POLISH POLAR RESEARCH
vol. 36, no. 3, pp. 281–295, 2015 doi: 10.1515/popore-2015-0017

Genetic variation of *Colobanthus quitensis* from King George Island (Antarctica)

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Abstract: Antarctic pearlwort (*Colobanthus quitensis*) is one of the flowering plant species considered native to maritime Antarctica. Although the species was intensively analyzed towards its morphological, anatomical and physiological adaptation to local environment, its genetic variability is still poorly studied. In the presented study, a recently developed retrotransposon-based DNA marker system (iPBS – inter Primer Binding Site) was applied to assess the genetic diversity and differentiation of *C. quitensis* populations from King George Island (South Shetland Islands, West Antarctic). A total of 143 scoreable bands were detected using 7 iPBS primers among 122 plant specimens representing 8 populations. 55 (38.5%) bands were found polymorphic, with an average of 14.3% polymorphic fragments per primer. Nine of all observed fragments were represented as a private bands deployed unevenly among populations. Low genetic diversity (on average H_e = 0.040 and I = 0.061) and moderate population differentiation (F_{ST} = 0.164) characterize the analyzed material. Clustering based on PCoA revealed, that the populations located on the edges of the study area diverge from the central populations. The pattern of population differentiation corresponds well with their geographic location and the characteristics of the sampling sites. Due to the character of iPBS markers, the observed genetic variation of populations may be explained by the genome rearrangements caused by mobilization of mobile genetic elements in the response to various stress factors. Additionally, this study demonstrates the usefulness of iPBS markers for genetic diversity studies in wild species.

Key words: Antarctica, *Colobanthus quitensis*, genetic diversity, iPBS.

Introduction

Plants of the polar regions have developed a number of mechanisms which enable them to grow and develop in harsh environmental conditions (*e.g.* Giełwanowska *et al.* 2015; Kellmann-Sopyła *et al.* 2015). Morphological and physiologi-

cal adaptations of these organisms were analyzed on examples of many species, including Antarctic pearlwort (*Colobanthus quitensis* (Kunth) Bartl., Caryophyllaceae), which together with Antarctic hairgrass (*Deschampsia antarctica* Desv., Poaceae) are the only two flowering plant species considered native to maritime Antarctica. Although *C. quitensis* and *D. antarctica* were intensively analyzed towards their morphological, anatomical and physiological adaptations to local climatic conditions (*e.g.* Bravo *et al.* 2001; Bravo and Griffith 2005; Giełwanowska *et al.* 2008; Ruhland and Krna 2010), we still have limited knowledge about the genetic variability of these species. Our knowledge concerning the genetic diversity of these plants is based mainly on a small number of publications devoted to *D. antarctica* (*e.g.* Chwedorzewska *et al.* 2004; Chwedorzewska and Bednarek 2008, 2011; van de Wouw *et al.* 2008; Volkov *et al.* 2010). *Colobanthus quitensis* is also poorly studied, as our understanding of its genetic composition and characteristics is based on outmoded methods like isoenzymatic analyses (Lee and Postle 1975), or very limited sample size (Gianoli *et al.* 2004; Acuña-Rodríguez *et al.* 2014).

Flowering plants have various mechanisms which enable them to respond to biotic stress or changes in environmental conditions (Bruce *et al.* 2007). Some of them may lead to genetically determined phenotypic variation. One of the mechanisms responsible for the formation of genetic variation is associated with the presence of transposable elements (TE) (Kalendar *et al.* 2000; Piacentini *et al.* 2014). These mobile genetic elements have a significant impact on the organization, plasticity and evolution of genomes (Frost *et al.* 2005). TE are also one of the major factors responsible for adaptation of genome to changing environmental conditions, and also take part in response to stress (Schrader *et al.* 2014). Due to the specific character of the TE (ubiquitous distribution, high copy number, widespread chromosomal dispersion), a number of multiplex DNA-based marker systems was developed on the basis of their sequences (*e.g.* Kalendar *et al.* 1999; Shedlock and Okada 2000; Schulman *et al.* 2004), which allowed to track genetic variability. Unfortunately, their application is limited to the species for which transposon sequences are known.

