Molecular Characterization of Vascular Plant Species from Antarctica (Deschampsia antarctica and Colobanthus quitensis) and Some Temperate and Tropical Cereal Crops Using Inter-Primer Binding Site (iPBS) Molecular Markers

Olivet Delasi GLEKU

olivetdelasi@gmail.com

Department of Agricultural Genetic Engineering, Faculty of Agricultural Sciences and Technologies, Institute of Natural and Applied Sciences, Nigde Omer Halisdemir University, 51240, Türkiye

Sedat SERCE

Department of Agricultural Genetic Engineering, Faculty of Agricultural Sciences and Technologies, Institute of Natural and Applied Sciences, Nigde Omer Halisdemir University, 51240, Türkiye

Research Article

Keywords: Antarctica, Molecular Characterization, Cereals, iPBS markers, Polymorphism, Vascular plants

Posted Date: January 10th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-3845412/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Molecular Biology Reports on December 2nd, 2025. See the published version at https://doi.org/10.1007/s11033-025-11324-2.

Abstract

Antarctica vascular species are of vital importance for plant breeding and ecosystem studies due to their extreme environmental adaptabilities. However, limited molecular studies exist especially comparing Antarctica vascular species and other cereal species from the *Poaceae* family. In this study, the two vascular plant species; *Deschampsia antarctica* and *Colobanthus quitensis* found in the Antarctic ecosystem were molecularly characterized using 45 inter primer binding site (iPBS) retrotransposon markers. The Antarctica species were sampled from various locations in the Antarctica region during a scientific expedition. Five temperate cereal species; *Avena sativa, Secale cereale, Hordeum vulgare, Triticum durum*, and *Triticum aestivum*, and one tropical cereal species, *Sorghum bicolor* were also included for comparative analysis. A total of 23 individuals in two replicates all from the *Poaceae* family were assessed in this study. The iPBS markers produced 1506 scorable reproducible bands of which 1226 bands were polymorphic. The dendrogram based on the UPGMA clustering and principal coordinate analysis (PCoA) classified the speices into three distinct clusters. High variabilities existed between the Arctic, temperate and tropical species indicating their geographical diversity and adaptations. The results of the present study provides basis for further research on these species to identify sources of variation for climate resilience and resistance breeding for sustainable agriculture. Further study is recommended to assess the phylogenetic relationship among these species.

Introduction

The Antarctic Peninsula lies north of the Antarctic Circle and is the most northern part of Antarctica. The climate in the region is considered to be one of the mildest in the continent with the temperatures in January being the warmest, averaging 1 to 2°C (34 to 36°F), and the coldest in June, averaging − 15 to -20°C (5 to -4°F) (Cortés-Antiquary et al, 2021). The Antarctic ecosystem favours only a few highly tolerant plant species that are adapted to this environment due to its extremely cold and dark climatic conditions (Cortés-Antiquary et al, 2021). *Deschampsia antarctica* Desv. (Antarctic hair grass) and *Colobanthus quitensis (Kunth)* Bartl. (Caryophyllaceae) (Antarctic pearl tea) are the two most popular vascular species that have colonized the ecosystem over the years. The Antarctic hair grass is much more abundant and widely distributed in Antarctica than the pearl wort (Smith, 2003). However, *Colobanthus quitensis* has a wider geographical distribution than *Deschampsia antarctica* (from Mexico down to Antarctica), though only found on sites with similar conditions along its distribution (i.e., cold, and well-watered areas) (Smith 2003).

Giełwanowska et al. (2015) sampled 8 populations of *Deschampsia antarctica* and *Colobanthus quitensis* from King George Island in Antarctica and analyzed 122 plant specimens with 7 iPBS primers, that produce 143 scorable bands with the mean inter-population polymorphism of 14.3%. In another study, Koc et al. (2018) estimated the pattern of genetic variation in *Colobanthus quitensis* relating to their geographic distribution and postglacial history using nine *Colobanthus quitensis* populations sourced from Chile and the Maritime Antarctic. The genetic polymorphism between individuals and genetic differentiation among populations was observed to be low (9.57%). Androsiuk et al. (2021) conducted a different study using retrotransposon-based iPBS markers to trace the genetic variation of *Deschampsia antarctica* sourced from nine sites of the Arctowski oasis on King George Island (Western Antarctic). The genotyping results of 165 samples from nine populations gave 125 amplification products, of which 15 amplicons representing 12% were polymorphic with each population having an average polymorphic fragments of 5.6% (Androsiuk et al. (2021).

Avena sativa is a widely cultivated and important cereal grain usually consumed as oatmeal or rolled oats, as well as in an assortment of baked goods, such as bread, cookies, and granolas. Their nutritional value and health benefits are well known, as they are a good source of dietary fiber, specifically beta-glucans, which are believed to reduce cholesterol levels and protect the heart (Ahmad, 2014).

Rye, *Secale cereale*, a member of the wheat clade, *Triticeae*, is widely grown as a grain, a cover crop, and a forage crop, and closely related to barley and wheat. It is a widely cultivated cereal commonly found in Central and Eastern Europe, particularly along the German-French border and north of the Alps. Rye is characterized by a number of health benefits, including losing weight, managing blood sugar levels better, reducing inflammation, supporting heart health, improving digestion, being rich in vitamins and minerals, and having a low glycemic index as well as a probiotic effect (Jonsson et al. 2018). A study conducted in Portugal, revealed that rye possesses diverse storage proteins mainly due to their overall genetic variation of the fourteen different population used (Ribeiro et al. 2012). Moreover, the beneficial characteristics of *S. cereale* can also be used to improve certain characteristics of other useful plants. This is useful in breeding studies to produce the most efficient cultivars of *S. cereale*.

