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Assessment of Morphological Variation in Irish *Brassica oleracea* Species

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Abstract

This study aimed at assessing the morphological variation in 25 accessions of an Irish collection of *Brassica oleracea* using 44 morphological traits. The morphological traits showed a pronounced variation among accessions. Vegetative preflowering and maturity traits revealed higher variation than seedling traits. Of the 44 morphological descriptors used, 28 were significantly different and proved useful in examining diversity and differentiating among accessions studied. Principal component analysis (PCA) showed variation among accessions, where 87.24% of the total variation in the morphological data was separated on the first five components, indicating a high degree of correlation among traits studied. Cluster analysis grouped the 25 accessions into 4 main groups, corresponding to kales, cauliflowers, cabbages and sprouts, and also showed a sort of correlation between clustering pattern and eco-geographical distribution of the accessions. Furthermore, this study identified 9 highly diverse accessions, providing opportunities for optimising parental sources in future breeding programs to develop new or more productive *Brassica* varieties. Morphological traits were deemed useful for assessing the diversity and relationships in Irish *Brassica oleracea* species.

Keywords: *Brassica oleracea*, morphological variation, relationships, trait

1. Introduction

The genus *Brassica* L. is one of the most economically important genera in the tribe Brassiceae, which in turn belongs to the family Brassicaceae (Rakow, 2004). This genus comprises a diverse group of species including major vegetable and oilseed crops with a wide range of agronomic traits (Rich, 1991; Christopher et al., 2005). It contains six economically important species with great genetic and morphological diversity which are cultivated worldwide (Saha et al., 2008). Three of these species are diploid (*Brassica oleracea*, 2n = 18; *Brassica rapa*, 2n = 20; *Brassica nigra*, 2n = 16), and three are amphidiploid (*Brassica napus*, 2n = 38; *Brassica juncea*, 2n = 36; *Brassica carinata*, 2n = 34). *Brassica* species play an important role in agriculture and horticulture, as well as contributing both to the economy and health of populations around the world (King, 2005; Zhao, 2007).

Brassica oleracea L. is an important vegetable crop species which includes many vegetable cultivars called cole crops (Katz, 2003). These cole crops comprise cabbage (*Brassica oleracea* subspecies *capitata*), cauliflower (*Brassica oleracea* subspecies *botrytis*), brussels sprout (*Brassica oleracea* subspecies *gemmifera*), broccoli (*Brassica oleracea* subspecies *italica*), Kale and collards (*Brassica oleracea* subspecies *acephala*), and kohlrabi (*Brassica oleracea* subspecies *gongylodes*). Cole crops have extreme morphological characteristics. Examples of such morphologies include the enlarged infloresences of cauliflower and broccoli; the enlarged stems of kohlrabi and marrowstem kale; the enlarged single apical bud of cabbage; and the several axillary buds of brussels sprout (Paterson et al., 2001; Hong et al., 2008).

Assessment of diversity is an essential component in germplasm characterisation and conservation, which in turn are essential to the continued maintenance and improvement of agricultural production and, thus, to sustainable development and poverty alleviation (Karp et al., 1997). Morphological variation in plant species has been described for traits controlled by a single or multiple gene systems. However, the variation patterns in the morphological traits are considered to be the result of both genetic and environmental attributes (Rohlf et al., 1990; Rohlf, 2000). Morphological traits have been used to assess the genetic variation and relationships among populations of different plant species, for example, mungbean (Arshad et al., 2009; Tantasawat et al., 2010),

soybean (Iqbal et al., 2008), rice (Bibi et al., 2009), eggplant (Tümbilen et al., 2011) and *Brassica* (Rabbani et al., 1999; Kop et al., 2003; Balkaya et al., 2005).

Rabbani et al. (1999) used the morphological traits to assess the variation in oilseed mustard. 35 phenotypic traits were recorded for all the 52 accessions studied from seedling emergence up to crop harvest. The results revealed a considerable level of diversity among all tested accessions for various traits. Seedling traits showed less variation, while the largest variation was found for flowering and maturity stage traits. Generally, a low correlation was observed among different traits, however, some of the related characters were significantly correlated with each other. The results also indicated that oilseed mustard in Pakistan has narrow genetic base and experiences a high level of genetic erosion.

Balkaya et al. (2005) determined the differences regarding morphological variation of white head cabbage (*Brassica oleracea* var. *capitata* subvar. *alba*) genetic resources collected from different regions of Turkey. Their cluster analysis based on 12 quantitative and 10 qualitative variables identified 10 groups. Morphological variability was high among the white head cabbage genotypes of Turkey. The genotypes also displayed a wide diversity of characters for selection and combination of interesting genotypes for important characters to obtain improved varieties.

Although significant progress has been made in the past decade, *Brassica* biodiversity loss has not been halted in the island of Ireland. The status of many of Irish habitats and species is judged to be poor (NBP, 2011). Moreover, information on the genetic diversity and relationships in Irish *Brassica oleracea* species is currently very limited. Therefore, the main aim of this study was to assess the morphological variation and character association in Irish *Brassica oleracea* accessions based on morphological traits, thus providing a correct taxonomic ranging and a precise characterisation of these accessions.

2. Materials and Methods

2.1 Plant Material

Twenty-Five accessions of Irish *Brasssica oleracea* were obtained from the germplasm collection maintained at the Horticultural Research Institute (HRI), Wellsbourne, United Kingdom (Table 1). These accessions were chosen based on their sampling site covering a broad and diverse geographic range of the island of Ireland. However, the selected accessions represented 4 subspecies within *Brassica oleracea* species (*Brassica oleracea capitata, Brassica oleracea acephala, Brassica oleracea botrytis* and *Brassica oleracea gemmifera*).

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	*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

2.2 Experimental Design and Plant Growth

Seeds were sown in PPE modules of 12 x 12 cells filled with seedling compost (Shamrock) in the greenhouse facilities at the Teagasc Horticultural Research centre in Kinsealy, Dublin, Ireland. Four weeks after germination, the seedlings were transplanted to larger PPE pots containing compost in the greenhouse. After a further 5 weeks, the modules were removed and placed on a bench outside the greenhouse in order to harden the seedlings off for 2 weeks. Land plots were prepared within the field trial site at Teagasc Kinsealy. Prior to transplanting, the seedlings were treated with Dursan (an insecticide for the control of caterpillars, cabbage root fly and cutworms). The seedlings were then transferred into 5 blocks in the field, with 5 accessions per block in a randomized scheme (Figure 1). Sowing and growing the accessions at different time points are shown in Figure 2.