A new and versatile method of organism genotyping based on the use of transposon sequences was recently developed by Kalendar *et al.* (2010). The iPBS method (inter Primer Binding Site) is based on the virtually universal presence of a tRNA complement as a reverse transcriptase primer binding site (PBS) in LTR (Long Terminal Repeat) retrotransposons. The iPBS technique has been introduced as a powerful DNA fingerprinting technology without the need for prior sequencing (Kalendar *et al.* 2010). iPBS may be therefore a useful tool for tracking genetic variation in non-model plant species, such as *Colobanthus quitensis*, for which data resources on the structure of the genome are limited.

Antarctic pearlwort, due to its broad distribution range spanning from Mexico (17˚N) to the southern Antarctic Peninsula (68°S) and from 0 to 4200 m a.s.l., undergoes various selection forces, which shape both its morphological and genetic

variability (Moore 1970; Smith 2003; Gianoli *et al.* 2004). Even in small geographic scale, in which considerable differences in microclimate or diverse soil and moisture conditions can be observed, significant molecular changes in plant genomes may occur, due to activation of the transposable elements (Kalendar *et al.* 2000). In the case of the *C. quitensis*, King George Island from South Shetlands archipelago, seems to be an interesting area to study the role of transposable elements in generation of genetic variation.

The aims of the study were (*i*) to verify whether *C. quitensis* populations from King George Island growing in diverse microhabitat conditions show genetic variation and (*ii*) to test the suitability of iPBS markers for their potential application in the studies of genetic variation shaped by stressful environmental conditions.

Material and methods

Study site and sampling. — The research material consisted of 122 specimens of *C. quitensis* representing eight sampling sites (referred later as populations) from King George Island (South Shetland Islands), located in Arctowski oasis (population 1, 2, 5 and 8) and in the area of ASPA (Antarctic Specially Protected Area) 128 (population 3, 4, 6 and 7) (Table 1; Fig. 1). Each population was represented by 8 to 31 specimens. Plants were collected in 2010 and stored at -20°C until DNA extraction.

Number and choice of sampling sites was based on the following factors: abundance of nutrients in the soil and share of the granulometric fractions (Table 1), exposure to sunlight and to strong winds, distance from the sea (direct effect of sea water spray or sea water).

DNA extraction and iPBS genotyping. — The DNA from specimens representing each population was extracted (Syngen Plant DNA Mini Kit). The quality of DNA was verified on 1% (w/v) agarose gel and visualized by staining with 0.5 mg/ml ethidium bromide, while amount and purity of DNA samples was assessed spectrophotometrically.

Initially, according to the procedure described in Kalendar *et al.* (2010), we screened 12 iPBS primers and their combinations for *C. quitensis*., from which seven gave polymorphic, clearly identifiable and repeatable bands, and therefore were selected for further analyses. The polymerase chain reaction (PCR) was performed with the seven iPBS primers: 2076, 2085, 2224, 2228, 2231, 2240 and 2378, applied individually (2085, 2224, 2228, 2231, 2240 and 2378) or in combination of two primers (2076×2085) (Table 2). For iPBS amplification, PCR was performed in 20 µl reaction volume containing 2.0 µl of PCR buffer (100 mM $(NH_4)_2SO_4$, 200 mM Tris-HCl pH 8.5, 20 mM $MgSO_4$, 1% Triton X-100); 200 µM of dNTP; 1.0 µM of primer for 12–13 nt primers (for primer combinations, 1 µM total concentration) or 0.6 µM for 18 nt primers; 1 u of RUN *Taq* DNA polymerase

Table 1

Description of the sampling sites, number of collected plants of the *Colobanthus quitensis* and chemical characteristics and granulometric composition of soil (Regional Agrochemical Station in Olsztyn, Poland, authorized by Polish Centre of Accreditation).

