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Genetic diversity and population structure of *Brassica oleracea* germplasm in Ireland using SSR markers

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ABSTRACT

The most economically important *Brassica oleracea* species is endangered in Ireland, with no prior reported genetic characterization studies. This study assesses the genetic diversity, population structure and relationships of *B. oleracea* germplasm in Ireland using microsatellite (SSRs) markers. A total of 118 individuals from 25 accessions of Irish *B. oleracea* were genotyped. The SSR loci used revealed a total of 47 alleles. The observed heterozygosity (0.699) was higher than the expected one (0.417). Moreover, the average values of fixation indices (*F*) were negative, indicating excess of heterozygotes in all accessions. Polymorphic information content (PIC) values of SSR loci ranged from 0.27 to 0.66, with an average of 0.571, and classified 10 loci as informative markers (PIC > 0.5) to differentiate among the accessions studied. The genetic differentiation among accessions showed that 27.1% of the total genetic variation was found among accessions, and 72.9% of the variation resided within accessions. The averages of total heterozygosity (H_T) and intra-accession genetic diversity (H_S) were 0.577 and 0.442, respectively. Cluster analysis of SSR data distinguished among kale and Brussels sprouts cultivars. This study provided a new insight into the exploitation of the genetically diverse spring cabbages accessions, revealing a high genetic variation, as potential resources for future breeding programs. SSR loci were effective for differentiation among the accessions studied.

1. Introduction

The genus *Brassica* L., belonging to the family Brassicaceae, contains six economically important species cultivated worldwide [1]. These species are *B. oleracea*, *B. rapa*, *B. nigra*, *B. napus*, *B. juncea*, and *B. carinata*. *B. oleracea* L. is an important vegetable crop species, including many economic cultivars called cole crops [2]. The cole crops include cauliflower (*B. oleracea* subsp. *botrytis*), cabbage (*B. oleracea* subspecies *capitata*), Brussels sprout (*B. oleracea* subsp. *gemmifera*), kale and collards (*B. oleracea* subsp. *acephala*), broccoli (*B. oleracea* subsp. *italica*), and kohlrabi (*B. oleracea* subsp. *gongylodes*). The evaluation of genetic diversity within crop species is vital for establishing efficient conservation and breeding practices [3–7] in order to develop new and more productive crops that are resistant to diseases and adapted to changing environments.

Many studies have assessed the genetic diversity and relationships of *Brassica* species worldwide [8–12]. However, there are no reported molecular genetic studies to our knowledge on the endangered *B. oleracea* species in Ireland that requires a long-term commitment to ensure that

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important endangered genetic resources are conserved and that existing collections are properly characterised, stored and maintained either in situ or ex situ as appropriate [13]. Those endangered *B. oleracea* germplasms have been collected from different locations throughout Ireland in 1980s, and maintained at the Horticultural Research Institute (HRI) in the United Kingdom. Their use is still very limited due to the lack of genetic characterisation and poor phylogenetic studies [13]. Therefore, our novel current study aimed to evaluate the genetic variation and relationships of a core collection of those endangered Irish *B. oleracea* genetic resources in order to improve their utilisation and conservation strategies.

Molecular markers proved to be powerful tools for evaluating genetic variation and relationships in plant species. Among these are simple sequence repeats (SSRs), alternatively known as microsatellite markers, which have been successfully used for assessing the genetic variability and distinguishing among closely related *Brassica* genotypes [8–12,14,15], because of their codominance, high polymorphism and ability to reveal a high number of alleles for each locus, resulting in a high degree of variability and reproducibility [16].

