Genetic diversity and evolution of Brassica genetic resources: from morphology to novel genomic technologies – a review

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Abstract

Brassica species have an economic and medicinal importance. Estimation of the amount and distribution of genetic diversity within Brassica species is essential for establishing efficient management, conservation and breeding practices. This review discusses the taxonomy, gene pool, and Brassica-derived phytochemicals and their nutraceutical importance. It also surveys the recently advanced studies of the genetic diversity and phylogenetic studies of Brassica species at the level of morphological, cytological, biochemical and molecular markers that have proven to be useful for evaluating the genetic variation, taxonomic relationships and species identity, and could be useful for improving Brassica crops through future promising breeding programmes.

Keywords: biochemical and molecular markers, Brassica, genetic diversity, karyotype, morphological traits, relationships

Introduction

The plant genus Brassica L. belongs to the tribe Brassiceae, which in turn belongs to the largest family Brassicaceae (Rakow, 2004; El-Esawi, 2012). Brassicaceae includes 380 genera and 3000 species (Heywood, 1993), whereas Mabberley (1997) recorded 365 genera and 3250 species. Judd et al. (1999) also recorded 419 genera and 4130 species, while Warwick et al. (2006) recorded 338 genera and 3709 species belonging to this family. Brassica species play an essential role in horticulture and agriculture, as well as contributing to the health of populations around the world (Rakow, 2004; El-Esawi, 2012; El-Esawi et al., 2012a). They are important sources of vegetable oil, vegetables and condiments (Zhao, 2007). Brassicaceae produce glucosinolates that are broken down to isothiocyanates that limit tumour development and provide protection against human cancers and heart diseases (King, 2005; El-Esawi, 2012). Several biotic and abiotic factors affect Brassicaceae growth (Relf and McDaniel, 2009; Consentino et al., 2015; El-Esawi et al., 2015; Jourdan et al., 2015). Moreover, disease resistant Brassica varieties are required in future breeding programmes in order to improve their agricultural production and conservation strategies. Knowledge of the amount and distribution of genetic variability within a species is vital for establishing efficient conservation and breeding practices (Avisé, 1994; Chaveerach et al., 2007; El-Esawi, 2008, 2012). It helps plant breeders develop, through selection and breeding, new or more productive crops, that are resistant to pests and diseases and highly adapted to changing environments. It also provides information for domestication and designing sampling protocols (Bretting and Widrlechner, 1995; Yu et al., 2001). Therefore, this review discusses the taxonomy, gene pool and Brassica-derived phytochemicals and their nutraceutical importance.
It also highlights the recent and current knowledge of the application of morphological, cytological, biochemical and molecular markers in the genus *Brassica* L. that help understand its genetic variability, phylogeny, conservation and breeding system as a basis for further research to improve the crop and develop new varieties.

**Brassica-derived phytochemicals and their nutraceutical importance**

*Brassica* vegetables are valuable sources of nutrients and health-promoting phytochemicals possessing nutraceutical and antioxidant activity such as vitamins, carotenoids, dietary fibre, phenolics, soluble sugars, minerals and glucosinolates (Wagner et al., 2013). Brassicaceae produces glucosinolates which are broken down to isothiocyanates that limit tumour development and provide protection against a range of human cancers and heart diseases (King, 2005; El-Esawi, 2012). Glucosinolates and other sulphur-containing metabolites act as anti-cancer agents due to their ability to induce detoxification enzymes in mammalian cells and to decrease the rate of tumour development. Isothiocyanates are modulators of Phases 1 and 2 enzyme activity and neutralize cancer-causing chemicals that damage cells by interfering with tumour growth (King, 2005). *Brassica* secondary products have antioxidant, antiviral and antibacterial effects as well as inducing the immune system and modulating steroid metabolism (King, 2005). Moreover, *Brassica*-derived phytochemicals may counteract different genetic pathways and exhibit chemopreventive activity (Wagner et al., 2013).

