C. R. Biologies 339 (2016) 163–170

Contents lists available at ScienceDirect

Comptes Rendus Biologies

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Molecular biology and genetics/Biologie et génétique moléculaires

AFLP analysis of genetic diversity and phylogenetic relationships of *Brassica oleracea* in Ireland

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ARTICLE INFO

Article history:
Received 7 February 2016
Accepted after revision 16 March 2016
Available online 4 May 2016

Keywords:
AFLP
Brassica oleracea
Genetic diversity
Relationships

ABSTRACT

Brassica oleracea L. is one of the most economically important vegetable crop species of the genus *Brassica* L. This species is threatened in Ireland, without any prior reported genetic studies. The use of this species is being very limited due to its imprecise phylogeny and uncompleted genetic characterisation. The main objective of this study was to assess the genetic diversity and phylogenetic relationships of a set of 25 Irish *B. oleracea* accessions using the powerful amplified fragment length polymorphism (AFLP) technique. A total of 471 fragments were scored across all the 11 AFLP primer sets used, out of which 423 (89.8%) were polymorphic and could differentiate the accessions analysed. The dendrogram showed that cauliflowers were more closely related to cabbages than kales were, and accessions of some cabbage types were distributed among different clusters within cabbage subgroups. Approximately 33.7% of the total genetic variation was found among accessions, and 66.3% of the variation resided within accessions. The total genetic diversity (H_T) and the intra-accessional genetic diversity (H_S) were 0.251 and 0.156, respectively. This high level of variation demonstrates that the Irish *B. oleracea* accessions studied should be managed and conserved for future utilisation and exploitation in food and agriculture. In conclusion, this study addressed important phylogenetic questions within this species, and provided a new insight into the inclusion of four accessions of cabbages and kales in future breeding programs for improving varieties. AFLP markers were efficient for assessing genetic diversity and phylogenetic relationships in Irish *B. oleracea* species.

1. Introduction

Brassica oleracea L. is one of the most economically important vegetable crop species of the genus *Brassica* L. in the tribe Brassiceae, which in turn belongs to the family Brassicaceae [1]. This species includes many important cultivars called cole crops [2], comprising cabbage (*B. oleracea* subspecies *capitata*), cauliflower (*B. oleracea* subsp. *botrytis*), Brussels sprout (*B. oleracea* subsp. *gemmifera*), broccoli (*B. oleracea* subsp. *italica*), Kale and collards (*B. oleracea* subsp. *acephala*), and kohlrabi (*B. oleracea* subsp. *gongylodes*). Assessment of genetic diversity, population structure, and relationships is very essential for crop characterisation and conservation, which in turn are important to the continued maintenance and enhancement of agricultural production, leading to

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http://dx.doi.org/10.1016/j.crvi.2016.03.002
1631-0691/

sustainable development and global food security [3,4]. Although the phylogeny and genetic diversity of *Brassica* species have been significantly investigated in the past decade [4–7], there are no reported genetic studies to our knowledge on *B. oleracea* species in the small island of Ireland [8], whose economy is depending largely on the agricultural production of highly agronomic crops in order to meet the needs of the increasing number of populations. Large numbers of those *B. oleracea* genetic resources have been collected from different environments and locations throughout Ireland in 1980s, and deposited at the Horticultural Research Institute (HRI) in the United Kingdom. The use of those GenBank resources is being very limited due to their uncompleted characterisation, imprecise phylogeny, and the unmanageable large numbers of accessions that are poor and in danger [8].

Since investigation of the phylogenetic relationships and genetic variation based on morphological and cytological traits could be influenced by environmental factors [9–11], molecular markers have been established and proven to be powerful tools for assessing the genetic diversity and phylogenetic relationships in plants. These molecular markers include AFLP (Amplified Fragment Length Polymorphism) technique developed by Vos et al. [12]. Although it is expensive, AFLP technique proved to be very effective and powerful when compared to other molecular techniques such as Restriction Fragment Length Polymorphism (RFLP) and Random Amplified Polymorphic DNA (RAPD), due to its ability to detect various polymorphisms in various genomic regions that allow the differentiation of closely related species as well as its highly reproducible data and larger numbers of amplified products generated in a single reaction [13]. This method has been successfully used for investigating phylogeny and genetic diversity in *Brassica* and many other plant species for example, sesame [14], common bean [15], potato [16] and *Brassica* [4–7,17]. However, those studies did not cover the Irish *B. oleracea* species. Consequently, the main objective of the current study was to use the powerful AFLP technique to assess the genetic diversity and phylogenetic relationships of a set of the Irish *B. oleracea* genetic resources deposited at the Horticultural Research Institute (HRI), United Kingdom.

2. Materials 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), United Kingdom (Table 1). These accessions were chosen based on their sampling site covering a diverse geographical range of the island of Ireland. The selected 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), following the procedures described by manufacturers. Five DNA samples were prepared from each of the 25 accessions studied and were subjected to AFLP analysis.

2.3. AFLP analysis

Sixteen AFLP primer sets were selected from the literature [18,19] and used in a screening test for

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

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