effective for distinguishing among genotypes.

4. Conclusions

This study aimed to assess the genetic variation present in the iPBS fingerprints generated from selected ecotypes of *Ambrosia artemisifolia* L. The analysis confirmed that the iPBS technique is easily applicable, highly reproducible, and effective in assessing genetic diversity. The study observed a high level of polymorphism in this specie, when iPBS primers were employed.

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