Leroy et al. [14] used four microsatellite primers to characterise *B. oleracea* accessions. Among the 136 reproducible fragments generated, 25 (18.4%) fragments were common for all *Brassica*, 27 (19.9%) were unique, and 84 (61.7%) were phylogenetically informative. Flannery et al. [15] assessed polymorphisms in *Brassica*, *Arabidopsis*, *Camelina*, *Raphanus* and *Sinapis* using 10 plastid SSR primer sets. Eight loci were polymorphic, and separated the individuals of Brassicaceae into taxon-specific groups (*Arabidopsis*, *Camelina*, *Sinapis* and *Brassica* genera). Louarn et al. [8] also evaluated 59 *B. oleracea* cultivars for microsatellite polymorphisms. All SSR markers, except one, produced a polymorphic information content (PIC value) of 0.5 or above. Ofori et al. [9] evaluated the genetic diversity in European winter *B. rapa* using microsatellie markers, and found that the majority of genetic variation (83%) resided within cultivars. Furthermore, Moghaddam et al. [10] studied the genetic variability among 32 rapeseed cultivars based on SSRs markers, and reported that the polymorphic information content (PIC) of SSRs loci varied from 0.60 to 0.91. All of these studies confirmed that microsatellite markers are very useful for assessing genetic diversity and relationships in *Brassica* species. Therefore, this current study aimed to evaluate the genetic diversity and phylogenetic relationships in the endangered *B. oleracea* cultivars in Ireland using microsatellite markers.

2. Material and methods

2.1. Plant material

Twenty-five accessions of Irish *B. oleracea* were obtained from the germplasm collection maintained at the Horticultural Research Institute (HRI), Wellesbourne, United Kingdom (Table 1). These accessions were selected based on their sampling site covering a diverse geographical range of Ireland. The accessions represented 4 subspecies within *B. oleracea* species (*B. oleracea capitata*, *B. oleracea acephala*, *B. oleracea botrytis and B. oleracea gemmifera*).

2.2. DNA extraction

Genomic DNA was isolated from 3-week-old leaf tissue using DNeasy Plant Mini Kit (Qiagen, United Kingdom),

Table 1
Accession numbers, crop names, and collection sites of the accessions of *Brassica oleracea* studied.

No.	Accession number	Subspecies	Accession name	Crop name	Geographical origin
1	HRIGRU 4502	*Brassica oleracea acephala*	Marrow Stem	Fodder kale	Kildare
2	HRIGRU 4503	*Brassica oleracea acephala*	Thousand Head	Fodder kale	Kildare
3	HRIGRU 7229	*Brassica oleracea acephala*	Cut and Come Again	Kale	Tipperary
4	HRIGRU 7556	*Brassica oleracea acephala*	Cut and Come Again	Kale	Cork
5	HRIGRU 7227	*Brassica oleracea acephala*	Raggedy Jack	Kale	Sligo
6	HRIGRU 4492	*Brassica oleracea botrytis*	Winter Roscoff	Winter cauliflower	Dublin
7	HRIGRU 4565	*Brassica oleracea botrytis*		Winter cauliflower	Cork
8	HRIGRU 4495	*Brassica oleracea botrytis*	Winter Roscoff	Winter cauliflower	Ballykea
9	HRIGRU 4579	*Brassica oleracea capitata*	Flat Dutch	Cattle cabbage	Donegal
10	HRIGRU 4561	*Brassica oleracea capitata*	Flat Dutch	Cattle cabbage	Galway
11	HRIGRU 4508	*Brassica oleracea capitata*	Flat Dutch	Cattle cabbage	Ballina
12	HRIGRU 4506	*Brassica oleracea capitata*	Flat Dutch	Cattle cabbage	Ballinrobe
13	HRIGRU 4585	*Brassica oleracea capitata*	Flat Dutch	Common cabbage	Donegal
14	HRIGRU 4586	*Brassica oleracea capitata*	Flat Dutch	Common cabbage	Mayo
15	HRIGRU 4497	*Brassica oleracea capitata*	Flat Dutch	Cabbage	Roscommon
16	HRIGRU 4498	*Brassica oleracea capitata*	Flat Dutch	Cabbage	Roscommon
17	HRIGRU 4588	*Brassica oleracea capitata*	Flat Dutch	Cabbage	Donegal
18	HRIGRU 5915	*Brassica oleracea capitata*	Flat Dutch	Cabbage	Limerick
19	HRIGRU12532	*Brassica oleracea capitata*	Delaway Cabbage	Cabbage	Mayo
20	HRIGRU 4566	*Brassica oleracea capitata*		Spring cabbage	Cork
21	HRIGRU 4564	*Brassica oleracea capitata*		Spring cabbage	Cork
22	HRIGRU 4571	*Brassica oleracea capitata*		Spring cabbage	Cork
23	HRIGRU 5914	*Brassica oleracea capitata*	Spring Greens	Spring cabbage	Limerick
24	HRIGRU 4491	*Brassica oleracea gemmifera*		Brussels sprout	Dublin
25	HRIGRU 4494	*Brassica oleracea gemmifera*		Brussels sprout	Dublin

following the procedures described by manufacturers. Three to five DNA samples were prepared from each of the 25 accessions studied and were subjected to SSRs analysis.