Population	Characteristic of the sampling site	No. of individuals	Chemical characteristics							Granulometric fractions (%)				
			pH [H_2O]	C [%]	N [%]	C:N	P [mg/100g]	K [mg/100g]	Mg [mg/100g]	< 0.002 mm	0.002–0.005	0.005–0.02	0.02–0.05	0.05–2.0
1.	**Penguin Rookery**: 10 m.a.s.l.,100–120 m from the sea shore; soil with high amounts of organic matter from the penguin fresh guano; area abundantly covered by lichens species as well as by *Colobanthus quitensis* and *Deschampsia antarctica* which grow intensively during the vegetative season. Habitat humid and sheltered from the wind.	8	5.55^{a}	4.87^{c}	0.69^{c}	7.20^{a}	31.07^{d}	62.73^{d}	26.85^{a}	0.00^{a}	0.19^{ab}	1.49^{a}	2.42^{a}	95.91^{e}
2.	**Rakusa Point**: just near the sea shore, 0.5–1 m.a.s.l., 1–2 m above the sea level; within the range of ocean waves during the stormy weather; soil with high amounts of organic matter from the penguin guano; many lichen species and *Deschampsia antarctica* together with *Colobanthus quitensis* (however less frequent than in location of population 1). Habitat humid and exposed	13	5.70^{a}	8.91^{d}	0.89^{d}	9.84^{ab}	27.01^{c}	35.75^{c}	39.20^{bc}	0.00^{a}	0.06^{a}	1.29^{a}	2.03^{a}	96.62^{e}
3.	**III Moraine of Ecology**: 40 m.a.s.l., 400 m from the sea, the oldest moraine; both lichens and *Colobanthus quitensis* with *Deschampsia antarctica* are spotted in this area, the plant growth is less intensive (2.5–6.5 cm); within the range of sea water aerosols during the stormy weather. Habitat dry and exposed	12	5.84^{ab}	3.74^{bc}	0.21^{ab}	19.92^{c}	24.79^{c}	25.21^{b}	49.27^{cd}	0.29^{ab}	0.70^{c}	3.58^{b}	5.43^{bc}	90.00^{cd}

Table 1 – *continued*.

4.	**II Moraine of Ecology**: 35 m.a.s.l., 400 m from the sea, both lichens and *Colobanthus quitensis* with *Deschampsia antarctica* are found in this area; size of vascular plants 3.5–7.5 cm; growth is quite intensive; the flowering is abundant. Habitat dry and exposed with minor influence of animal rookeries both penguins and seals.	12	6.55^{cd}	0.63^{a}	0.02^{a}	34.59^{d}	6.54^{a}	11.71^{a}	53.41^{d}	3.13^{d}	3.42^{e}	13.77^{d}	17.24^{e}	62.45^{a}
5.	**Puchalski**: 110 m.a.s.l., 500 m from the sea, in the neighborhood of W. Puchalski grave; typical Antarctic tundra; old penguin rookery; vascular plants, mosses and lichens form dense mat; vascular plants growth slowly and reach the size of 1.5 –2.5 cm, habitat dry and exposed	23	5.47^{a}	4.92^{c}	0.29^{b}	16.22^{bc}	25.15^{c}	29.20^{bc}	27.18^{a}	0.05^{a}	0.55^{bc}	3.05^{b}	4.67^{b}	91.68^{d}
6.	**Jersak Hills**: 200 m.a.s.l.; in the vicinity of the glacier; far away from the coast 700 m; rocky area with little amount of soil; flora dominated by lichens; *Colobanthus quitensis* and *Deschampsia antarctica* are rare, and found as individual specimens, habitat dry and exposed, soil very poor with low content of organic matter	10	6.19^{bc}	2.03^{ab}	0.10^{a}	20.64^{c}	5.63^{a}	12.21^{a}	53.56^{d}	0.64^{bc}	1.37^{d}	5.37^{c}	6.97^{c}	85.67^{b}
7.	**The Youngest Moraine of Ecology**: 0.5 m.a.s.l.; 20 m from the sea, the youngest moraine; in the vicinity of the lagoon; very moist area, within the range of direct influence of sea water, with minor influence of animal rookeries both penguins and seals.	13	6.89^{de}	0.85^{a}	0.02^{a}	50.82^{e}	10.63^{b}	13.51^{a}	31.43^{ab}	3.76^{e}	4.05^{f}	13.53^{d}	13.46^{d}	65.21^{a}
8.	***Arctowski* Station**: 0.5 m m.a.s.l.; 50 m from the sea, the ground transformed due to continuous human influence (road, water tank); humid and shelter from the wind	31	6.97^{e}	0.62^{a}	0.02^{a}	37.24^{d}	3.58^{a}	19.65^{ab}	56.66^{d}	0.88^{c}	1.51^{d}	5.31^{c}	5.50^{bc}	86.80^{bc}