Barley, *Hordeum vulgare*, a member of the *Poaceae* family is a versatile cereal grain widely grown and used globally, making it an important staple in regions with challenging climates due to its hardiness, diverse environmental adaptability (El-Hashash and El-Absy, 2019). It is also recognized for its nutritional and health benefits, as it is a good source of dietary fiber, vitamins, and minerals and used in various health food products and supplements (Ross, 2005; Rattan and Kaur, 2022).

Bread wheat, *Triticum aestivum*, is a hexaploid species (2n = 6x = 42) of the *Poaceae* family and one of the most widely cultivated important cereal crops globally, from temperate to subtropical climates. It grows with a slender, upright stem and long, narrow leaves, small inconspicuous flowers called wheat ears and spikes. *Triticum aestivum* is available in several varieties and cultivars, each with unique characteristics and adaptations (Islam, 2012). *Triticum durum*, commonly known as durum wheat or hard wheat, is the other species of wheat used in the study. It is widely cultivated and used for several culinary purposes. Its protein content is higher than that of *Triticum aestivum*, a property that makes it well-suited for making pasta products like spaghetti, macaroni, and couscous. Durum wheat is characterized by a yellow endosperm (the starchy portion of the wheat kernel) which imparts its distinctive colour to pasta products. The protein and gluten content also contribute to the desirable texture and consistency of pasta. The gluten in durum wheat is however different from that in *Triticum aestivum* as it is more elastic and less extensible, making it ideal for pasta processing but less suitable for bread. Durum wheat is a good source of complex carbohydrates, dietary fiber, protein, and various essential nutrients such as B vitamins and minerals. (Tidiane Sall, 2019)

Sorghum, *Sorghum bicolor* L., is the fifth most globally produced cereal with over 7,000 varieties of the *Poaceae* family, native to Africa but domesticated across the globe (de Morais Cardoso et al., 2017). It is a versatile and important crop that plays a vital role in the diets and economies of many countries, as

well as its relevance in global food security and agricultural sustainability in regions with challenging growing conditions (de Morais Cardoso et al. 2017). Four samples of Sorghum bicolor cultivated in Türkiye were used in this study.

The *Poaceae* family, popularly referred to as grasses, is one of the largest families in the plant kingdom consisting of a wide range of ecological and economic important plants (Peterson PM, 2013). Few studies on the above outlined *Poaceae* species focus on their adaptations to extreme environmental conditions such as that of the Antarctica ecosystem. Also, there are limited studies on the molecular diversity of Antarctica vascular species in comparison with other species of the *Poaceae* family. This study focused on some major plant species from this family collected from varied ecosystems to access their molecular characteristics on ecosystem basis while comparing them collectively as an entity. The Antarctica plant species are well adapted to extreme weather conditions as opposed to the temperate and tropical species. This can serve as a source for harnessing and introgression stress tolerant genes from the Antarctica plants species into the temperate and tropical species.

Molecular markers are nucleotide sequences and can be investigated through the polymorphism present between the nucleotide sequences of different individuals. Molecular markers are very relevant in breeding, biodiversity applications, forensics and map-based cloning of genes as a result of their use in giving recognition to a specific sequence of DNA in a pool of unknown DNA. The iPBS amplification procedure basically requires the availability of a tRNA complement as a reverse transcriptase primer binding site (PBS) in LTR (long term repeat) retrotransposons. This procedure is germane to endogenous retroviruses and retroviruses, Gypsy and Copia LTR retrotransposons and also to non-autonomous LARD and TRIM elements, both in the plant and animal kingdom as opposed to erstwhile retrotransposon isolation procedures. This approach has proven to be a robust technology in DNA fingerprinting even for a novice in DNA sequencing. All organisms with retrotransposons containing PBS sites complimentary to tRNA can employ this procedure for apt results (Guo et al., 2014; Haghighi and Hancock, 1992; Kalendar et al, 2010; Nadeem, M.A., et al 2018). The iPBS is a cheap procedure to perform since it requires just the basic laboratory facilities to conduct and for fingerprinting and genetic similarity studies in plants, they have been proven to be a sturdy marker system. The iPBS process provides answers to the limitations of erstwhile processes because of its potential to both isolate LTR retrotransposons in almost all organisms as well as its direct use as a marker system. However, it is stated that, one of the limitations to iPBS marker system is the use of low number primers to define the diversity of species population, especially when dealing with a large geographical area and long distance related plant species. In the case of long distance plant species or species of different climatic origins and adaptation, it is especially important to use high number of primers for the characterization. These informed the use of 45 primers for this study.

Materials and Methods

Plant Materials

The samples used in this study are members of the *Poaceae* family collected from three different ecosystems (Arctic, Temperate and Tropical) (Table 1). The Antarctica plant materials (*Deschampsia antarctica* and *Colobanthus quitensis*) were sampled from the Arctic ecosystem (Table 2) during the 3rd Turkish Antarctic Expedition in 2019. These plant species were maintained at the Niğde Ömer Halisdemir University's Faculty of Agricultural Sciences and Technologies (Fig. 1). The temperate (*Avena sativa, Secale cereale, Hordeum vulgare, Triticum durum* and *Triticum aestivum*) and tropical (*Sorghum bicolor*) species were obtained from the Faculty of Agricultural Sciences and Technologies, Nigde Omer Halisdemir University, Türkiye. A total of 23 species consisting of seven *Deschampsia antarctica*, one *Colobanthus quitensis*, one *Avena sativa*, one *Secale cereale*, one *Hordeum vulgare*, four *Triticum durum*, four *Triticum aestivum* and four *Sorghum bicolor* samples were used in this study.