2.3. SSRs analysis

Initially, a set of 17 SSR primer sets specific to *Brassica* chromosomes were selected based on the analysis of the relevant literature [11,12,17,18] and were screened for polymorphisms using three different cabbage accessions. Following screening, 12 primer sets revealed polymorphism and were used to analyse all the accessions (Table 2).

The PCR reactions were performed in a final volume of 25 μL containing 2 μL of genomic DNA (25 ng/μL), 1.5 μL of forward primer (50 ng/μL), 1.5 μL of reverse primer (50 ng/μL), 12.5 μL of GoTaq® green master mixture and 7.5 μL of water (nuclease free). The same reaction mixture without genomic DNA was set up to serve as a negative control. The PCR reactions were then amplified in a DNA thermocycler (G-Storm GS1 thermal cycler, GRI Ltd., Gene House, Essex, United Kingdom) programmed as follows: (1) 94 °C for 2 min (initial denaturation); (2) 94 °C for 1 min (denaturation), (3) 54 °C for 1 min (annealing), 72 °C for 1 min (extension) per 35 cycles; (4) 72 °C for 20 min (final extension), then 4 °C (infinitive). The amplified PCR products were resolved on 2% (w/v) agarose gels stained with ethidium bromide. A 50 bp DNA ladder was used as a DNA molecular size standard. Bands were detected and photographed using a UVP gel documentation system (Ultra-Violet Products Ltd., Cambridge, United Kingdom).

2.4. Data analysis

Because of the codominance of the markers, microsatellites were scored as homozygotic and heterozygotic genotypes. The SSR data were analysed using GelCompar II version 6.0 Applied Maths, GenAlEx version 6 [19] and POPGENE version 1.31 software packages. The dendrogram was constructed based on Nei's genetic distance using UPGMA [20]. The partitioning of total genetic diversity into within- and among accession components was examined using Nei's [20,21] genetic diversity statistics. The polymorphic information content (PIC) of each SSR marker was also calculated using the following formula:

$$\mathrm{PIC}_i = 1 - \sum_{j=1}^{n} P_{ij}^2$$

where PIC_i is the polymorphic information content of a marker I, and P_{ij} is the frequency of the jth pattern for marker I, and the summation extends over n patterns.

3. Results

3.1. SSR markers and alleles scored

The 12 nuclear SSR markers used in this study were single-locus and polymorphic. These SSR primer pairs (loci) revealed a total of 47 alleles (Table 2). The alleles generated ranged in size from 50 to 276 bp. The number of alleles amplified by each primer pair (locus) varied from 2 to 5, with an average value of 3.92 alleles per locus. The primer pair Ol12-E03 amplified the lowest number of alleles (2), with sizes ranging from 160 to 190 bp. The primer pairs Ol10-A03a, Ol10-F11a and Ra2-E03 amplified the highest number of alleles (5), with sizes ranging from 50 to 160 bp, 64 to 240 bp and 110 to 245 bp, respectively. A total of 4 unique alleles were detected at 3 SSR loci. The accession of winter cauliflower HRIGRU4495 had a unique allele with a size of 220 bp at locus Ol12-F11 (Table 2), whereas the accession of kale HRIGRU7227 had 3 unique

Table 2
SSR primer sets, number and size of alleles amplified in the 25 accessions of *Brassica oleracea* studied.