a, b, c, d, e – the same letters means lack of statistically significant differences (Fischer's LSD multiple range test, $p < 0.05$).

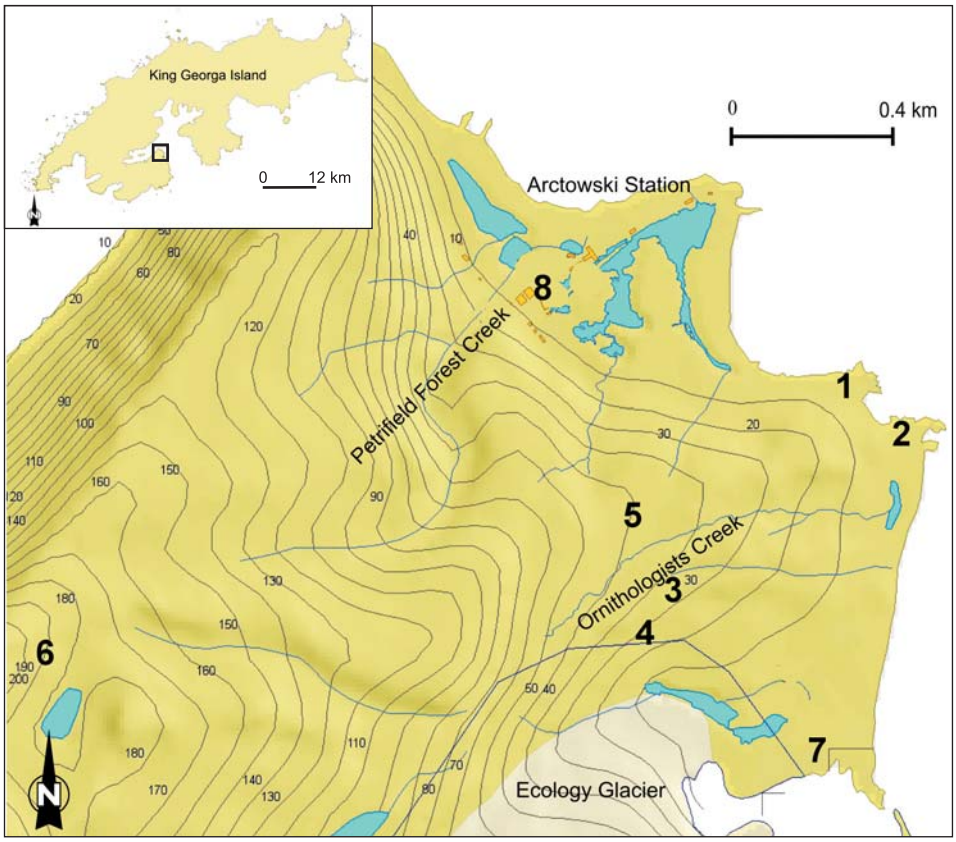

Fig. 1. Study area showing sampling sites of *Colobanthus quitensis* on King George Island. Numbers of sites according to Table 1.