2 plants per plot: 7 plots per accession = 14 plants in total per accession
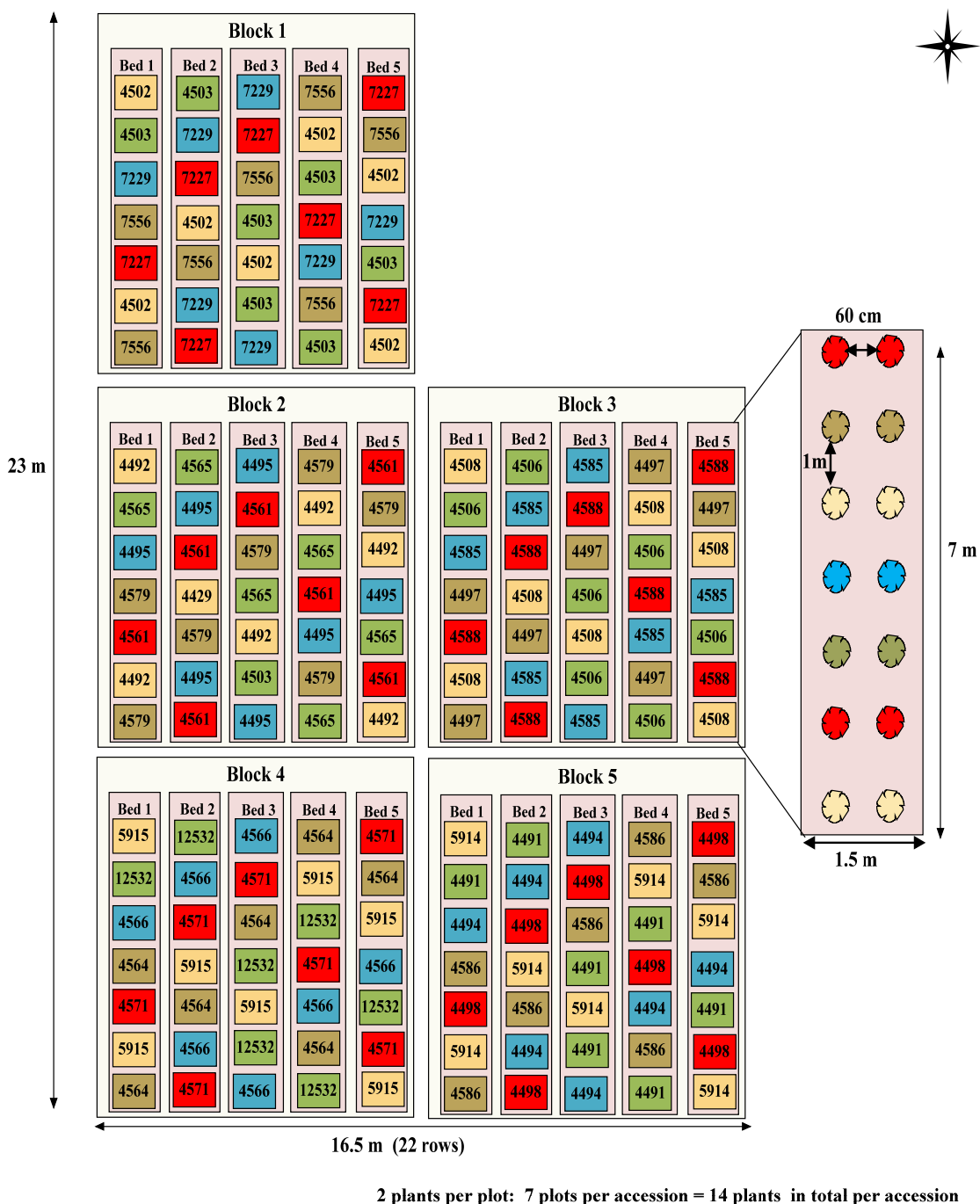
Figure 1. Randomised planting scheme used for transplanting *Brassica oleracea* accessions (the numbers in the blocks refers to the accessions numbers)
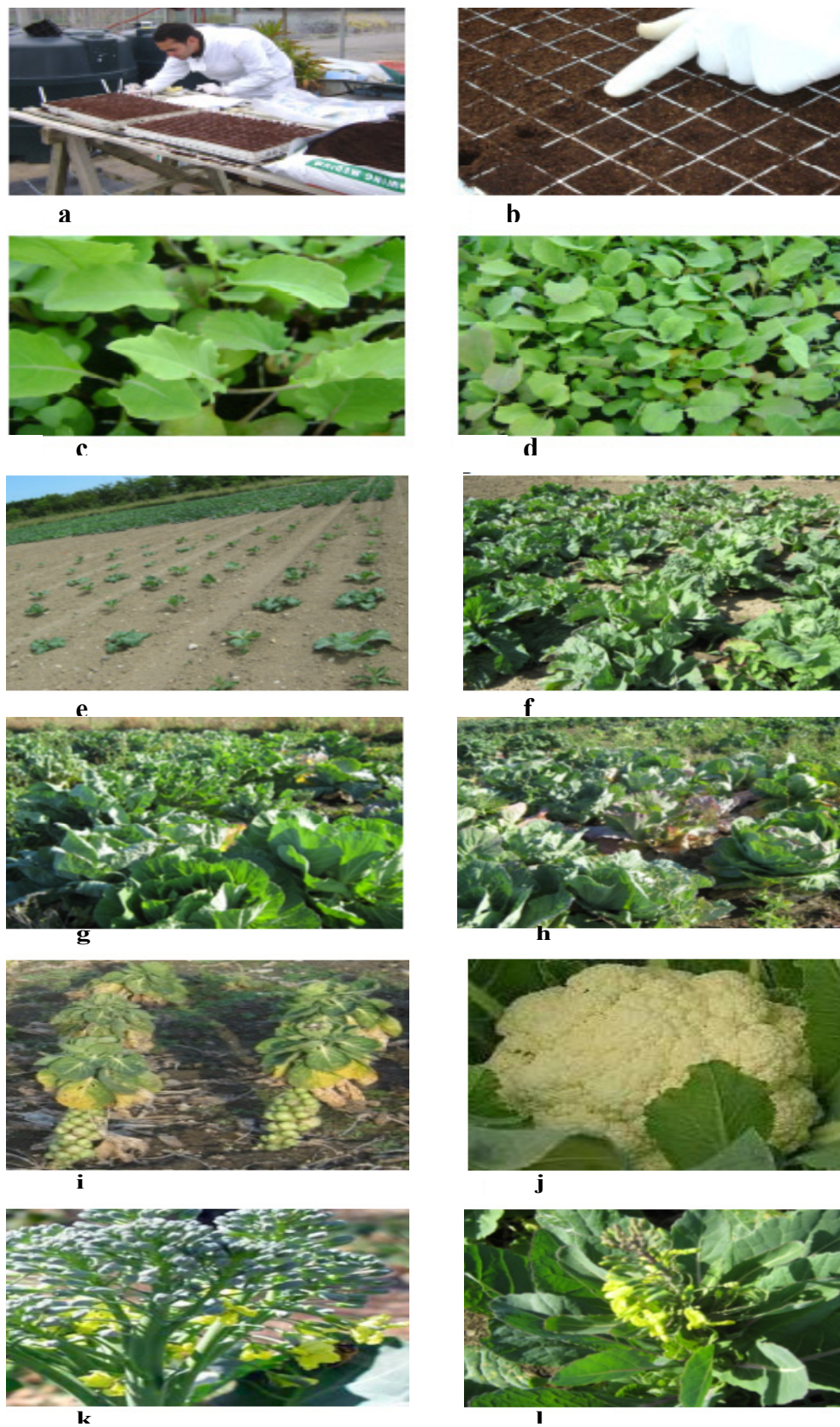
Figure 2. Sowing *Brassica* accessions at Teagasc Kinsealy (**a**, **b**, March 2009), seedling stage (**c**, **d**, April 2009), transplanting into field site (**e**, June 2009), growth of the plants (**f**, August 2009; **g**, **h**, September 2009), mature pre-flowering stage (**i**, **j**, October 2009), and flowering stage (**k**, **l**, December 2009)