SSR primer sets (loci)	Sequences	Size range (bp)	Total number of alleles	Number of unique alleles
Ol10-A03a	F: CTGGTTTTCTCCTTCATCAG R: CTGTGTAGCTTTTAGTCTTT	50–160	5	0
Ol10-F11a	F: TTTGGAACGTCCGTAGAAGG R: CAGCTGACTTCGAAAGGTCC	64–240	5	1
Ol10-H02	F: AACAGGAAGAAACGACGAGG R: AGAGAGCCATGAGAAGCACC	98–260	4	0
Ol11-G11	F: GTTGCGGGCGAAACAGAGAAG R: GAGTAGGCGATCAAACCGAG	70–210	4	0
Ol11-H02	F: TCTTCAGGGTTTCCAACGAC R: AGGCTCCTTCATTTGATCCC	96–250	4	2
Ol12-E03	F: CTTGAAGAGCTTCCGACACC R: GACGGCTAACAGTGGTGGAC	160–190	2	0
Ol12-F11	F: AAGGACTCATCGTGCAATCC R: GTGTCAGTGGCTACAGAGAC	100–276	4	1
Ol13-C12	F: AGAGGCCAACAAAGAACACC R: GAAGCAGCACCAGTGACAAG	84–196	3	0
Ra2-E03	F: AGGTAGGCCCATCTCTCTCC R: CCAAAACTTGCTCAAAACCC	110–245	5	0
Ra2-E11	F: GGAGCCAGGAGAGAAGAAGG R: CCCAAAACTTCCAAGAAAAGC	74–208	3	0
Na12-C08	F: GCAAACGATTTGTTTACCCG R: CGTGTAGGGTGATCTATGATGGG	68–190	4	0
Na14-C12	F: CACATTTTGGTTCAATTCGG R: TACGACCTGGTTTCGATTC	92–198	4	0

alleles with sizes of 240 bp, 210 bp and 248 bp at the loci Ol10-F11a and Ol11-H02, respectively (Table 2).

3.2. Genetic diversity and accession-level homozygosity

As shown in Table 3, the observed heterozygosity (H_o) varied from 0.467 in the accession of spring cabbage HRIGRU4571 to 0.80 in the accessions of kale HRIGRU7227, cauliflowers HRIGRU4492 and HRIGRU4495, cattle cabbage HRIGRU4508, cabbages HRIGRU4588 and HRIGRU5915, and spring cabbage HRIGRU5914, with an average of 0.699. The expected heterozygosity (H_e) ranged from 0.333 in the accessions of cauliflower HRIGRU4565 and cabbage HRIGRU4497 to 0.556 in the accession of spring cabbage HRIGRU4566, with an average of 0.417. Moreover, the effective number of alleles per locus (A_e) varied from 1.597 in the accession of cabbage HRIGRU4497 to 2.394 in the accession of spring cabbage HRIGRU4566, with a mean of 1.906 (Table 3). The mean number of alleles per locus (A) ranged from 1.8 to 2.8 with an average of 2.096. These results indicated that the SSR loci in *B. oleracea* accessions analysed presented uneven allele frequencies (A_e = 1.906). The average fixation indices values (F) were lower than zero for all accessions studied (Table 3). These negative values indicated an excess of heterozygotes.

Table 4 shows the estimates of genetic diversity at the level of each subspecies analysed in this study. In *B. oleracea capitata* (cabbages), the values of observed and expected heterozygosity were 0.693 and 0.548, respectively, whereas the inter-accession genetic differentiation as measured by F_{st} showed that 24.3% of the total genetic variation was found among accessions of *B. oleracea capitata* (cabbages) and 75.7% of the variation resided within these accessions. In *B. oleracea botrytis* (cauliflowers), the values of observed and expected heterozygosity were 0.689 and 0.446, respectively, whereas the inter-accession genetic differentiation showed that 11.1% of the total genetic variation was found among accessions of *B. oleracea botrytis*, and 88.9% of the variation resided within them. Moreover, in *B. oleracea acephala* (kales), the values of observed and expected heterozygosity were 0.693 and 0.459, respectively, and the inter-accession genetic differentiation showed that 11% of the total genetic variation was found among accessions of *B. oleracea acephala* (kales), and 89% of the variation resided within them. In *B. oleracea gemmifera* (sprouts), the values of observed and expected heterozygosity were 0.733 and 0.536, respectively, whereas the inter-accession genetic differentiation showed that 9.8% of the total genetic variation was found among accessions of *B. oleracea gemmifera*, and 90.2% of the microsatellites variation resided within them (Table 4).