(A&A Biotechnology); and 30–40 ng of template DNA. The PCR was performed using the following protocol: 1 cycle at 94°C for 3 min., followed by 30 cycles (15 s at 94°C, 60 s at 50–54°C (see Table 2), 60 s at 68°C) and final extension at 72°C for 5 min). In the case of PCR reactions where the primer combination 2076×2085 was used, the applied annealing temperature was 54°C. Amplification products were analyzed by gel electrophoresis in 1.5% (w/v) agarose with 1× TBE electrophoresis buffer at 100 V for 2 h, and visualized by staining with 0.5 μg/ml ethidium bromide.

Data analysis. — All bands that could be reliably read were treated as single dominant loci and scored either present (1) or absent (0) across genotypes. On the base of the obtained binary matrix of amplification products (bands) the following genetic parameters were estimated: total number of bands per population (N_B), percentage of polymorphic bands (P), Shannon's Information index (I) and expected heterozygosity (H_e). Nei's pairwise genetic distances (Nei 1972) among all analysed populations were also estimated. The matrix of genetic distances was

Table 2

iPBS primers applied in the study and their specification

Primer Number	Sequence	Tm(°C)	Number of amplified bands
2076	5'-GCTCCGATGCCA-3	54[1]	24[2]
2085	5'-ATGCCGATACCA-3	50	25
2224	5'-ATCCTGGCAATGGAACCA-3	52	23
2228	5'-CATTGGCTCTTGATACCA-3'	54	14
2231	5'- ACTTGGATGCTGATACCA-3'	52	16
2240	5'-AACCTGGCTCAGATGCCA-3'	53	18
2378	5'-GGTCCTCATCCA-3'	53	23
		Total	143

1 – number of bands scored when iPBS2076 was used in combination with primer iPBS2085;
2 – annealing temperature applied in PCR with combination of primers 2076×2085

used to perform Principal Coordinates Analysis (PCoA) analysis to investigate patterns of genetic subdivision of analysed populations of *Colobanthus quitensis.* All calculations mentioned above were performed with the GenAlEx 6.5 software (Peakall and Smouse 2012). The data were also tested for presence of population structure by AMOVA (Analysis of Molecular Variance) using Arlequin 3.5 software (Excoffier 2005). For that analysis, the iPBS data were treated as haplotypic, comprising a combination of alleles at one or several loci (Excoffier 2005). The significances of the fixation indices were tested using a non-parametric permutation approach (Excoffier *et al.* 1992).

Results

Efficiency of iPBS primers. — Our analysis of *Colobantus quitensis* populations from King George Island, using 7 iPBS primers/primers combination, yielded 143 clearly distinct amplification products (Table 2). The highest number (25) of bands was revealed by the iPBS2085 primer, whereas the lowest number (14) was scored for the iPBS2228 primer. The average number of bands per primer was 20.43. Out of the all identified loci, 55 (38.5%) were polymorphic. Of a total of 143 bands scored, 9 (6.3%) amplification products were represented as private bands – *i.e.* observed only in one population and absent in the others. The highest number of private alleles (4) was found in the population 3. Also, a high number of private alleles (3) was observed for the population 8. In the case populations 2 and 6, one private allele was found in each population. Populations 1, 4, 5 and 7 had no private alleles.

Genetic diversity and differentiation. — The iPBS markers revealed the presence of genetic polymorphism among individual specimens within populations, and low level of genetic variation between populations (Table 3). The num-

Table 3

Population genetic characteristics for analysed populations of *Colobanthus quitensis*.

Population	N_B	P	I	H_e
1.	111	4.90%	0.027	0.018
2.	127	19.58%	0.072	0.045
3.	123	16.08%	0.062	0.039
4.	115	9.09%	0.046	0.031
5.	118	11.19%	0.049	0.032
6.	119	17.48%	0.095	0.064
7.	118	11.19%	0.045	0.029
8.	130	24.48%	0.089	0.057
Mean over loci and populations	120.125	14.25%	0.061	0.040

ber of iPBS bands ranged from 111 for population 1, to 130 for population 8. The highest number of polymorphic bands was scored for population 8 (24.5%), whereas the lowest polymorphism was observed for population 1 (4.9%). The genetic variation was assessed with two parameters: Shannon's Information index (I) and expected heterozygosity (H_e), and in the both cases the highest values were observed for population 6 from Jersak Hills, while the lowest value was for population 1 (Penguin Rookery).