2.3 Evaluation of Morphological Traits

Forty-four discriminating descriptors were selected from the list of morphological descriptors for *Brassica* and *Raphanus* published by the International Board for Plant Genetic Resources (IBPGR, 1990). These descriptors were considered to be suitable for *Brassica oleracea*, and were used in this study to characterize 10 plants of each of the 25 accessions studied. The descriptors selected included 5 descriptors at the seedling stage, 32 descriptors at the mature pre-flowering stage and 7 descriptors at the flowering stage (Table 2).

Table 2. Morphological traits and descriptor states applied to all accessions of *Brassica oleracea* studied (IBPGR, 1990)

IBPGR Descriptor No.	Trait	Descriptor state
4.1 Seedling		
4.1.1	Hypocotyl colour	Observe at 5-leaf stage
		1, White; 2, Pale green; 3, Green; 4, Pink; 5, Red; 6, Purple; 7, Other
4.1.2	Seedling leaf colour	1, White green; 2, Yellow green; 3, Light green; 4, Green; 5, Dark green; 6, Purple green; 7, Purple; 8, Other
4.1.3	Seedling leaf (marginal incisions)	0, Entire; 1, Crenate; 2, Dentate; 3, Serrate; 4, Undulate; 5, Doubly dentate; 6, Other
4.1.4	Seedling pubesence	0, Glabrous; 1, Very sparse; 3, Sparse; 5, Intermediate; 7, Abundant
4.1.5	Juvenile development	3, Slow; 5, Intermediate; 7, Fast
4.2 Vegetative preflowering mature		
4.2.2	Plant growth habit	1, Shortened nonbranching stem supporting leafy rosette; 2, Shortened nonbranching stem terminating in leafy head; 3, Extremely shortened branching stems forming leafy crown; 4, Elongate and/or enlarged nonbranching stem (tubers) supporting leaves and/or head; 5, Elongate nonbranching stem supporting enlarged compact axillary buds; 6, Elongate branching stems supporting leaves and/or heads; 7, Elongate nonbranching stem terminating in enlarged floral or prefloral apex (curd); 8, Elongate branching stems terminating in enlarged floral or prefloral apices;
		9, Enlarged root; 10, Other
4.2.9	Lodging	3, Low; 5, Intermediate; 7, High
4.2.11	Number of leaves (estimated)	3, Few; 5, Intermediate; 7, Many
4.2.16	Leaf blade shape in outline	1, Orbicular; 2, Elliptic; 3, Obovate; 4, Spathulate; 5, Ovate; 6, Lanceolate;
		7, Oblong; 8, Other
4.2.17	Leaf division (margin)	0, Entire; 1, Crenate; 2, Dentate; 3, Serrate; 4, Undulate; 5, Doubly dentate; 6, Other
4.2.18	Leaf division (incision)	1, Entire; 2, Sinuate; 3, Lyrate; 4, Lancerate; 5, Other
4.2.19	Leaf apex shape	2, Acute; 4, Intermediate; 6, Rounded;
		8, Broadly Rounded
4.2.24	Leaf colour	1, Yellow green; 2, Light green; 3, Green; 4, Dark green; 5, Purple green; 6, Purple; 7, Other
4.2.25	Leaf hairiness	0, Absent; 3, Sparse; 5, Intermediate; 7, Abundant
4.2.26	Leaf bloom	0, Absent, glossy; 3, Low; 5, Intermediate; 7, High, glaucous
4.2.32	Petiole section	3, Round; 5, Semiround; 7, Flat
4.2.33	Petiole and/or midvein colour	1, White; 2, Light green; 3, Green; 4, Purple; 5 Red; 6 Other
4.2.34	Heading habit	Observe at harvest, head-forming types only
		0, Nonheading; 5, Semiheading; 7, Heading
4.2.35	Head shape in longitudinal section	1, Triangular; 2, Ovate; 3, Obovate; 4, Elliptic; 5, Spheric; 6, Cylindric; 7, Transverse elliptic; 8, Other