Table 1
The list of plant materials used in the study

No.	Species	Common name	Cultivar
1	*Deschampsia antarctica*	Antarctic hair grass	Livingston
2	*Deschampsia antarctica*	Antarctic hair grass	King George
3	*Deschampsia antarctica*	Antarctic hair grass	Ardley
4	*Deschampsia antarctica*	Antarctic hair grass	Galindez
5	*Deschampsia antarctica*	Antarctic hair grass	Ridge
6	*Deschampsia antarctica*	Antarctic hair grass	Lagotellerie 5
7	*Deschampsia antarctica*	Antarctic hair grass	Lagotellerie 200
8	*Colobanthus quitensis*	Antarctic pearlwort	Livingston
9	*Avena sativa*	Oat	Kahraman
10	*Secale cereale*	Rye	Aslım 95
11	*Hordeum vulgare*	Barley	Narende
12	*Triticum durum*	Durum wheat	Zenit
13	*Triticum durum*	Durum wheat	Svevo
14	*Triticum durum*	Durum wheat	Kızıltan-91
15	*Triticum durum*	Durum wheat	Dylan
16	*Triticum aestivum*	Wheat	Reis Triticum
17	*Triticum aestivum*	Wheat	Ahmet Ağa
18	*Triticum aestivum*	Wheat	Cendere
19	*Triticum aestivum*	Wheat	Altinöz
20	*Sorghum bicolor*	Sorghum	Beydari
21	*Sorghum bicolor*	Sorghum	Uzun
22	*Sorghum bicolor*	Sorghum	E. Sumaç
23	*Sorghum bicolor*	Sorghum	Leoti

Table 2
The sampling sites of the Antarctica species

Sample	Sampling date	Location	Elevation (m)
Ardley	05.02.2019	62° 12' 31.97" G; 58° 56' 46.31" B	5
King George	06.02.2019	62° 10' 11.07" G; 58° 51' 03.88" B	5
Livingston	07.02.2019	62° 38' 35.71" G; 62° 38' 35.71" B	25
Galindez	09.02.2019	65° 14' 54,40" G; 64° 14' 50,40" B	15
Ridge	22.02.2019	67° 40' 18,80" G; 67° 03' 02,85" B	200
Lagotellerie	23.02.2019	67° 53' 13.43" G; 67° 24' 12.53" B	190
Lagotellerie	23.02.2019	67° 52' 54,73" G; 67° 23' 43,66" B	5

Genomic DNA Extraction

Genomic DNA was extracted from sprouts of plant species using the standard CTAB protocol. The DNA purity was checked by Quawell (model Q5000 UV-VIS) spectrophotometer while the quality was verified on a 1.8% agarose gel using 0.5× TBE buffer at run time of 45minutes. The gel was checked using Gel Doc™ XR + gel imaging system (Bio-Rad).

Polymerase Chain Reaction (PCR)

Samples were subjected to PCR analysis in a SENSOQUEST Lab cycler (96 gradient Thermocycler) at a run time of 2 hours 30 minutes. The PCR products were ran on a 1.8% agarose gel using 0.5× TBE buffer at 120 V/cm for 2 hours. 1 kb DNA ladder ; GeneRuler DNA Ladder Mix (Thermo Scientific), was loaded into the first and last well on the gel as a guide. The gels were ethidium bromide stained and bands visualized using the Gel Doc™ XR + gel imaging system

(Bio-Rad). All PCR and electrophoresis analysis were repeated twice and only clear sharp bands scored. The PCR components for iPBS primer and PCR conditions are listed in the Tables 3 and 4 respectively.

Table 3
PCR components for iPBS primers used in the study

Contents	Volumes 18 bp	Volumes 12/13 bp
DNA (5 ng/µl)	5 µl	5 µl
10X PCR buffer	2.5 µl	2.5 µl
$MgCl_2$ (25mM)	2ul	2 µl
dNTPs (10 mM)	0.375 µl	0.375 µl
iPBS primer	3 µl	5 µl
Dream Tag polymerase (5U/µl)	0.2 µl	0.2 µl
dH_2O	11.925 µl	9.925 µl
Total	25 µl	25 µl

Table 4
PCR amplification conditions used in the study

Steps	Temperature	Duration	Cycles
1.Initial denaturation	95°C	3 minute	1
2.Denaturation	95°C	15 second	
3.Annealing	50–63°C	60 second	35
4.Extension	72°C	2 minute	
5.Final extension	72°C	7 minute	1

iPBS Retrotranspons Markers

iPBS markers are characterized by primers that produce sharp, clear and repeatable polymorphic band profiles for all plant species. 45 iPBS (inter Primer Binding Site) retrotransposon markers developed by Kalendar et al. (2010) were used in this study. The reproducibility of primer band profiles were verified based on comparison and having two replicates of each sample for the analysis. Gels were studied to identify and confirm iPBS amplicons (bands) in all replicates. The reaction conditions used for each primer was according to by Kalendar et al. (2010).

Statistical Analysis

The molecular data obtained from the iPBS molecular analysis were documented in binary form (1- indicating the presence of a band, and 0- absence of band) to generate a binary matrix. Only reproducible repeats were used in the statistical analysis. The analysis was conducted considering each accession as an entity. NTSYSpc program version 2.11V (Exeter Software, Setauket, NY) was used for statistical analysis according to procedure of SAS program. A similarity matrix was generated using similarity coefficients, and the matrix used for multivariate analysis; Cluster analysis using the UPGMA (Unweighted Pair Group Method using Arithmetic Average) method was used to construct dendrograms and the principal coordinate analysis (PCoA) for plotting the genotypes on the first three dimensions using the G3D.

Results

Polymorphism Revealed by iPBS Primers.