The AMOVA analysis revealed that most of the described genetic variation occurred among individuals within populations (83.57%), whereas remaining 16.43% of variation was attributed to variation between populations (Table 4).

In order to estimate the genetic differentiation between *C. quitensis* populations, pairwise genetic distance values were calculated (Table 5). Values of that parameter ranged from 0.002 to 0.027 (on average 0.014). On the basis of genetic distance values, the analyzed populations were subjected to grouping based on

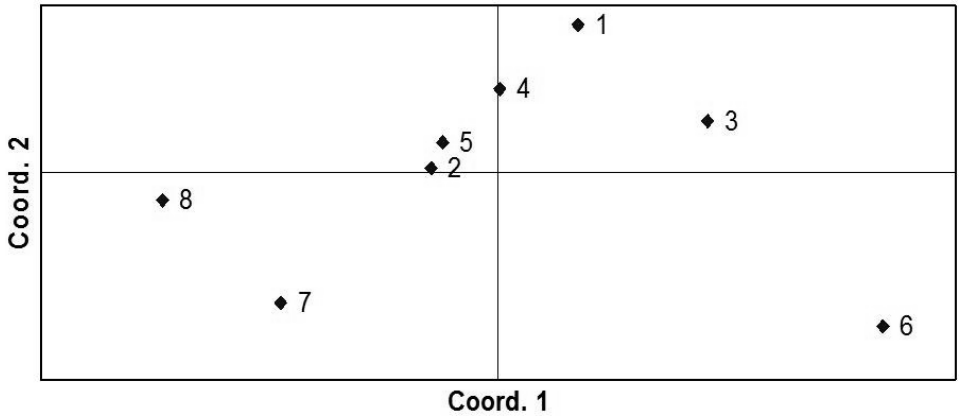

Fig. 2. Principal coordinates analysis (PCoA) based on Nei Genetic distances between eight *Colobanthus quitensis* populations.

Table 4

Partitioning of diversity found in *Colobanthus quitensis* from all analysed populations using AMOVA (F_{ST} = 0.164).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	7	81.629	0.588	16.43
Within populations	114	340.773	2.989	83.57
Total	121	422.402	3.577	

Significance tests (1023 permutations); p <0.001.

Table 5

Pairwise genetic distances between analysed *Colobanthus quitensis* populations.

	1	2	3	4	5	6	7	8
1								
2	0.013							
3	0.011	0.012						
4	0.011	0.014	0.007					
5	0.013	0.011	0.007	0.002				
6	0.021	0.020	0.012	0.018	0.017			
7	0.019	0.013	0.017	0.015	0.011	0.021		
8	0.018	0.013	0.019	0.011	0.008	0.027	0.010	

PCoA. This revealed that 74.68% of variation is explained by the first three components (33.98%, 22.95% and 17.75%, respectively). Figure 2 illustrates the projection of the analyzed populations on the first two axes. The grouping revealed by PCoA shows that the most distinct characteristics are represented by populations 6 and 8, and to a lesser extent, by populations 7 and 3, departed from the others along the Coord. 1. The remaining populations 1, 2, 4, and 5 can be merged into one group, where populations 2 and 5 seem to share the highest similarity.