4.2.36	Head-forming leaf overlap at terminal region	1, Leaves curled outwards; 2, Erect; 3, Curled inwards; 4, Slight overlap; 5, Strong overlap
4.2.37	Head cover from subtending leaves	3, Exposed; 5, Intermediate; 7, Covered
4.2.38	Primary colour of outer head leaves	1, Yellow green; 2, Pale green; 3, Green; 4, Dark green; 5, Red green; 6, Red or purple
4.2.39	Head size in relation to plant size	3, Small; 5, Intermediate; 7, Large
4.2.40	Head solidity	3, Low (soft, loose); 5, Intermediate; 7, High (firm, hard)
4.2.41	Head length (cm)	Measure median transverse section
4.2.42	Head diameter (cm)	Measured at widest point
4.2.47	Stem length in head, estimate	3, Short; 5, Intermediate; 7, Long
4.2.48	Primary colour inside cut	1, White; 2, Yellow; 3, Pale green; 4, Green; 5, Red green; 6, Red or purple
4.2.49	Tendency of head to split	3, Low; 5, Intermediate; 7, High
4.2.50	Head holding ability	3, Low; 5, Intermediate; 7, High
4.2.51	Relative time of maturity	3, Early; 5, Midseason; 7, Late
4.2.61	Leaf retention on stem	3, Low; 5, Intermediate; 7, High
4.2.63	Number of shoots or enlarged buds	3, Few; 5, Intermediate; 7, Many
4.2.66	Size of enlarged vegetative buds	3, Small; 5, Intermediate; 7, Large
4.2.67	Firmness of enlarged vegetative buds	3, Loose; 5, Intermediate; 7, Firm
4.2.68	Colour of enlarged vegetative buds or shoots	1, Light green; 2, Green; 3, Dark green; 5 Red/Purple green; 6 Red/purple
4.2.73	Floral apex branching pattern	1, Single flower raceme; 2, Enlarged stem with terminally branched raceme; 3, Loosely branched small terminal heads; 4, Terminal head with smaller heads on axillary shoots; 5, Compact head of regularly packed subheads; 6, Single compact head of irregularly packed subheads; 7, Other
4.3 Flowering plant characteristics		
4.3.2	Days to flowering	Number of days from sowing to when 50% of plants first flower
4.3.7	Flower stalk colour	Observed at base of stem
		1, White; 2, Yellow green; 3, Green; 4, Red green; 5, Red; 6, Purple; 7, Other
4.3.10	Flower stalk length, estimate	3, Short; 5, Intermediate; 7, Long
4.3.11	Flower stalk internode length	3, Short; 5, Intermediate; 7, Long
4.3.15	Flowering plant hairiness	0, Absent; 3, Few hairs; 5, Intermediate; 7, Hairy
4.3.16	Flower colour variability in the crop	3, Uniform; 5, Intermediate; 7, Highly variable, many colours
4.3.17	Petal colour	1, White; 2, Pale yellow; 3, Yellow; 4, Orange yellow; 5, Pink; 6, Red; 7, Purple; 8, Other

2.4 Data Analysis

The morphological data were standardised and converted to z-scores to avoid bias in the analysis due to scale differences in the variables (Legendre and Legendre, 1998). The standardised dataset was then used to estimate the genetic diversity and relationships among the accessions which were evaluated by Jaccard similarity index, cluster analysis, factor analysis, analysis of variance and correlation coefficients. These multivariate analyses were made using the software package SYSTAT for Windows, version 7.0 copyright (C) 1997, SPSS INC. A dendrogram was constructed through the average linkage-joining rule.

3. Results

3.1 Variation of Morphological Traits and Their Statistical Parameters

Of the 44 morphological traits analyzed in all the 25 accessions studied, 42 were polymorphic and therefore further used for the diversity analysis (Table 3). However, the hypocotyl colour and seedling leaf (marginal incisions) traits had no taxonomic value among the 25 accessions studied. Many traits showed a high variability among the accessions studied, for example, petiole section, petiole and midvein colour, leaf colour, primary colour of outer head leaves, and leaf division (incision). The minimum, maximum, range, mean, standard deviation, percent and

coefficient of variation were estimated for morphological traits analyzed in all the 25 accessions studied (Table 3). The quantitative characters measured exhibited broad variability. Head length, for example ranged from 0 to 22 cm, head diameter ranged from 0 to 19.5 cm, and days to flowering ranged from 240 to 307. Standard deviation (SD) and coefficient of variance (CV%) showed high values for most traits, e.g. head length (SD= 8.457; CV= 0.606) and head diameter (SD= 7.541; CV= 0.612). The coefficient of variance (CV%) showed a low value for days to flowering (CV= 0.078).

Table 3. Statistical parameters for quantitative and qualitative traits of *Brassica oleracea* accessions (see Table 1 for identification of accessions numbers)

Quantitative traits	Min.	Max.	Range	Mean	SD	CV
Head length	0	22	22	13.96	8.457	0.606
Head diameter	0	19.5	19.5	12.320	7.541	0.612
Days to flowering	240	307	67	263.96	20.491	0.078
Qualitative characterisation						

Traits	Descriptor state	Percent	Accessions numbers
Seedling leaf colour	Light green	44%	1, 2, 7, 9, 10, 11, 12, 19, 20, 21, 22
	Green	48%	3, 4, 5, 6, 8, 15, 16, 17, 18, 23, 24, 25
	Purple green	8%	13, 14
Seedling pubesence	Glabrous	44%	6, 7, 8, 10, 11, 12, 15, 16, 18, 24, 25
	Very sparse	40%	1, 2, 3, 4, 9, 13, 14, 17, 21, 22
	Sparse	16%	5, 19, 20, 23
Juvenile development	Fast	16%	3, 5,11,18
	Intermediate	84%	The rest of the accessions
Plant growth habit	Shortened nonbranching stem supporting leafy rosette	12%	3, 4, 5
	Elongate and/or enlarged nonbranching stem supporting leaves and/or head	8%	1, 2
	Elongate nonbranching stem supporting enlarged compact axillary buds	8%	24, 25
	Elongate nonbranching stem terminating in enlarged floral or prefloral apex	12%	6, 7, 8
	Shortened nonbranching stem terminating in leafy head	60%	The rest of the accessions studied
Lodging	Intermediate	16%	1, 2, 24, 25
	High	84%	The rest of the accessions
Number of leaves	Intermediate	8%	1, 2
	Many	92%	The rest of the accessions
Leaf blade shape in outline	Elliptic	28%	1, 2, 3, 4, 5, 19, 23
	Oblong	12%	6, 7, 8
	Orbicular	60%	The rest of the accessions
Leaf division (margin)	Crenate	8%	24, 25
	Dentate	92%	The rest of the accessions
Leaf division (incision)	Lancerate	16%	13, 14, 19, 23
	Lyrate	84%	The rest of the accessions
Leaf apex shape	Intermediate	40%	1, 2, 3, 4, 5, 6, 7, 8, 19, 23
	Rounded	60%	The rest of the accessions
Leaf colour	Dark green	12%	7, 24, 25
	Purple green	4%	10
	Green	84%	The rest of the accessions
Leaf hairiness	Sparse	4%	5
	Absent	96%	The rest of the accessions
Leaf bloom	Absent, glossy	8%	24, 25
	Intermediate	92%	The rest of the accessions
Petiole section	Semiround	12%	4, 24, 25
	Flat	12%	6, 7, 8
	Round	76%	The rest of the accessions