Several clear and scorable bands were detected in the polymorphic analysis for all 45 primers used. The band sizes range from 50bp to 3000bp. The observed data matrix constructed based on the presence or absence of iPBS bands from 45 primers revealed a total of 1506 scorable bands, of which 1226 were polymorphic (83.9%). The scorable bands range from 25 (iPBS2380) to 42 (iPBS2231) while the polymorphic bands range from 9 (iPBS2085) to 40 (iPBS2231) (Table 5).

Table 5
Summary of Amplified Products by iPBS Marker System of Plant Samples from *Deschampsia antarctica*, *Colobanthus quitensis*, *Sorghum bicolor*, *Avena sativa*, *Seale cereale*, *Hordeum vulgare*, *Triticum aestivum* and *Triticum durum*

Primer	Scored band sizes (bp)	Number of total scored bands	Number of total polymorphic bands	Percentage of Polymorphism (%)
2074	100–3000	39	12	30.77
2075	50–3000	33	15	45.45
2077	150–3000	41	14	34.15
2079	100–3000	36	24	66.67
2081	180–3000	34	21	61.76
2085	150–3000	33	9	27.27
2095	250–3000	32	20	62.50
2228	400–3000	27	11	40.74
2229	350–3000	28	18	64.29
2230	300–3000	32	32	100.00
2231	80–3000	42	40	95.24
2232	300–3000	34	34	100.00
2237	200–3000	34	31	91.18
2238	100–3000	35	35	100.00
2239	80–3000	40	34	85.00
2246	480–3000	28	26	92.86
2249	380–3000	32	30	93.75
2251	200–3000	35	34	97.14
2252	380–3000	37	37	100.00
2255	300–3000	34	30	88.24
2257	300–3000	34	33	97.06
2272	220–3000	35	32	91.43
2273	300–3000	27	22	81.48
2274	250–3000	31	30	96.77
2276	100–3000	36	36	100.00
2277	150–3000	26	23	88.46
2373	100–3000	37	35	94.59
2374	200–3000	33	27	81.82
2375	200–3000	32	30	93.75
2376	150–3000	30	28	93.33
2377	100–3000	38	30	78.95
2380	300–3000	25	21	84.00
2381	180–3000	40	34	85.00
2382	220–3000	34	20	58.82
2383	250–3000	32	32	100.00
2385	220–3000	31	17	54.84
2387	100–3000	40	39	97.50
2390	200–3000	29	27	93.10
2391	100–3000	33	30	90.91
2392	100–3000	34	27	79.41
2393	300–3000	27	18	66.67

Primer	Scored band sizes (bp)	Number of total scored bands	Number of total polymorphic bands	Percentage of Polymorphism (%)
2398	280–3000	33	30	90.91
2399	100–3000	34	30	88.24
2400	150–3000	40	39	97.50
2402	380–3000	29	29	100.00
Total / Mean	---	**1506**	**1226**	**83.85**

Similarity Matrix and Diversity of Varieties

The bands were used to evaluate pairwise comparisons of the genotypes. Similarity and diversity of the species was performed using the pairwise similarity index comparison matrix (Table 6). The pairwise similarity indexes of the species varied between 90–99%. Among the Antarctica species *(Deschampsia antarctica* and *Colobanthus quitensis)* populations, the lowest similarity was observed in *Colobanthus quitensis* (0.95) whiles the highest similarity matrix was observed in all seven *Deschampsia antarctica* species (0.99). Among the temperate species (*Avena sativa, Secale cereale, Hordeum vulgare, Triticum durum* and *Triticum aestivum*) populations, the lowest similarity was observed in *Triticum aestivum's* Altinöz (0.92) whiles the highest similarity matrix was observed in *Triticum aestivum's* Svevo, Kiziltan-91, Dylan, Ahmet Aga and Cendere species (0.99). The similarity indexes among the tropical species (*Sorghum bicolor*), ranges from (0.90) in Uzun to (0.99) in E. Sumac and Leoti species. Cluster analysis based on the similarity indexes was performed (Fig. 2) to reveal the ecological relatedness of the individual genotypes. The results revealed a closer relationship between the Antarctica species and the temperate species than with the tropical species (Fig. 2). This relatedness was confirmed by the PCoA results (Fig. 3) except for *Colobanthus quitensis* which was grouped with samples from the temperate zone in the PCoA. The otherwise behaviour of *Colobanthus quitensis* could be due to its diverse geographical distribution and possible genetic mutations.

Table 6
Pairwise Similarity Index Comparison Matrix of the Samples (*Deschampsia antarctica*, *Colobanthus quitensis*, *Sorghum bicolor*, *Avena sativa*, *Seale cereale*, *aestivum* and *Triticum durum*)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	2
1	1.00																			
2	0.99	1.00																		
3	0.99	0.99	1.00																	
4	0.99	0.99	0.99	1.00																
5	0.99	0.99	0.99	0.99	1.00															
6	0.99	0.99	0.99	0.99	0.99	1.00														
7	0.99	0.99	0.99	0.99	0.99	1.00	1.00													
8	0.95	0.95	0.95	0.95	0.95	0.95	0.95	1.00												
9	0.95	0.96	0.96	0.96	0.96	0.96	0.96	0.96	1.00											
10	0.95	0.95	0.95	0.95	0.95	0.95	0.96	0.95	0.98	1.00										
11	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.94	0.96	0.97	1.00									
12	0.95	0.96	0.95	0.95	0.95	0.96	0.96	0.95	0.97	0.97	0.97	1.00								
13	0.95	0.95	0.95	0.95	0.95	0.95	0.96	0.95	0.97	0.97	0.97	0.99	1.00							
14	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.94	0.96	0.97	0.97	0.99	0.99	1.00						
15	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.94	0.97	0.97	0.97	0.99	1.00	0.99	1.00					
16	0.95	0.96	0.95	0.95	0.95	0.95	0.95	0.94	0.97	0.97	0.98	0.99	0.99	0.98	0.99	1.00				
17	0.95	0.96	0.96	0.95	0.95	0.95	0.95	0.94	0.97	0.97	0.97	0.99	0.99	0.99	0.99	0.99	1.00			
18	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.94	0.96	0.97	0.97	0.99	0.99	0.98	0.99	0.99	0.99	1.00		
19	0.94	0.93	0.93	0.93	0.93	0.93	0.94	0.92	0.94	0.94	0.95	0.96	0.96	0.96	0.96	0.96	0.97	0.97	1.00	
20	0.92	0.92	0.91	0.91	0.92	0.92	0.92	0.91	0.92	0.92	0.92	0.93	0.92	0.92	0.92	0.93	0.92	0.92	0.91	1
21	0.91	0.91	0.90	0.90	0.91	0.91	0.91	0.90	0.91	0.91	0.91	0.92	0.91	0.91	0.91	0.91	0.91	0.91	0.90	0
22	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.92	0.92	0.92	0.92	0.92	0.92	0.91	0.92	0.92	0.92	0.91	0
23	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.92	0.91	0.92	0.91	0.92	0.91	0.91	0.91	0