Discussion

A very characteristic feature of maritime Antarctic ice-free areas are mosaics of microhabitats (Chwedorzewska *et al.* 2015), extremely differentiated by abiotic features *e.g.*, water conditions (Nędzarek *et al.* 2014), salinity or nutrient content of soil (Rakusa-Suszczewski and Nędzarek 2002). At the King George Island, a number of diversified habitats can be found which vary considerably in microclimatic conditions, as well as soil moisture and nutrient content. These three characteristics appear to be the main factors responsible for successful growth and propagation of plants, especially in the harsh Antarctic environment. Eight sampling sites of *C. quitensis* chosen for this study represent microhabitats which can be found in maritime Antarctic. Some of them appear to create relatively good conditions for plant growth. Such locations are characterized by

good soil conditions (high content of nutrients, optimal moisture), and relatively stable microclimate (shelter from the wind with good exposure to sunlight). Alternatively, there are locations characterized by very poor soils or over-manuring, exposed to strong winds or direct influence of sea water (occasionally flooded with salty water). There are also transitional sites and locations under strong influence of human activity.

Regardless of the nature of the stress factor, the reaction of the organism is directed to the development of mechanisms enabling it to survive in the stress conditions. On the genetic level, one of the main mechanisms associated with response to stress factors is the activation of the transposable elements (TE) (McClintock 1984; Capy *et al.* 2000; Schrader *et al.* 2014; Makarevitch *et al.* 2015). Increasing numbers of TE copies in response to the stress factors has been thought to be associated with decreased fitness through increased lethality (Wilke *et al.* 1992; Charlesworth *et al.* 1994; Stapley *et al.* 2015). However, it was proved for many plant species that the tendency of TE to insert into repetitive DNA mitigates their deleterious potential (SanMiguel *et al.* 1996; Kalendar *et al.* 1999; Suoniemi *et al.* 1997; Ramsay *et al.* 1999). Furthermore, it was observed that a rapid mutational process in plants caused by TE during environmental stress, could be adventitious for the particular group of organisms, by rapidly increasing genotypic variation, which may be associated with adaptation for abiotic stress (Wessler 1996; Kalendar *et al.* 2000; Finatto *et al.* 2015). The development of iPBS technique allowed for tracking genomic changes induced by TE in species for which genomic information is limited: *Prunus armeniaca* (Baránek *et al.* 2012), *Malus x domestica* (Kuras *et al.* 2013), *Cicer* species (Andeden *et al.* 2013), *Psidium guajava* (Mehmood *et al.* 2013), *Myrica rubra* (Chen and Liu 2014). However, so far, iPBS genotyping has not been applied to the analysis of genetic variation shaped in environmental stress gradient.

Genetic characterization of *C. quitensis* with the application of iPBS markers revealed that on average 14.25% of the observed amplification products were polymorphic, whereas in previous studies the mean level of polymorphism for iPBS markers reached 85.7% for guava accessions (Mehmood *et al.* 2013), or 86.3% for grape varieties (Guo *et al.* 2014), or even 97.4% for *Myrica rubra* (Chen and Liu 2014). Only Baránek *et al.* (2012) reported lower level of polymorphism for iPBS markers (4.88%), but their analyses aimed at genetic identification of clones of the apricot cultivar.

Previous studies on genetic characteristics of *C. quitensis* pointed at its low genetic diversity and differentiation even among spatially isolated populations. Lee and Postle (1975), on the basis of results obtained for nine izoenzymatic systems, reported "virtually no genetic variation" for the two *C. quitensis* populations originated from West Falkland Island and Tierra del Fuego. Analyses of Gianoli *et al.* (2004) performed on two populations of *C. quitensis*, one from the Andes of central Chile and the other from maritime Antarctic, pointed at only 1.17% of sequence divergence within ITS regions 1 and 2, which was an evidence for rela-

tively high genetic similarity of populations studied despite the significant geographic distance between them.