Petiole and/or midvein colour	Green	8%	24, 25
	Purple	4%	19
	Light green	88%	The rest of the accessions
Heading habit	Nonheading	20%	1, 2, 3, 4, 5
	Heading	80%	The rest of the accessions
Head shape in longitudinal section	Spheric	80%	6,7,8,9,10,11,12,13,14,15,16,17,18, 19,20,21,22,23,24,25
Head-forming leaf overlap at terminal region	Curled inwards	12%	6, 7, 8
	Strong overlap	68%	9,10,11,12,13,14,15,16,17,18,19,20, 21,22,23,24,25
Head cover from subtending leaves	Intermediate	20%	6, 7, 8, 24, 25
	Covered	60%	9,10,11,12,13,14,15,16,17,18,19,20, 21,22,23
Primary colour of outer head leaves	Green	64%	6,8,9,10,12,13,14,15,16,17,18,19,20,21,22,23
	Dark green	12%	7, 24, 25
	Red green	4%	11
Head size in relation to plant size	Small	8%	24, 25
	Intermediate	12%	6, 7, 8
	Large	60%	9,10,11,12,13,14,15,16,17,18,19,20, 21,22,23
Head solidity	High (firm, hard)	80%	6,7,8,9,10,11,12,13,14,15,16,17,18, 19,20,21,22,23,24,25
Stem length in head, estimate	Intermediate	80%	6,7,8,9,10,11,12,13,14,15,16,17,18, 19,20,21,22,23,24,25
Primary colour inside cut	White	52%	6, 7, 8, 12, 15, 16, 17, 18, 19, 20, 21, 22, 23
	Pale green	28%	9, 10, 11, 13, 14, 24, 25
Tendency of head to split	Low	36%	15, 16, 17, 18, 19, 20, 21, 22, 23
	Intermediate	44%	6, 7, 8, 9, 10, 11, 12, 13, 14, 24, 25
Head holding ability	High	80%	6,7,8,9,10,11,12,13,14,15,16,17,18, 19,20,21,22,23,24,25
Relative time of maturity	Late	32%	1, 2, 3, 4, 5, 6, 7, 8
	Midseason	68%	The rest of the accessions
Leaf retention on stem	Low	8%	24, 25
	Intermediate	4%	5
	High	88%	The rest of the accessions
Number of shoots or enlarged buds	Many	8%	24, 25
Size of enlarged vegetative buds	Large	8%	24, 25
Firmness of enlarged vegetative buds	Firm	8%	24, 25
Colour of enlarged vegetative buds or shoots	Green	4%	25
	Dark green	4%	24
Floral apex branching pattern	Single compact head of irregularly packed subheads	12%	6, 7, 8
Flower stalk colour	Green	12%	8, 24, 25
	Red green	20%	1, 2, 3, 4, 5
	Yellow green	68%	The rest of the accessions
Flower stalk length, estimate	Intermediate	32%	1, 2, 3, 4, 5, 6, 7, 8
	Long	8%	24, 25
	Short	60%	The rest of the accessions
Flower stalk internode length	Intermediate	32%	1, 2, 3, 4, 5, 6, 7, 8
	Long	8%	24, 25
	Short	60%	The rest of the accessions
Flowering plant hairiness	Few hairs	8%	24, 25
	Absent	92%	The rest of the accessions
Flower colour variability in the crop	Intermediate	4%	25
	Uniform	96%	The rest of the accessions
Petal colour	Yellow	32%	1, 3, 4, 9, 11, 15, 18, 24
	Pale yellow	68%	The rest of the accessions

Most of accessions were of green seedling leaf color (48%) (Table 3). Also light green color of seedling leaf is represented by a high percentage (30%). While only the 2 accessions of common cabbage were of purple green seedling leaf color. Most of accessions were of glabrous seedling pubescence, intermediate juvenile development, shortened nonbranching stem terminating in leafy head, high lodging, many number of leaves, orbicular leaf blade shape, dentate leaf margin, lyrate leaf incision, rounded leaf apex, green leaf colour, absent leaf hairiness, intermediate leaf bloom, rounded petiole section, spheric head shape, strong head forming leaf overlap, covered head, green primary colour of outer head leaves, high head solidity, intermediate stem length in head, white primary colour inside cut, high holding ability, high leaf retention on stem, yellow green flower stalk, short flower stalk length, absent flowering plant hairiness and pale yellow petals (Table 3).

3.2 Cluster Analysis

Morphological data differentiated all the accessions of *Brassica oleracea* studied. The phenogram constructed using each accession as an OTU (operational taxonomic unit) was performed on the basis of the collected morphological data of the 25 accessions of *Brassica oleracea* (Figure 3). This phenogram exhibited that all the genetic distance among the examined accessions was around 65%, and showed 4 major groups. The first group contained the 2 accessions of brussels sprout. The second group contained all accessions of kale and fodder kale. The third group was homogenous and contained all accessions of winter cauliflower. The fourth group split into 4 subgroups. Furthermore, the accessions of most cabbage types were distributed among different clusters within these subgroups. None of the studied cabbage types formed a distinct cluster except the 2 accessions of common cabbage which were separated in one cluster within the third subgroup. The accessions of cabbages HRIGRU5915 and HRIGRU4588 were very similar to the accessions of cabbages HRIGRU4497 and HRIGRU4498.
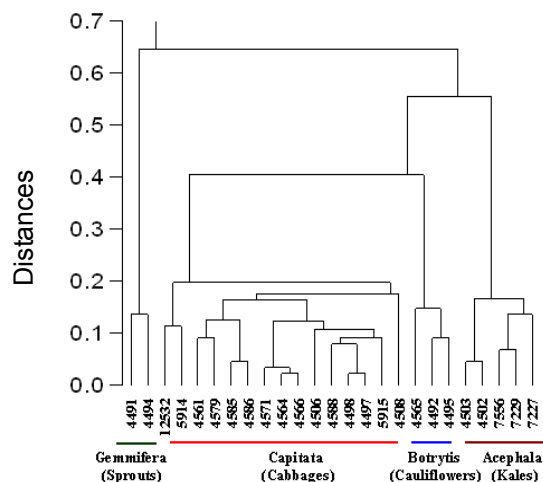

Figure 3. Dendrogram showing the relationship among 25 accessions of *Brassica oleracea* based on morphological data