Principal Coordinate Analysis and Dendrogram Cluster Analysis iPBS Markers

The unweighted pair-group method with arithmetic mean (UPGMA) dendrogram classified the species into 3 clusters (Fig. 2). The first cluster consists of the tropical species, the second and largest group composed of temperate species together with *Colobanthus quitensis*, whiles the third group consisted of the *Deschampsia antarctica* species (Fig. 2). These classifications were substantiated by the PCoA of which the *Deschampsia antarctica* accessions were differentiated together. The temperate accessions also clustered together with *Colobanthus quitensis*, and *the* tropical species also formed one cluster (Fig. 3). For the PCoA, the first three coordinates cumulatively accounted for 74.1% of the total genetic diversity based on their eigenvalues of approximately equal to 1 (Table 7). The PCo-1(D1) accounted for 38.7, PCo-2 (D2) 27.2% and PCo-3 (D3) 8.3% of percentage of genetic diversity (Fig. 3 and Table 7).

Table 7
The eigenvalue, percentage and cumulative variation three dimensional view of the amplified products by iPBS marker system after the principle coordinate analysis for the plant samples

Dimensions	Eigenvalue	Percentage (%)	Cumulative
1	0.430	38.72	38.72
2	0.302	27.15	65.87
3	0.092	8.27	74.14
4	0.055	4.91	79.05
5	0.045	4.06	83.11
6	0.040	3.56	86.67
7	0.022	1.96	88.63
8	0.018	1.66	90.29
9	0.017	1.55	91.84
10	0.014	1.30	93.14
11	0.012	1.12	94.27
12	0.012	1.04	95.31
13	0.009	0.82	96.13
14	0.009	0.79	96.92
15	0.007	0.68	97.59
16	0.007	0.64	98.24
17	0.006	0.56	98.80
18	0.005	0.47	99.27
19	0.003	0.25	99.52
20	0.002	0.20	99.72
21	0.002	0.17	99.89
22	0.001	0.11	100.00
23	0.000	0.00	> 100%
	----	-----	
Sum	1.111	100.00	
Average root	0.0483		

Discussions

Molecular diversity studies in plant genetic resources assists plant breeders in developing new and improved cultivars with desirable traits for farmers. Molecular markers are very necessary in plant breeding and are currently in global use for multiple purposes. Retrotransposons are transposable elements which form a major part of the plant genome. Genomic variations occur because of the repetitions making them a unique source of molecular markers. Molecular characterization of plants requires marker systems with very high reproducibility and high polymorphism, even among closely related genotypes. For this purpose, reproducibility of the iPBS retrotransposon markers in these species were tested using repeated polymerase chain reaction procedure. Retrotransposon (iPBS) markers have been used in studying various crops for phylogenetic and genetic diversity assessments due to their user-friendliness and genotype resolution systems. Studies in plants clearly demonstrated the effectiveness of iPBS retrotransposons as molecular tools. However, this marker system has not been used in the comparative evaluation of the molecular diversity of Antarctica vascular species and other cereals in the temperate and tropical ecosystems. This study investigated the diversity of Antarctica's vascular species *Deschampsia antarctica and Colobanthus quitensis* as well as *Avena sativa*, *Seale cereale*, *Hordeum vulgare*, *Triticum* and *Sorghum bicolor* species using iPBS markers. 45 iPBS primers employed in this study produced a

total of 1506 scorable bands, of which 1226 were polymorphic representing (83.9%). This clearly demonstrated the high reproducibility nature of the iPBS retrotransposons markers. The results suggest that the iPBS marker system can be used to study the genetic diversity of these species. The species studied in this research covered three different ecosystems (Arctic, Temperate and Tropical) and the resultant clustering and principal coordinate analysis revealed the species clustering together according to their ecosystems except for *Colobanthus quitensis* which was grouped with species from the temperate zone. The behaviour of *Colobanthus quitensis* can be attributed to the diverse nature of its distribution across geographic regions from temperate zones (Mexico) to the Arctic zone (Antarctica) (Smith, 2003).

A study by Yildiz et al. (2022) examined the genetic diversity and population structure of 38 accessions of carrots (*Daucus aspiaceae*) and one accession of a related genus with 21 iPBS markers, obtained 309 clear and unambiguous bands with 306 (99.0%) polymorphic. This indicate a high level of polymorphism in the *Daucus* germplasm reflecting a very good level of genetic variation that may be massively used with *Daucus* germplasm for breeding purposes. The dendrogram based on the UPGMA clustering algorithm and a principal coordinate analysis (PCoA) indicated the presence of four clusters with few accessions intermixed amongst clusters. Genetic diversity was observed among the species studied just as was in this present study revealing the potency of the iPBS marker system as a universal marker system for any kind of species.