Application of iPBS markers also pointed at *C. quitensis* low genetic diversity ($H_e = 0.040$) and its rather moderate population differentiation ($F_{ST} = 0.164$). In our case, the highest H_e values observed in populations 6 and 8 may be explained by the rise in genetic variation due to highest activity of TE in response to intense environmental stress. Contrary, the lowest genetic variation was found in population 1 which grows in conditions which can be regarded as the optimal for vegetation (rich and humid). This area is densely covered not only by *C. quitensis,* but also by *D. antarctica* and mosses. The results of AMOVA analysis showed that the most of the genetic variation (83.57%) is portioned within populations, whereas only 16.43% describe differences between populations. High level of genetic variation detected within populations may be a result of independent mutation events in individuals from particular locations, caused by the TE activation by the environmental stress. Rather moderate genetic differentiation of populations reflects on one side the reproductive biology of the *C. quitensis,* which is an autogamous species capable for asexual reproduction (Moore 1970; Smith 2003), and on the other side the lack of sufficient physiographic barriers for gene flow. Analogous partition of genetic variation was described also for *D. antarctica* (Chwedorzewska and Bednarek 2008) which seems to share with *C. quitensis* not only the same habitats, but also strategy of reproduction favoring self-fertilization and/or vegetative propagation in response to harsh polar conditions (Holdegeregger *et al.* 2003).

The observed geographic pattern of genetic variation of *C. quitensis* revealed by iPBS markers may also reflect the genetic variation associated with TE and developed in response to diverse microhabitat conditions characteristics for each sampling site. The individual molecular character of population 6 corresponds well with the unique characteristic of Jersak Hills location. In this site, due to the intense influence of various abiotic stress factors (poor soil, bad water conditions and exposure to strong winds), the high genetic variation developed, probably as a result of TE mobilisation, instead of expected genetic erosion caused by intense selection processes (Table 3). By analogy, high H_e value and distant position of population 8 may reflect the TE mobilisation as the response to strong influence of sea spray (salt stress, the highest pH of the soil among all analysed sampling sites) and/or to environment disturbance by human activity (*e.g.* vegetation trampling and/or diasporas transfer on shoes from other locations). In the case of population 7 its genetic variation may have been modified by strong selection from high level of fresh (melting snow) and sea water (the area is within the range of direct influence of sea aerosols, temporarily flooded with sea water). In the case of populations 1, 2, 4 and 5, their quite close placement and low and moderate H_e values may reflect less intense mobilization of TEs, due to less diversifying influence of local environmental conditions. These locations may be described as areas with good conditions for germination due to very rich soil – attributed to ongoing or presently

abounded bird colonies (populations 1, 2 and 5), and favourable microclimate (population 4). In the case of population 3, exposition to strong winds as well as indirect influence of sea water, may independently shape its genetic variation. According to our observations, the exposure of particular site to strong winds causing the water stress appears to be one of the main factors limiting the plant growth in the study area. Even a shallow hollow in the ground appears to be a sufficient shelter from the winds. In such case, plants are intensively green, larger and generally appear healthy, whereas when exposed to direct wind, they are smaller, often with yellow leaves and a large share of necromass.

It has to be emphasized here that so far, there is no answer to the question, whether the adaptation in polygenic traits (believed to be involved in adaptation to marginal environments) is a result of many mutations of small phenotypic effect, or a result of a few large mutations (Orr 2005). Nevertheless, the application of the molecular markers based on the TE, like iPBS, appears to be very helpful in assessment of the scale of genome rearrangements arising in response to abiotic stress. Furthermore, although the particular *C. quitensis* populations are not very different from each other, according to the iPBS data, we obtained an interesting geographic pattern of genetic variation, corresponding with the data describing soil properties of the sampling sites. The iPBS markers proved to be an efficient DNA fingerprinting method in the absence of initial genomic information. Moreover, the observed pattern of genetic differentiation of analysed populations confirmed our expectations that genetic variation pattern revealed by iPBS markers may be influenced by the abiotic stress, and thus shaped in the response to local environment conditions.

Acknowledgements. — Part of this research was supported by the Polish Ministry of Scientific Research and Higher Education grant 2013/09/B/NZ8/03293. We thank anonymous reviewers for friendly, helpful, constructive criticism and remarks which have greatly improved our manuscript.

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Received 9 April 2015
Accepted 10 August 2015