3.3 Jaccard Binary Similarity Coefficients (Sj)

The overall mean similarity index for *Brassica oleracea* accessions calculated by the Jaccard similarity index (Sj) ranged from 0.051 to 0.976 with an average of 0.503 (Table 4). The highest similarity indices (0.976) were between the accessions of the same crop variety and geographical region, e.g. cabbage HRIGRU4497 and HRIGRU4498 from Roscommon. The second indices (0.952) were also between the accessions of the same group and geographical region, e.g. spring cabbages HRIGRU4566 and HRIGRU4564 from Cork, and spring cabbages HRIGRU4564 and HRIGRU4571 from Cork. The Sj similarity between the accessions of fodder kales HRIGRU4502 and HRIGRU4503 from Kildare was about 0.93. The accession of common cabbage HRIGRU4585 from Donegal had a high similarity index (0.907) with the accession of common cabbage HRIGRU4586 from Mayo. The Sj similarity between the accessions of common cabbage HRIGRU4586 and cabbage HRIGRU5915 was about 0.64. The lowest similarity indices and the highest variation were found between the accessions of kale HRIGRU7227 from Sligo and brussels sprout HRIGRU4494 from Dublin. The highest genetic distance in the cabbage types was found between the accessions of cabbage HRIGRU12532 and cattle cabbages HRIGRU4579, HRIGRU4561 and HRIGRU4508.

Table 4. Sj Jaccard binary similarity coefficients calculated from the morphological data for 25 accessions of *Brassica oleracea* (see Table 1 for identification of accessions numbers)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	1																								
2	0.930	1																							
3	0.750	0.729	1																						
4	0.750	0.729	0.867	1																					
5	0.660	0.640	0.804	0.729	1																				
6	0.277	0.281	0.317	0.339	0.281	1																			
7	0.277	0.281	0.277	0.297	0.242	0.745	1																		
8	0.277	0.281	0.317	0.339	0.281	0.822	0.708	1																	
9	0.292	0.277	0.292	0.292	0.221	0.407	0.383	0.383	1																
10	0.239	0.242	0.239	0.239	0.206	0.414	0.414	0.390	0.844	1															
11	0.258	0.242	0.297	0.258	0.242	0.390	0.390	0.367	0.766	0.745	1														
12	0.258	0.262	0.258	0.258	0.224	0.464	0.439	0.439	0.766	0.783	0.783	1													
13	0.239	0.242	0.258	0.258	0.206	0.390	0.344	0.367	0.804	0.745	0.673	0.745	1												
14	0.239	0.242	0.258	0.258	0.206	0.390	0.344	0.367	0.804	0.745	0.673	0.745	0.907	1											
15	0.254	0.239	0.292	0.292	0.239	0.456	0.383	0.431	0.714	0.694	0.729	0.804	0.660	0.660	1										
16	0.239	0.242	0.277	0.277	0.242	0.464	0.390	0.439	0.694	0.708	0.708	0.822	0.673	0.673	0.976	1									
17	0.258	0.262	0.297	0.297	0.242	0.439	0.367	0.414	0.729	0.673	0.673	0.783	0.708	0.708	0.844	0.864	1								
18	0.239	0.224	0.317	0.277	0.262	0.439	0.367	0.414	0.694	0.673	0.783	0.822	0.673	0.640	0.886	0.864	0.822	1							
19	0.258	0.262	0.258	0.258	0.242	0.390	0.367	0.367	0.596	0.577	0.577	0.673	0.608	0.608	0.660	0.673	0.745	0.640	1						
20	0.258	0.262	0.258	0.258	0.242	0.414	0.390	0.390	0.729	0.708	0.673	0.783	0.673	0.673	0.766	0.783	0.783	0.745	0.708	1					
21	0.277	0.281	0.277	0.277	0.224	0.414	0.390	0.390	0.766	0.708	0.673	0.783	0.708	0.708	0.766	0.783	0.822	0.745	0.673	0.952	1				
22	0.277	0.281	0.277	0.277	0.224	0.414	0.390	0.390	0.804	0.745	0.673	0.783	0.708	0.745	0.766	0.783	0.822	0.745	0.673	0.907	0.952	1			
23	0.258	0.262	0.297	0.297	0.281	0.439	0.367	0.414	0.596	0.577	0.547	0.708	0.673	0.673	0.694	0.708	0.708	0.708	0.783	0.745	0.708	0.708	1		
24	0.091	0.078	0.091	0.120	0.064	0.203	0.221	0.221	0.273	0.277	0.277	0.258	0.239	0.239	0.273	0.258	0.239	0.258	0.169	0.221	0.221	0.221	0.186	1	
25	0.064	0.065	0.064	0.092	0.051	0.188	0.206	0.206	0.239	0.262	0.242	0.242	0.224	0.224	0.239	0.242	0.224	0.224	0.155	0.206	0.206	0.206	0.171	0.766	1

3.4 Principal Component Analysis (PCA)

Principal component analysis (PCA) showed variations among the accessions studied (Table 5). The first 5 varieties of the principal component analysis accounted for 87.24% of the total variation among the 25 accessions. The first component (PC1) explained 37.88% of the total variation, while PC2, PC3, PC4 and PC5 contributed 27.97%, 11.89%, 5.13% and 4.38% of the total variation, respectively.

Table 5. Matrix of eigenvectors and values of the principal components for the morphological traits of 25 accessions of *Brassica oleracea*

| | Principal components | | | | |
	PC1	PC2	PC3	PC4	PC5
Variance Explained by Components	15.908	11.747	4.993	2.156	1.839
Percent of Total Variance Explained	37.88	27.97	11.89	5.13	4.38
Accumulated Eigenvectors	37.875	65.844	77.733	82.866	87.244
Seedling leaf colour	0.102	0.080	-0.078	0.416	0.305
Seedling pubesence	-0.162	-0.341	-0.344	0.695	-0.115
Juvenile development	-0.264	-0.177	-0.255	-0.123	0.701
Plant growth habit	-0.091	0.478	0.821	-0.038	-0.185
Lodging	0.450	-0.668	0.161	0.143	0.468
No. of leaves (estimated)	0.591	0.082	0.062	0.220	0.644
Leaf blade shape in outline	-0.212	-0.016	0.959	0.136	0.067
Leaf division (margin)	0.017	-0.984	0.155	-0.027	-0.012
Leaf division (incision)	0.306	-0.159	-0.157	0.740	-0.207
Leaf apex shape	0.708	0.200	-0.438	-0.367	0.098
Leaf colour	0.115	0.549	0.121	-0.165	0.000
Leaf hairiness	-0.399	-0.097	-0.170	0.370	0.541
Leaf bloom	0.017	-0.984	0.155	-0.027	-0.012
Petiole section	-0.142	0.405	0.862	0.046	0.131
Petiole and/or midvein colour	0.092	0.518	-0.154	0.446	-0.242