Aydın et al. (2020) also studied genetic variation at intraspecies and interspecies levels among 112 yeast strains belonging to eight species previously obtained from fermented foods using eight iPBS markers. The eight iPBS primers used in the study produced 278 bands, all of which were polymorphic with an average of 34.75 polymorphic fragments per primer with 0.23 and 10.11 as an average polymorphism information content and resolving power values respectively. The genetic percentage of polymorphic loci for each species ranged from 19.23–71.21%, Nei's gene diversity from 0.085 to 0.228, Shannon's information index ranged from 0.125 to 0.349. The value of gene flow and genetic variation among the populations was also found to range from 0.09 to 0.85, revealing higher genetic variation among the species. The UPGMA analysis revealed considerable genetic variability in the yeast strains as they are clustered according to their species and revealed the intraspecific variation. Every individual iPBS primer gave an adequate discrimination of species. This results also confirm that iPBS marker system is a good marker system to be employed in estimating the genetic variation of yeast strains.

Germplasm characterization is a vital phase in crop breeding based on this premise, Yildiz et al. (2020) characterized the genetic diversity and the population structure of a collection of 94 pepper accessions from these groupings (*C. annuum, C. chinense*, and *C. frutescens*) using iPBS markers. With 20 iPBS primers, a total of 172 bands (mean = 8.6 bands/primer), representing ~ 92% polymorphism with individual polymorphism at 83%, 69%, and 80% respectively for *C. annuum, C. chinense*, and *C. frutescens* subsets were obtained. The iPBS markers was observed to clearly differentiate the taxa. Which was confirmed by clear segregation of all the *C. annuum* accessions together from the other pepper species by cluster analyses. Few sub clusters were observed among the other pepper species, including groupings with accessions of both *C. frutescens* and *C. chinense.* At the interspecies level, 3 clustering methods clearly discriminated *C. annuum* from *C. frutescens* and *C. chinense*, this indicates the diversity of the species confirming the precision and high power of the iPBS marker system in the genetic diversity of species.

The study of Pour et al., 2019 on Adonis reported very high levels of polymorphism with iPBS markers, just us is found in this study confirming that they are an excellent marker system for detecting genetic variation in plant germplasm. In their study 62 species were collected from different regions in Türkiye and 83 iPBS primers were used for the molecular analysis however only 10 primers were finally accessed with enough polymorphic bands, a total of 204 alleles were produced by these 10 primers, with an average polymorphism percentage of 99.50% and a polymorphic information content ranging from 0.16 to 0.39. More specifically, cluster analysis according to unweighted pair-group mean average (UPGMA), revealed four main clusters of the 62 Adonis ecotypes. In the principal coordinate analysis, the first three principal coordinates accounted for 81.51% of the total variation which is higher the results obtained in the current study where the first three PCoAs accounted for 74.1% of the total variability. A Bayesian analysis of the genetic structure of the germplasm used in the study revealed four subpopulations, with expected heterozygosity measurements of 0.2634 and population differentiation measurements of 0.2154. iPBS markers were found to be effective in the identification of genetic diversity among the species of Adonis. In this our study, Bayesian analysis was not performed and so no comparison can be made.

Furthermore, the efficiency of iPBS markers in detecting genetic differentiation in African Gnetum species was determined by Doungous et al. (2020) using 21 iPBS markers on 14 African Gnetum accessions. Based on the six best selected primers, 103 bands were generated in *G. africanum*, 95 in *G. buchholzianum*, and 24 in *G. latispicum*. Two major clusters were obtained using cluster analysis, *G. africanum* was grouped together within the first group, *while G. buchholzianum* and *G. latispicum* were grouped together within the second group. Jaccard's similarity coefficient revealed a close relationship between *G. buchholzianum* and *G. latispicum*. The study on African Gnetum concluded that, the iPBS marker system revealed genetic differentiation and could provide an avenue to evaluate genetic diversity, conservation, taxonomy, and evolution.

Finally, to assess potato genotype genetic diversity, Demirel et al. (2018) screened 16 potato genotypes using 45 iPBS retrotransposon markers. For fingerprinting the whole collection of accessions, 17 primers were chosen due to their strong, reproducible, and polymorphic bands. In total, 290 scorable bands and 224 polymorphic bands were obtained. The number of polymorphic bands per primer ranged from 6 to 21 with an average of 17.1. The number of bands per primer ranged from 10 to 26 with an average of 17.1. The polymorphism rate per primer ranged between 46.2 and 100.0% with an average of 77.2%. The mean polymorphism information content (PIC) of iPBS primers ranged from 0.12 to 0.31 per primer and the average similarity coefficient of 0.73 between potato genotypes with a range from 0.61 to 0.93. Thus, it was concluded that iPBS retrotransposons serve as powerful DNA markers for fingerprinting large samples of potato germplasm.

There is a vivid correlation between these results and those of the present study based on similar high polymorphisms, clustering and PCoA results. (Figs. 2 and 3).

Conclusion

Molecular studies on Antarctica's vascular species have been carried out mainly on species from few locations and without comparison with species from any other ecosystem, but in this study the genetic diversity of *Deschampsia antarctica* species from seven different locations were assessed, one *Colobanthus quitensis*, one *Avena sativa*, one *Secale cereale*, one *Hordeum vulgare*, four *Triticum aestivum*, four *Triticum durum* and four *Sorghum bicolor* using an iPBS retrotransposons marker system. According to the results obtained, 27 to 100% polymorphic bands were found per primer, representing 83.9% polymorphism. The temperate species showed a greater level of variation and diversity, whilst being closely related to the arctic species especially *Colobanthus quitensis*. The high rate of polymorphisms and the high number of bands recorded per assay using a single primer indicate that the iPBS is a very informative marker system that can be used for studying populations, taxonomy, conservation, and domestication of species. These results further elucidate the usefulness of iPBS marker system in plant breeding studies.