Anti-inflammatory properties of *Brassica*-derived phytochemicals have been reported (Juge et al., 2007). These useful effects may be mediated through the stimulation of antioxidants and the prohibition of proinflammatory signalling pathways via regulation of different transcription factors that may be further controlled by miRNAs and epigenetic modifications (Wang et al., 2009a, b; Wagner et al., 2013). Moreover, *Brassica*-derived phytochemicals show antiviral and anti-infective activity (Yanaka et al., 2009). The kappa B (nuclear factor kB) transcription factor plays an important role in inflammatory processes, and is an attractive target to treat inflammation-related diseases (Wagner et al., 2013). *Brassica*-derived phytochemicals may also mediate anti-inflammatory effects through an interaction with reduced redox regulators such as thioredoxin, glutathione, or redox factor 1, resulting in changes of the reducing milieu needed for the correct DNA binding (Heiss and Gerhäuser, 2005; Wagner et al., 2013). Nrf2 is a transcription factor playing an essential role in regulating inflammation and chemoprevention (Wagner et al., 2013). Under basal conditions, Nrf2 is bound to its cytosolic inhibitor (Keap1) (Surh, 2005). In the presence of isothiocyanates, Nrf2 could be activated through two distinct cellular signalling pathways, leading to the liberation of Nrf2 from its inhibitor Keap1. The liberated Nrf2 is transferred to the nucleus and binds together with many cofactors, including small Maf proteins (MafF, MafG and MafK). *Brassica*-derived phytochemicals also influence epigenetic mechanisms. Epigenetic aberrations, occurring in the early stages of carcinogenesis, represent an initial process of cancer development. Phytochemicals may intervene in this process to prevent cancer (Gerhauser, 2013; Wagner et al., 2013).

**Taxonomy and gene pool of Brassica species and their wild relatives**

The genus *Brassica* L. is one of the most economically important genera in the tribe Brassicaceae, which in turn belongs to the family Brassicaceae (Cruciferae) (Rakow, 2004). There are several different opinions regarding the number of genera and species included in the Brassicaceae. Heywood (1993) recorded about 380 genera and 3000 species in this family, whereas Mabberley (1997) recorded 365 genera and 3250 species. Judd et al. (1999) also recorded 419 genera and 4130 species, while Warwick et al. (2006) recorded 338 genera and 3709 species belonging to this family. The genus *Brassica* L. 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). The cytogenetic relationship of the six main economically important species of the *Brassica* genus was depicted in the U triangle (UN, 1935). Three of these species are diploid (*B. oleracea*, 2n = 18; *B. rapa*, 2n = 20; *B. nigra*, 2n = 16), and three are amphidiploid (*B. napus*, 2n = 38; *B. juncea*, 2n = 36; *B. carinata*, 2n = 34). *Brassica* species are characterized by a wide range of adaptations and abilities to adapt to a wide range of habitats and growing environments (King, 2005; Hong et al., 2008).

*B. oleracea* is an important vegetable crop species which includes many vegetable cultivars called cole crops (Katz, 2003). These cole crops comprise cabbage (*B. oleracea* subspecies *capitata*), cauliflower (*B. oleracea* sub-species *botrytis*), brussels sprout (*B. oleracea* subspecies *gemmifera*), broccoli (*B. oleracea* subspecies *italica*), Kale and collards (*B. oleracea* subspecies *acephala*), and kohlrabi (*B. oleracea* subspecies *gongylodes*). The cole crops have extreme morphological characteristics. Examples of such morphologies include the enlarged inflorescences 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 (Hong et al., 2008). *B. oleracea* generally grows slowly and has a large storage capacity for...
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nutrients, which accounts for its adaptation to diverse natural habitats. It has a recent history of cultivation (Gomez-Campo and Prakash, 1999; Navabi, 2009).

Brassica rapa, commonly known as field mustard or turnip mustard, is a crop species widely cultivated as a leaf vegetable, a root vegetable and an oilseed. There are three well-defined groups of B. rapa, based on their morphological characteristics (CFIA, 1999): (1) the oleiferous or oil-type rape, often referred to Polish rape or summer turnip rape, of which canola is a specific form containing low erucic acid in its oil and low glucosinolate content in its meal protein; (2) the leafy type B. rapa, comprising the chinensis group (pak-choi, celery mustard), the pekinensis group (Chinese cabbage) and the perviridis group (tender-green); and (3) the rapifera type B. rapa, including the rapifera group (turnip, rapini) and the ruvo group (turnip broccoli, Italian turnip) (CFIA, 1999). Rakow (2004) also stated that seven varieties of vegetable B. rapa types are known, and these are: var. campestris, var. pekinensis, var. chinensis, var. para-chinensis, var. narinosa, var. japonica and var. rapa. The var. pekinensis is adapted to a cooler climate. The var. chinensis is a leaf vegetable which differentiated from oilseed rape types of middle China, var. para-chinensis is a derivative of the var. chinensis, and var. campestris is the most primitive leaf vegetable. The var. narinosa has a high cold tolerance and is similar to var. chinensis in its adaptation. The var. japonica is a leaf vegetable of Japan. The var. rapa (turnip) is cultivated all over the world as a vegetable and as fodder for animals (Rakow, 2004).