Heading habit	0.954	0.193	0.206	0.022	-0.003
Head shape in longitudinal section	0.954	0.193	0.206	0.022	-0.003
Head-forming leaf overlap at terminal region	0.972	0.181	-0.116	0.006	-0.039
Head cover from subtending leaves	0.996	-0.007	0.007	0.006	-0.032
Primary colour of outer head leaves	0.866	0.351	0.204	-0.064	0.075
Head size in relation to plant size	0.976	-0.199	0.037	0.000	-0.033
Head solidity	0.954	0.193	0.206	0.022	-0.033
Head length	0.945	-0.301	-0.020	-0.007	-0.015
Head diameter	0.938	-0.317	-0.028	0.010	-0.038
Stem length in head, estimate	0.954	0.193	0.206	0.022	-0.003
Primary colour inside cut	0.631	0.473	-0.131	-0.050	0.106
Tendency of head to split	0.796	0.360	0.349	-0.032	0.088
Head holding ability (bolting resistance)	0.954	0.193	0.206	0.022	-0.003
Relative time of maturity	-0.841	-0.137	0.511	0.015	0.078
Leaf retention on stem	0.154	-0.911	0.208	-0.154	-0.198
Number of shoots or enlarged buds	-0.017	0.984	-0.155	0.027	0.012
Size of enlarged vegetative buds	-0.017	0.984	-0.155	0.027	0.012
Firmness of enlarged vegetative buds	-0.017	0.984	-0.155	0.027	0.012
Colour of enlarged vegetative buds or shoots	-0.017	0.959	-0.154	0.004	0.022
Floral apex branching pattern	-0.034	0.042	0.988	0.048	0.108
Days to flowering	-0.949	-0.020	0.268	0.029	-0.024
Flower stalk colour	-0.959	0.144	-0.127	0.001	0.025
Flower stalk length, estimate	-0.627	0.735	0.241	0.034	0.067
Flower stalk internode length	-0.627	0.735	0.241	0.034	0.067
Flowering plant hairiness	-0.017	0.984	-0.155	0.027	0.012
Flower colour variability in the crop	-0.008	0.699	-0.100	0.097	-0.029
Petal colour	-0.256	0.070	-0.286	-0.544	0.222

The values of the principal component analysis for all morphological traits were shown in Table 5. The traits, which showed the greatest variability on the first component, were leaf apex shape (0.708), heading habit (0.954), head shape in longitudinal section (0.954), head-forming leaf overlap at terminal region (0.972), head cover from subtending leaves (0.996), primary colour of outer head leaves (0.866), head size in relation to plant size (0.976), head solidity (0.954), head length (0.945), head diameter (0.938), stem length in head estimated (0.954), tendency of head to split (0.796) and head holding ability (0.954).

Leaf colour (0.549), petiole and/or midvein colour (0.518), number of shoots or enlarged buds (0.984), size of enlarged vegetative buds (0.984), firmness of enlarged vegetative buds (0.984), colour of enlarged vegetative buds or shoots (0.959), flower stalk length estimated (0.735), flower stalk internode length (0.735), flowering plant hairiness (0.984) and flower colour variability in the crop (0.699) were highly and positively correlated with the second component.

The traits which showed the greatest influence in the third component, were plant growth habit (0.821), leaf blade shape in outline (0.959), leaf margin (0.155), leaf bloom (0.155), petiole section (0.862), relative time of maturity (0.511), leaf retention on stem (0.208), floral apex branching pattern (0.988) and days to flowering (0.268). The fourth component separated three traits; seedling leaf colour (0.416), seedling pubesence (0.695) and leaf incision (0.740). Juvenile development (0.701), lodging (0.468), number of leaves estimated (0.644), leaf hairiness (0.541) and petal colour (0.222) showed the greatest variability on the fifth component. The values of the principal components of flower stalk colour, leaf margin, leaf bloom, leaf retention on stem traits were low (Table 5). However, these traits exhibited some degree of variation among the accessions of *Brassica oleracea*.

3.5 Analysis of Variance (ANOVA)

Of the 44 morphological traits examined in this study, 28 were significantly different among the accessions of *Brassica oleracea* (Table 6). These 28 significant descriptors were deemed useful and can be used for further characterisation of the *Brassica* accessions. The regression coefficients (r^2) values ranged from 0.041 to 0.55 with an average of 0.31.

Table 6. One-way ANOVA for the morphological traits among 25 accessions of *Brassica oleracea*

Dependent variable (traits)	r^2 (regression coefficients)	Probability (P)
Seedling leaf colour	0.041	0.026
Seedling pubesence	0.088	0.179
Juvenile development	0.074	0.001*
Plant growth habit	0.154	0.000**
Lodging	0.234	0.001*
No. of leaves (estimated)	0.164	0.000**
Leaf blade shape in outline	0.059	0.011
Leaf division (margin)	0.309	0.000**
Leaf division (incision)	0.082	0.001*
Leaf apex shape	0.271	0.317
Leaf colour	0.118	0.000**
Leaf hairiness	0.107	0.000**
Leaf bloom	0.317	0.000**
Petiole section	0.133	0.000**
Petiole and/or midvein colour	0.120	0.000**
Heading habit	0.509	0.003*
Head shape in longitudinal section	0.490	0.003*
Head-forming leaf overlap at terminal region	0.484	0.001*
Head cover from subtending leaves	0.483	0.018
Primary colour of outer head leaves	0.388	0.000**
Head size in relation to plant size	0.459	0.001*
Head solidity	0.392	0.003*
Head length	0.421	0.703
Head diameter	0.389	0.443
Stem length in head, estimate	0.324	0.003*
Primary colour inside cut	0.253	0.125
Tendency of head to split	0.230	0.326
Head holding ability (bolting resistance)	0.244	0.003*
Relative time of maturity	0.253	0.072
Leaf retention on stem	0.536	0.000**
Number of shoots or enlarged buds	0.540	0.000**
Size of enlarged vegetative buds	0.498	0.000**
Firmness of enlarged vegetative buds	0.448	0.000**
Colour of enlarged vegetative buds or shoots	0.362	0.000**
Floral apex branching pattern	0.170	0.000**
Days to flowering	0.367	0.064
Flower stalk colour	0.343	0.001*
Flower stalk length, estimate	0.550	0.006
Flower stalk internode length	0.437	0.006
Flowering plant hairiness	0.497	0.000**
Flower colour variability in the crop	0.510	0.000**
Petal colour	0.065	0.072

* and ** are significantly different at the P<0.005 and P<0.001 respectively.