Declarations

Author contribution

SS conceived the research and sampled the Antarctica species and supervised the research. ODG conducted the research. SS and ODG analysed the data and drafted the manuscript. All authors read and approved the draft manuscript.

Acknowledgement

Authors acknowledge Dr Caner Yavuz for providing the *Sorghum bicolor* seeds, Ibrahim Köken for providing the Triticum species, and Prof Dr Mustafa Avcı for providing the *Avena sativa, Hordeum vulgare*, and *Secale cereale* seeds.

Conflict of Interest

Authors declare no conflict of interest.

References

1. Androsiuk P, Chwedorzewska KJ, Dulska J, Milarska S, Giełwanowska I (2021) Retrotransposon-based genetic diversity of *Deschampsia antarctica* Desv. from King George Island (Maritime Antarctic). Ecol Evol 11(1):648–663
2. Ahmad M, Dar ZA, Habib M (2014) A review on oat (*Avena sativa* L.) as a dual-purpose crop. Sci Res Essays 9(4):52–59
3. Aydın F, Özer G, Alkan M, Çakır İ (2020) The utility of iPBS retrotransposons markers to analyze genetic variation in yeast. Int J Food Microbiol 325:108647
4. Cortés-Antiquera R, Pizarro M, Contreras RA, Köhler H, Zúñiga GE (2021) Heat shock tolerance *in Deschampsia antarctica* Desv. cultivated in vitro is mediated by enzymatic and non-enzymatic antioxidants. Front Plant Sci 12:635491
5. de Morais Cardoso L, Pinheiro SS, Martino HSD, Pinheiro-Sant'Ana HM (2017) Sorghum (*Sorghum bicolor* L.): Nutrients, bioactive compounds, and potential impact on human health. Crit Rev Food Sci Nutr 57(2):372–390
6. Demirel U, Tındaş İ, Yavuz C, Baloch FS, Çalışkan ME (2018) Assessing genetic diversity of potato genotypes using inter-PBS retrotransposon marker system. Plant Genetic Resources 16(2):137–145
7. Doungous O, Kalendar R, Filippova N, Ngane BK (2020) Utility of iPBS retrotransposons markers for molecular characterization of African Gnetum species. Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology 154(5):587–592
8. El-Hashash EF, El-Absy KM (2019) Barley (*Hordeum vulgare* L.) breeding. Advances in Plant Breeding Strategies: Cereals. 5:1–45
9. Giełwanowska I, Androsiuk P, Chwedorzewska K, Szandar K (2015) Genetic variability of Colobanthus quitensis from King George Island (Antarctica). Pol Polar Res, (3):281–295
10. Guo DL, Guo MX, Hou XG, Zhang GH (2014) Molecular diversity analysis of grape varieties based on iPBS markers. Biochem Syst Ecol Feb 52(1):27–32
11. Haghighi K, Hancock JF (1992) DNA restriction fragment length variability in the genomes of highbush blueberry. HortScience Jan 1(1):44–47
12. Islam S, Haque MS, Emon RM, Islam MM, Begum SN (2012) Molecular characterization of wheat (*Triticum aestivum* L.) genotypes through SSR markers. Bangladesh J Agricultural Res 37(3):389–398
13. Jonsson K, Andersson R, Knudsen KE, Hallmans G, Hanhineva K, Katina K, Kolehmainen M, Kyrø C, Langton M, Nordlund E, Lærke HN (2018) Rye and health-Where do we stand and where do we go? Trends in Food Science and Technology Sep 79(1):78–87
14. Kalendar R, Antonius K, Smýkal P, Schulman AH (2010) iPBS: a universal method for DNA fingerprinting and retrotransposon isolation. Theor Appl Genet 121:1419–1430
15. Koc J, Androsiuk P, Chwedorzewska KJ, Cuba-Díaz M, Górecki R, Giełwanowska I (2018) Range-wide pattern of genetic variation in Colobanthus quitensis. Polar Biol 41:2467–2479
16. Nadeem MA, Nawaz MA, Shahid MQ, Doğan Y, Comertpay G, Yıldız M, Hatipoğlu R, Ahmad F, Alsaleh A, Labhane N, Özkan H (2018) DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. Biotechnol Biotechnol Equip Mar 4(2):261–285
17. Peterson PM (2013) Poaceae (Gramineae). Encyclopedia of Life Sciences, eLS
18. POUR AH, Karahan F, İlhan E, İlçim A, Haliloğlu K (2019) Genetic structure and diversity of Adonis L. (Ranunculaceae) populations collected from Turkey by inter-primer binding site (iPBS) retrotransposon markers. Turkish J Bot 43(5):585–596

19. Rattan SI, Kaur G (2022) Nutrition, Food and Diet in Health and Longevity: We Eat What We Are. Nutrients Dec 18(24):5376
20. Ribeiro M, Seabra L, Ramos A, Santos S, Pinto-Carnide O, Carvalho C, Igrejas G (2012) Polymorphism of the storage proteins in Portuguese rye (*Secale cereale L.*) populations. Hereditas Apr 149(2):72–84
21. Ross IA (2005) Hordeum vulgare. Humana Press, pp 235–261
22. Smith RL (2003) The enigma of *Colobanthus quitensis* and *Deschampsia antarctica* in Antarctica, 234–239
23. Tidiane Sall A, Chiari T, Legesse W, Seid-Ahmed K, Ortiz R, Van Ginkel M, Bassi FM (2019) Durum wheat (*Triticum durum* Desf.): Origin, cultivation, and potential expansion in Sub-Saharan Africa. Agronomy 9(5):263
24. Yildiz M, Kocak M, Nadeem MA, Cavagnaro P, Barboza K, Baloch FS, Keleş D (2020) Genetic diversity analysis in the Turkish pepper germplasm using iPBS retrotransposon-based markers. Turkish J Agric Forestry 44(1):1–14
25. Yildiz M, Arbizu C (2022) Inter-primer binding site (iPBS) retrotransposon markers provide insights into the genetic diversity and population structure of carrots (Daucus, Apiaceae). Turkish J Agric Forestry 46(2):214–223