3.3. Genetic structure and gene flow

The 12 SSR loci selected were statistically significant ($P < 0.001$) for discriminating among the 25 accessions studied (Table 5). The polymorphic information content (PIC) values based on SSR markers ranged from 0.27 (locus Ol12-E03) to 0.66 (locus Ol10-A03a) with an average of 0.571. The SSR marker Ol10-A03a had the highest PIC

Table 3
Sample sizes, estimates of genetic diversity, and average fixation index (F) in the 25 accessions of *Brassica oleracea* studied.

Accession number	H_o	H_e	A	A_e	F
Fodder kale HRIGRU 4502	0.733	0.433	2.2	1.989	−0.693
Fodder kale HRIGRU 4503	0.667	0.422	2.4	1.914	−0.581
Kale HRIGRU 7229	0.600	0.389	2.2	1.760	−0.542
Kale HRIGRU 7556	0.667	0.378	1.8	1.720	−0.765
Kale HRIGRU 7227	0.800	0.422	2.0	1.914	−0.896
Cauliflower HRIGRU 4492	0.800	0.467	2.4	2.143	−0.713
Cauliflower HRIGRU 4565	0.533	0.333	2.0	1.789	−0.601
Cauliflower HRIGRU 4495	0.800	0.400	1.8	1.800	−1.000
Cattle cabbage HRIGRU 4579	0.733	0.456	2.2	2.114	−0.607
Cattle cabbage HRIGRU 4561	0.733	0.411	2.0	1.874	−0.783
Cattle cabbage HRIGRU 4508	0.800	0.444	2.2	2.029	−0.802
Cattle cabbage HRIGRU 4506	0.667	0.378	1.8	1.720	−0.765
Common cabbage HRIGRU 4585	0.733	0.422	2.2	1.914	−0.737
Common cabbage HRIGRU 4586	0.733	0.389	1.8	1.760	−0.884
Cabbage HRIGRU 4497	0.533	0.333	1.8	1.597	−0.601
Cabbage HRIGRU 4498	0.667	0.378	2.0	1.791	−0.765
Cabbage HRIGRU 4588	0.800	0.400	1.8	1.800	−1.000
Cabbage HRIGRU 5915	0.800	0.422	2.0	1.914	−0.896
Cabbage HRIGRU 12532	0.600	0.433	2.2	1.989	−0.386
Spring cabbage HRIGRU 4566	0.667	0.556	2.8	2.394	−0.199
Spring cabbage HRIGRU 4564	0.667	0.456	2.4	2.103	−0.463
Spring cabbage HRIGRU 4571	0.467	0.344	1.8	1.637	−0.358
Spring cabbage HRIGRU 5914	0.800	0.400	1.8	1.800	−1.000
Brussels sprout HRIGRU 4491	0.733	0.544	2.6	2.263	−0.347
Brussels sprout HRIGRU 4494	0.733	0.422	2.2	1.914	−0.737
Mean ± standard deviation	0.699 ± 0.094	0.417 ± 0.054	2.096 ± 0.278	1.906 ± 0.191	−0.676 ± 0.216

H_o: the observed heterozygosity; **H_e**: the expected heterozygosity; **A**: the mean number of alleles per locus; **A_e**: the effective number of alleles per locus; **F**: Wright's F inbreeding coefficient (fixation index).

Table 4
Estimates of genetic diversity in the subspecies of *Brassica oleracea* studied based on SSR data.

Subspecies	The observed heterozygosity (H_o)	The expected heterozygosity (H_e)	The inter-accession genetic differentiation (F_{ST})
B. o. capitata (cabbages)	0.693	0.548	0.243
B. o. botrytis (cauliflowers)	0.689	0.446	0.111
B. o. acephala (kales)	0.693	0.459	0.110
B. o. gemmifera (sprouts)	0.733	0.536	0.098

Table 5
F-statistics, Nei's [21] genetic diversity indices, polymorphic information content, and estimates of inter-accession gene flow.