Brassica napus L., commonly known as canola or oilseed rape, is the amphidiploid (allo-tetraploid) of B. rapa and B. oleracea (Tsunoda, 1980; Rakow, 2004). It is the most productive oilseed species under cultivation. Both winter and summer annual forms of B. napus are grown as oilseeds in many countries of the world. Its high-yield potential might be related to the high photosynthetic rate per unit leaf area which is positively related to chloroplast number per unit leaf area and to chloroplast volume. There are also root-forming B. napus types, known as tuber-bearing swede or rutabaga, grown as vegetables and fodder for animals (Rakow, 2004).

Brassica nigra, commonly known as black mustard, is an annual weedy plant cultivated for its seeds. It is found growing wild as a weed in the cultivated fields in the Mediterranean region (Tsunoda, 1980). Plants of B. nigra can reach a height of up to 2 m and do not require vernalization for flower induction (Rakow, 2004). Brassica juncea L. is an amphidiploid species originated from crosses between B. rapa and B. nigra (Rakow, 2004). It has a great seed yield potential for semi-arid conditions, and is known to be more drought tolerant than rapeseed species (Rabbani et al., 1999) It is grown as an oilseed and leafy vegetable. Brassica carinata, or Ethiopian mustard, is an amphidiploid species originated from crosses between B. nigra and B. oleracea, and contains mustard oil (Rakow, 2004).

The genetic resources available for the breeding of Brassica crops are regulated by the boundaries of their primary, secondary and tertiary gene pools (Harlan, 1975; Branca and Cartea, 2011). Brassica oleracea represents the primary gene pool, but many studies have been achieved to assess the other gene pools and their important use (Branca and Cartea, 2011). Studies on pachytene chromosome morphology helped in investigating the secondary gene pool, and identified the basic genomes of Brassica (Branca and Cartea, 2011): AA (2n = 20) is for B. rapa, BB (2n = 16) for B. nigra and CC (2n = 18) for B. oleracea. Investigations on genomic libraries of B. napus and B. oleracea revealed shared fragments among A, B and C-genomes, suggesting their partial homology and the origin of the amphidiploid species B. napus, B. carinata and B. juncea from the parental diploid ones (Hosaka et al., 1990; Branca and Cartea, 2011). The phylogenetic studies explain the evolution of Brassica and allied genera from a common ancestor with n = 6 through increase in the number of chromosomes and partial homology of A, B and C genomes (Song et al., 1990; Branca and Cartea, 2011). Finally, the tertiary gene pool involves species and genera related to Brassica crops in 36 cytodemes such as Diplotaxis, Enarthrocarpus, Eruca, Erucastrum, Hirschfeldia, Rhychnosinapis, Sinapis, Sinapodendron and Trachystoma genera (Harbered, 1976; Branca and Cartea, 2011). These gene pools can confer favourable alleles and useful traits using special methods. Tissue culture techniques and protoplast culture helped in the introgression of beneficial genes overcoming genetic boundaries (Branca and Cartea, 2011).