Figures

Figure 1

Pictures of the species sampled from Antarctica

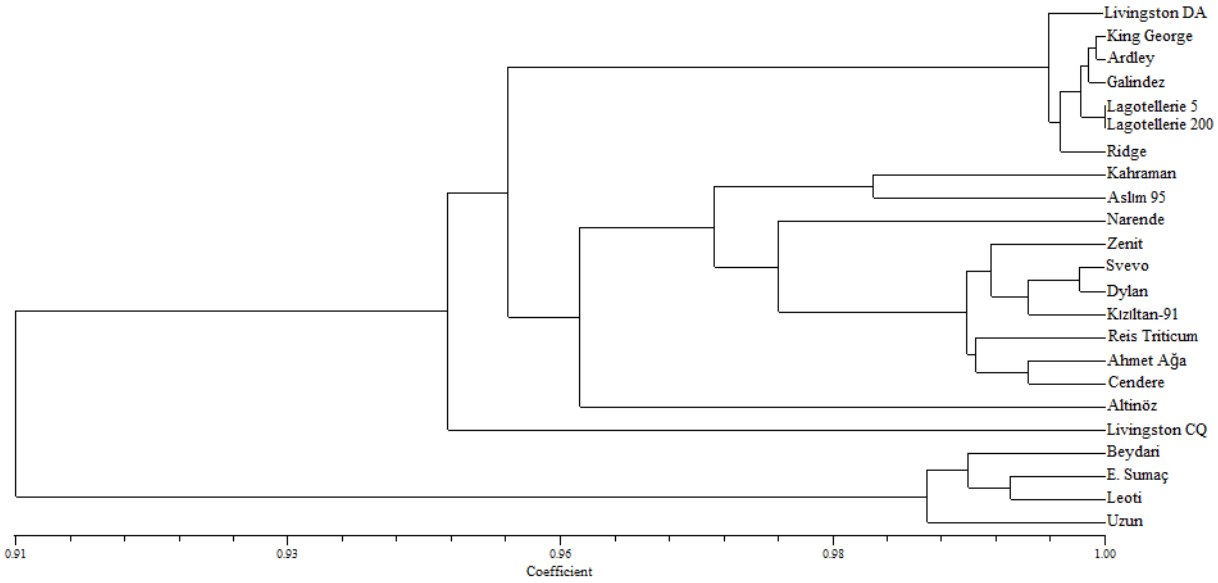

Figure 2

The UPGMA (Unweighted Pair Group Method using Arithmetic Average) dendrogram generated by the amplified products by iPBS marker system of plant samples from *Deschampsia antarctica*, *Colobanthus quitensis*, *Sorghum bicolor*, *Avena sativa*, *Seale cereale*, *Hordeum vulgare, Triticum aestivum* and *Triticum durum*

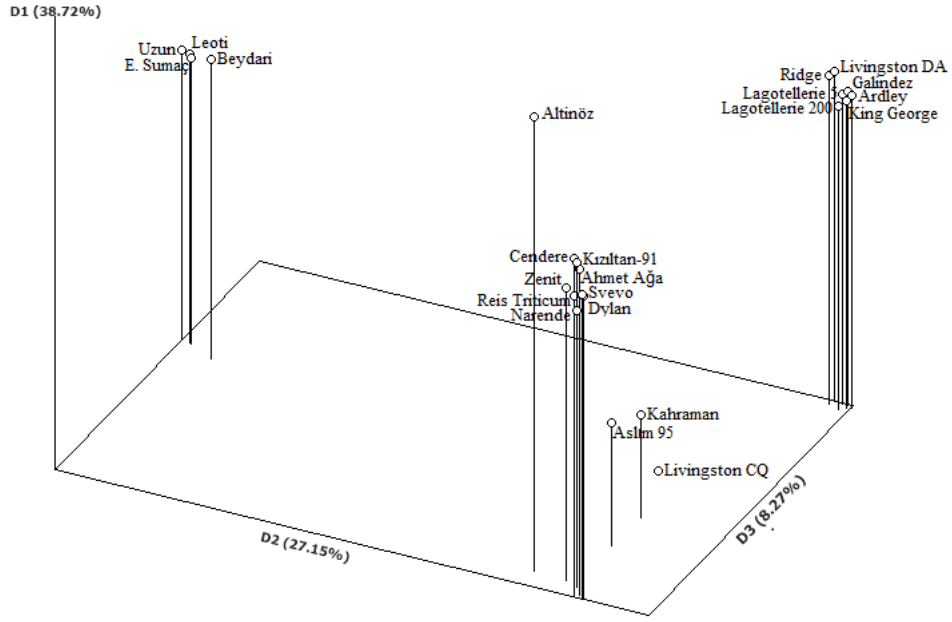

Figure 3

The three dimensional view of the amplified products by iPBS marker system after the principle coordinate analysis for the plant samples from *Deschampsia antarctica*, *Colobanthus quitensis*, *Sorghum bicolor*, *Avena sativa*, *Seale cereale*, *Hordeum vulgare, Triticum aestivum* and *Triticum durum*