SSR primer loci	*F*-statistics			Nei's genetic diversity indices			PIC	Nm_w	χ^2	*P*
	F_{IS}	F_{IT}	F_{ST}	H_T	H_S	D_{ST}				
Ol10-A03a	−0.545	−0.209	0.215	0.662	0.517	0.145	0.66	0.900	52.57	0.000*
Ol10-F11a	−0.684	−0.320	0.214	0.647	0.507	0.140	0.65	0.906	32.25	0.0004*
Ol10-H02	−0.850	−0.588	0.142	0.588	0.505	0.084	0.59	0.514	56.85	0.000*
Ol11-G11	−0.681	−0.319	0.214	0.642	0.504	0.138	0.62	0.900	32.04	0.000*
Ol11-H02	−0.544	−0.208	0.217	0.661	0.516	0.145	0.65	0.601	52.50	0.000*
Ol12-E03	1.000	1.000	0.632	0.267	0.098	0.169	0.27	0.145	77.84	0.000*
Ol12-F11	−0.736	−0.489	0.142	0.609	0.523	0.087	0.61	0.709	51.17	0.000*
Ol13-C12	−0.682	−0.318	0.212	0.640	0.503	0.136	0.62	0.905	31.50	0.0000*
Ra2-E03	−0.543	−0.207	0.217	0.662	0.517	0.145	0.65	0.600	52.57	0.000*
Ra2-E11	1.000	1.000	0.633	0.269	0.099	0.170	0.28	0.144	77.80	0.000*
Na12-C08	−0.680	−0.317	0.212	0.642	0.504	0.138	0.63	0.902	30.15	0.000*
Na14-C12	−0.544	−0.208	0.216	0.660	0.516	0.144	0.65	0.903	50.42	0.000*
Mean ± standard deviation	−0.374 ± 0.649	−0.100 ± 0.526	0.271 ± 0.170	0.577 ± 0.147	0.442 ± 0.161	0.137 ± 0.026	0.571 ± 0.145	0.676 ± 0.288	49.81 ± 16.49	0.0001*

F_{IS}: the fixation index related to non-random mating within accessions; **F_{IT}**: the mean inbreeding coefficient of a set of accessions; **F_{ST}**: the inter-accession genetic differentiation due to genetic drift; **H_T**: the total genetic diversity; **H_S**: the genetic diversity within accessions; **D_{ST}**: the genetic diversity among accessions; **PIC**: the polymorphic information content of a marker; **Nm_w**: the gene flow estimate according to Wright's (1951) equation; **χ^2**: Chi^2 to test F_{ST} for significant difference from zero; **P**: the probability value (significant F_{ST} at $P < 0.001$).
* Significant at $P < 0.001$

value, which was expected as it produced the highest number of alleles.

F-statistics revealed varying fixation indices among the loci (Table 5). The estimates of fixation indices (F_{is}) revealed 10 SSR loci (Ol10-A03a, Ol10-F11a, Ol10-H02, Ol11-G11, Ol11-H02, Ol12-F11, Ol13-C12, Ra2-E03, Na12-C08 and Na14-C12) with heterozygotes excess, as indicated by the negative average values of fixation indices. The two loci Ol12-E03 and Ra2-E11 exhibited heterozygote deficiency. The F_{is} values calculated ranged from –0.850 at locus Ol10-H02 to 1.0 at loci Ol12-E03 and Ra2-E11, with an average of –0.374. Moreover, the mean inbreeding coefficient (F_{it}) values of all accessions ranged from –0.588 at locus Ol10-H02 to 1.0 at loci Ol12-E03 and Ra2-E11, with an average of –0.1. The inter-accession genetic differentiation (F_{st}) ranged from 0.142 to 0.633 with an average of 0.271.

The estimates of accessions genetic structure using Nei's [21] genetic diversity statistics are shown in Table 5. The level and distribution of genetic variation and heterozygosity were estimated. The averages of total heterozygosity (H_T) and intra-accession genetic diversity (H_S) were 0.577 and 0.442, respectively. Moreover, the inter-accession genetic diversity (D_{ST}) varied from 0.084 to 0.170, with an average of 0.137. The number of migrants per generation based on Wright's equation (Nm_w) was 0.676 (Table 5).