3.6 Correlation Coefficients among the Morphological Traits

Pearson correlation coefficients were computed for all pairs of traits (data not shown). The highest correlation was between leaf margin and leaf bloom (R= 1), and flower stalk length estimated and flower stalk internode length (R=1). There was a high correlation between flowering plant hairiness trait and some of enlarged buds traits, e.g. firmness, size and number of enlarged buds (R=1). There was also a high correlation among head traits (heading habit, head shape in longitudinal section, head solidity, stem length in head and head holding ability) (R=1). The head holding ability (bolting resistance) was high in most of the accessions studied. On the contrary, leaf margin and leaf bloom were negatively correlated with flowering plant hairiness and most enlarged buds traits, e.g. number, size and firmness (R= -1). Days to flowering trait was also negatively correlated to most traits, e.g. number of leaves estimated, leaf apex shape, leaf retention on stem, stem length in head, head length, size and number of enlarged buds, and all head and enlarged buds traits.

4. Discussion

To conserve and utilise germplasm efficiently, it is essential to investigate the extent of diversity available. Morphological characterisation is an essential step in the characterisation and classification of crop germplasm because a breeding program mainly relies on the magnitude of morpho-phenological variability (Koffi et al., 2008). The morphological traits used in this study showed a pronounced variation among accessions. Seedling traits such as juvenile development and seedling leaf colour showed the lowest variation. The hypocotyl colour and seedling leaf (marginal incisions) traits had no taxonomic value among accessions of *Brassica oleracea* because these traits showed the same patterns within all of the accessions studied. However, they could be used to assess the variation among wild and cultivated species of genus *Brassica*. Vegetative preflowering and maturity traits such as petiole section, petiole and midvein colour, leaf colour, primary colour of outer head leaves, leaf division (incision), etc. exhibited a high level of variation among the accessions studied. These results were consistent with that reported by Rabbani et al. (1999).

The cluster analysis showed that the cauliflowers were more closely related to the cabbages than the kales were. However, the kales were more closely related to cauliflowers and cabbages than the brussels sprouts were. The accessions of most cabbage types were distributed among different clusters within cabbage subgroups, and this agreed with Balkaya et al. (2005), who reported that there was some degree of variation among Turkish white head cabbages. However, the cluster analysis from this current study is more reliable, as most of the accessions of each group and subgroup were collected from closely related geographical regions. Only a few accessions were scattered among the different subgroups. This showed a sort of correlation between clustering pattern and eco-geographical distribution of the accessions. This result agreed with that reported by Faltusová et al. (2011), but was not consistent with that reported by Balkaya et al. (2005). The difference in these data could be attributed to the differences in accessions or the traits used which could in turn be influenced by environmental factors such as geographical area, elevation of temperature, and soil fertility.

The output of the Jaccard binary similarity coefficient from our morphological data revealed that the highest similarity indices were between the accessions of the same crop variety and geographical region. This confirmed the correlation between grouping on morphological data and eco-geographical distribution of the accessions. However, this high similarity could be attributed to the low gene flow within the same crop variety. This study also suggested that the accessions of kale HRIGRU7227, common cabbage HRIGRU4586, spring cabbage HRIGRU5914, cattle cabbage HRIGRU4561, HRIGRU4508 and HRIGRU4579 and cabbage HRIGRU12532, HRIGRU5915 and HRIGRU4588, due to the highest variation found among them, could provide the opportunities for selecting as parental source in future breeding programs to develop new or more productive *Brassica* varieties.

In the analysis made to estimate the relative contributions of the different morphological traits studied towards the overall phenotypic variation among the 25 accessions, a total of 5 principal components (PCs) explained 87.24% of the total variation in all the 44 traits studied. This data indicated a high degree of correlation among traits of the accessions studied. About 38% of the total variability explained by the first PC alone was due chiefly to variations in enlarged apical buds (head) traits, indicating a very high degree of correlation among them. Furthermore, all enlarged vegetative buds and most of enlarged inflorescences (floral branching pattern) traits were separated on the second component (explained about 28% of the total variability), indicating a high degree of correlation among them. We could thus conclude that the traits of enlarged apical buds, enlarged vegetative buds and enlarged inflorescences proved to be useful markers for separating *Brassica oleracea* varieties. These observations agreed with the classification criteria of *Brassica oleracea* varieties reported by Paterson et al. (2001) and Hong et al. (2008), that are mainly based on extreme morphological characteristics such as the enlarged single apical bud of

cabbage, the several enlarged vegetative buds of brussels sprout and the enlarged inflorescences (floral branching pattern) of cauliflower.

One-way analysis of variance (ANOVA) of the morphological traits used showed that 28 traits were significantly different among the accessions studied, and can be used for further characterisation of *Brassica* accessions. However, the low percent of contribution of the morphological traits (31%) could be attributed to the low number of flowering traits used. Furthermore, most of accessions were of orbicular leaf blade shape, dentate leaf margin, rounded leaf apex, green leaf colour, intermediate leaf bloom, rounded petiole section, spheric head shape, strong head forming leaf overlap, covered head, green primary colour of outer head leaves, yellow green flower stalk, short flower stalk length and pale yellow petals. These results were relatively consistent with that reported by Katz (2003).

The correlation is a measure of the degree to which variables differ or a measure of the intensity of association (Rabbani et al., 1999). The correlations revealed by Pearson correlation matrix in this study suggest that when the flower stalk length estimated is used as a distinguishing character, the flower stalk internode length could be redundant for characterizing the accessions. Most of the highly correlated traits (e.g. heading habit, head solidity, stem length in head firmness, size and number of enlarged buds and flower stalk length estimated) are of economic importance and are identified as useful for *Brassica* breeding programs and taxonomic purposes.

5. Conclusion

This study differentiated all the accessions of *Brassica oleracea* studied, and identified 28 morphological traits which were significantly different among the accessions and can be used for further characterisation of *Brassica* accessions. It also identified 9 highly diverse accessions, providing opportunities for optimising parental sources in future breeding programmes to develop new or more productive *Brassica* varieties. Morphological traits proved useful in assessing the diversity and relationships of Irish *Brassica oleracea* germplasm.

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