



Molecular biology and genetics/Biologie et génétique moléculaires

# 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.

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## 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 microsatellite markers, and found that the majority of genetic variation (83%) resided within cultivars. Furthermore, Moghaddam et al. [10] studied the genetic variability among 32 rape-seed 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      | <i>Brassica oleracea acephala</i>  | Marrow Stem        | Fodder kale        | Kildare             |
| 2   | HRIGRU 4503      | <i>Brassica oleracea acephala</i>  | Thousand Head      | Fodder kale        | Kildare             |
| 3   | HRIGRU 7229      | <i>Brassica oleracea acephala</i>  | Cut and Come Again | Kale               | Tipperary           |
| 4   | HRIGRU 7556      | <i>Brassica oleracea acephala</i>  | Cut and Come Again | Kale               | Cork                |
| 5   | HRIGRU 7227      | <i>Brassica oleracea acephala</i>  | Raggedy Jack       | Kale               | Sligo               |
| 6   | HRIGRU 4492      | <i>Brassica oleracea botrytis</i>  | Winter Roscoff     | Winter cauliflower | Dublin              |
| 7   | HRIGRU 4565      | <i>Brassica oleracea botrytis</i>  |                    | Winter cauliflower | Cork                |
| 8   | HRIGRU 4495      | <i>Brassica oleracea botrytis</i>  | Winter Roscoff     | Winter cauliflower | Ballykea            |
| 9   | HRIGRU 4579      | <i>Brassica oleracea capitata</i>  | Flat Dutch         | Cattle cabbage     | Donegal             |
| 10  | HRIGRU 4561      | <i>Brassica oleracea capitata</i>  | Flat Dutch         | Cattle cabbage     | Galway              |
| 11  | HRIGRU 4508      | <i>Brassica oleracea capitata</i>  | Flat Dutch         | Cattle cabbage     | Ballina             |
| 12  | HRIGRU 4506      | <i>Brassica oleracea capitata</i>  | Flat Dutch         | Cattle cabbage     | Ballinrobe          |
| 13  | HRIGRU 4585      | <i>Brassica oleracea capitata</i>  | Flat Dutch         | Common cabbage     | Donegal             |
| 14  | HRIGRU 4586      | <i>Brassica oleracea capitata</i>  | Flat Dutch         | Common cabbage     | Mayo                |
| 15  | HRIGRU 4497      | <i>Brassica oleracea capitata</i>  | Flat Dutch         | Cabbage            | Roscommon           |
| 16  | HRIGRU 4498      | <i>Brassica oleracea capitata</i>  | Flat Dutch         | Cabbage            | Roscommon           |
| 17  | HRIGRU 4588      | <i>Brassica oleracea capitata</i>  | Flat Dutch         | Cabbage            | Donegal             |
| 18  | HRIGRU 5915      | <i>Brassica oleracea capitata</i>  | Flat Dutch         | Cabbage            | Limerick            |
| 19  | HRIGRU12532      | <i>Brassica oleracea capitata</i>  | Delaway Cabbage    | Cabbage            | Mayo                |
| 20  | HRIGRU 4566      | <i>Brassica oleracea capitata</i>  |                    | Spring cabbage     | Cork                |
| 21  | HRIGRU 4564      | <i>Brassica oleracea capitata</i>  |                    | Spring cabbage     | Cork                |
| 22  | HRIGRU 4571      | <i>Brassica oleracea capitata</i>  |                    | Spring cabbage     | Cork                |
| 23  | HRIGRU 5914      | <i>Brassica oleracea capitata</i>  | Spring Greens      | Spring cabbage     | Limerick            |
| 24  | HRIGRU 4491      | <i>Brassica oleracea gemmifera</i> |                    | Brussels sprout    | Dublin              |
| 25  | HRIGRU 4494      | <i>Brassica oleracea gemmifera</i> |                    | Brussels sprout    | Dublin              |

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