3.4. Cluster analysis

The dendrogram constructed based on Nei's [20] genetic distance using UPGMA showed the relationships among the 25 accessions of *B. oleracea* studied (Fig. 1). It

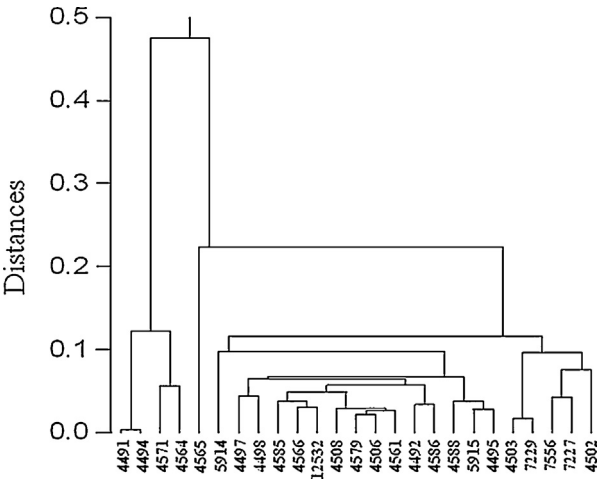

Fig. 1. UPGMA dendrogram based on Nei's [20] genetic distance, showing the relationship among 25 accessions of *Brassica oleracea* based on SSR data.

revealed two major groups. The first group included two distinct clusters; the first cluster contained the two accessions of Brussels sprout, whereas the second one included the two accessions of spring cabbage (HRIGRU4571 and HRIGRU4564). The second group splits into three subgroups; the first one contained the accession of winter cauliflower HRIGRU4565, whereas the second one included all the accessions of kales. The four accessions of cattle cabbage formed a distinct cluster in the third subgroup. The two accessions of winter cauliflower HRIGRU4492 and 4495 and all remaining accessions of cabbages were distributed among different clusters within the third subgroup (Fig. 1).

4. Discussion

Molecular characterization of plant genetic diversity and relationships using microsatellite markers is promising because of their codominance and ability to reveal a high number of alleles per polymorphic locus. This is the first comprehensive study that assesses genetic diversity, population structure, and relationships in Irish *B. oleracea* species using the powerful microsatellite technique. The 12 SSR loci used in this study were significantly polymorphic and useful for differentiation among the accessions studied. These results were in agreement with that reported by Raybould et al. [22] who revealed that all microsatellite loci were polymorphic, and displayed significant spatial differentiation of genetic variation in *B. oleracea*. Moreover, the 12 SSR loci revealed a total of 47 alleles with an average value of 3.92 alleles per locus. This average value was higher than that reported by Cui et al. [23] for *B. rapa* (2.91). Four unique alleles were detected for two accessions (winter cauliflower HRIGRU4495 and kale HRIGRU7227) at 3 SSR loci, representing 8.5% of the total number of alleles generated. This percentage was lower than that reported by Leroy et al. [14] for *B. oleracea* accessions (19.9%). This could be due to the difference in the SSR loci and/or the accessions assessed. The unique alleles could be used as markers to genetically distinguish among *Brassica* genotypes.

In the present study, the observed heterozygosity was higher than the expected one for the 25 accessions studied. However, the average values of fixation indices (F) were lower than zero, indicating an excess of heterozygotes in all accessions studied. The heterozygote excess observed could be attributed to the outcrossing breeding system and the low effective population size of the accessions studied. The expected heterozygosity ($H_e = 0.417$) and the mean number of alleles per locus ($A = 2.096$) in our results were lower than those reported by Ofori et al. [9] for *B. rapa* ($H_e = 0.507$ and $A = 3.58$). The difference in this data could be attributed to the differences in *Brassica* species or the methodology used.

In the present study, F-statistics revealed varying fixation indices among the loci. Ten SSR loci showed heterozygotes excess. However, the accessions analysed contained a high genetic diversity, but the distribution of this variation was not homogenous. The genetic differentiation among accessions as measured by F_{st} showed that 27.1% of the total genetic variation was due to differences among accessions, and 72.9% of the microsatellites variation resided within accessions. Therefore, the majority of the total genetic variation resided within accessions. This result agreed with that reported by Lázaro and Aguinagalde [24], Watson-Jones et al. [25] and Hintum et al. [26]. These results also correspond to the short-lived herbaceous plants, which gained a relatively high genetic variation, but most of this genetic diversity resided within accessions. The distribution of SSR variation within and among the accessions studied is the product of interactions among several evolutionary factors, including selection, effective population size and the ability of the species to disperse pollen and seeds.

The average value of the total heterozygosity (H_T) in our results was 0.577. This value was higher than that reported by Hintum et al. [26], Watson-Jones et al. [25] and Lázaro and Aguinagalde [24,27] for *B. oleracea* (0.249, 0.25, 0.338 and 0.294, respectively). The intra-accessional genetic diversity (H_S) was 0.44, whereas this value was higher than that reported by Hintum et al. [26] for *B. oleracea* (0.13). The differences in this data could again be attributed to the differences in *Brassica* accessions or the methodology used. The low levels of genetic differentiation among accessions ($F_{ST} = 0.271$, $\chi^2 = 49.81$, $P < 0.001$) and the inter-accession genetic diversity ($D_{ST} = 0.137$) were probably indicative of a relatively high gene flow, which was confirmed by the estimates of the number of migrants per generation based on Wright's equation ($Nm_w = 0.676$). However, this result confirmed the presence of a high percentage of cross-pollination in the plant.

Polymorphic information content (PIC) is considered as one of the important features of the molecular markers and could be used to assess the differentiation ability of the markers [28]. PIC values ranged from 0.27 to 0.66, and classified 10 SSR loci as informative markers ($PIC > 0.5$). The average PIC value of all SSR markers was 0.571, indicating the ability of the utilised markers to differentiate the *B. oleracea* accessions studied. However, this average value was lower than that reported by Moghaddam et al. [10] for *B. oleracea* (0.69), but higher than that reported by Cui et al. [23] for *B. rapa* (0.54). The difference in these data may be attributed to the differences in the *Brassica* species or the SSR loci used. The cluster analysis of our SSR data distinguished many cultivars. It showed that the kale and Brussels sprouts formed distinct clusters, but the cauliflowers could not be fully distinguished. Furthermore, the analysis showed that spring cabbages had a considerable level of genetic variation, and were distributed among different clusters. This study provided a new insight into the use of those promising genetically diverse spring cabbages accessions, revealing a high genetic variation, as potential resources for future breeding programs to develop new and more productive crops indeed. SSR markers showed that the gene pool of Irish *B. oleracea* has a high genetic variation. SSRs markers, revealed high polymorphism in this study, may also be used for genetic analysis studies in other related crops [29–35].

5. Conclusions

This study assessed the genetic diversity, population structure and relationships of *B. oleracea* germplasm across

Ireland using 12 microsatellite markers. SSR loci were found to be significantly polymorphic and effective for differentiation among the accessions studied. The observed heterozygosity was higher than the expected one for all the accessions, which had an excess of heterozygotes. The majority of the genetic variation resided within accessions. Those genetic features of Irish *B. oleracea* could provide insights and guidelines for protecting this species from extinction as well as developing its practical conservation strategies. This study also provided new interesting results and provides insight into the choice and use of the most variable spring cabbages accessions, identified here as potential resources for future breeding programs to develop new and more productive crops. SSR markers proved to be an effective platform for *Brassica* germplasm characterization and association mapping studies. Further analysis should analyse the variation of Irish accessions in relation to those found in Europe.

Authors' contributions

ME designed and performed the experiments, analyzed the data, and wrote the manuscript. KG contributed to the experimental design, data analysis, and the writing of the manuscript. PB contributed to the experimental design and the writing of the manuscript. RM contributed to the experimental design, data analysis and the writing of the manuscript. All authors read and approved the final version of the manuscript.

Disclosure of interest

The authors declare that they have no competing interest.

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