Different wild B. oleracea species with a chromosome number of n = 9 (including Atlantic B. oleracea) were collected. Four wild B. oleracea-related species were found in Sicily (B. rupestris, B. incana, B. villosa and B. macrocarpa) (Branca and Cartea, 2011). Each accession was divided into three parts, stored at the UPM (Spain), the University of Tohoku (Sendai, Japan) and also at seed banks of those countries, where the collection was done (Izmir, Thessaloniki, Greece, Bari, Italy, France, Kew, UK) (Branca and Cartea, 2011). In Europe, and under the aegis of the European Cooperative Program for Crop Genetic Resources Networks (ECPGR), a working group on Brassicas was established in 1991. One of the major efforts of this group has been to set up a European Brassica database (Bras-EDB), which was developed by the Center for Genetic Resources, Netherlands (Boukema and van Hintum, 1998; Branca and Cartea, 2011). This database involves cultivated plant materials as well as wild ones and comprises 36 collections from 22 countries and more than 19,600 accessions (Branca and Cartea, 2011).
Brassica collections were characterized for their broadening agricultural use, including assessing and utilizing genetic variation in B. carinata for its utilization as an oilseed crop (Branca and Cartea, 2011). The main aim was to create a core collection of four important Brassica species (B. oleracea, B. rapa, B. napus and B. carinata). This project was an important attempt to unify efforts on Brassica germplasm within the EU and it was complementary to the activities of the ECPGR Working Group on Brassica who also evaluated different wild species based on DNA analysis, morphological traits and quality aspects focused on oils and nutraceutical compounds. The role of the Working Group is to highlight the usefulness of the wild germplasm for breeding practices and their importance for improving Brassica crops, as well as to select the most appropriate accessions of the future European Genebank Integrated System (Astley et al., 2007; Branca and Cartea, 2011).

**Genetic diversity of Brassica species**

Genetic diversity is the variation of individual genotypes within and among species. It permits species to adjust to a changing environment, whether these changes are due to human or natural factors (Chaveerach et al., 2007; El-Esawi, 2008, 2012). The genetic composition of whole populations varies from place to place across a species range. These differences might emerge as a result of chance occurrences, such as the genetic composition of outspreading individuals which form a new population (founder effect), or changes in allele frequencies which result from chance crossings in very small populations (genetic drift) (Meffe and Carroll, 1994; Husband and Schemske, 1996; Falk et al., 2001). Differences among populations may also arise when the environment in different locations exposes the individuals to different conditions and optima for survival and reproduction (fitness). For these and other reasons, populations can diverge from one to another in their genetic profile. This divergence is especially strong and quick when there is a little gene flow among populations (e.g., limited dispersal of pollen or seeds or limited movement of animals across physiographic barriers) (Falk et al., 2001). Over evolutionary time, such among-population genetic differences could accumulate, resulting in the development of a new species (allopatric speciation).

Effective conservation of Brassica genetic resources requires a complementary approach that makes use of both ex situ and in situ conservation methods to maximize the genetic diversity available for use (Karp et al., 1997). In situ conservation is a method for conserving forest species and wild crop relatives and is based on the maintenance of the whole ecosystem in which the target taxa are present with other species (Hodgkin et al., 1995). In situ conservation helps evolution to continue, increases the amount of diversity which could be conserved, and strengthens the links between conservation workers and the communities who have maintained and used the resources (Karp et al., 1997).

**Ex situ** conservation aims at maintaining the accessions without change in their genetic constitution (Frankel et al., 1995). The methodologies used are designed to minimize the possibility of selection, mutation, random genetic drift or contamination. For many species, long-term ex situ conservation can be undertaken by storing seeds for long periods at low humidities and temperatures (Karp et al., 1997). The major world germplasm collections of Brassica are located in Centre for Genetic Resources (CGN, The Netherlands), Institute for Horticultural Plant Breeding (IVT, The Netherlands), Horticultural Research Institute (HRI, UK) and Gene Bank of Crop Research Institute (UK) (van der Meer et al., 1984). Genetic diversity can be estimated based on morphological, cytological, biochemical and molecular markers.

**Morphological traits**

Morphological variation in plant species has been described for traits which are controlled by a single or multiple gene systems. The greater the number of gene loci determining the trait, the more continuous the variability will be (Ayala, 1982; El-Esawi et al., 2012a). The expression of morphological traits is affected by the environment. Thus, the variation patterns in these morphological traits are considered to be the result of both environmental and genetic attributes. The differentiation of populations in a species is carried out by coding the measurements of quantitative traits and their evaluation based on different biological and mathematical assumptions using a number of computer softwares (Rohlf, 2000). Morphological traits have been used by different evolutionists to assess the genetic variation and phylogenetic relationships among populations of the plant species, for example, soybean (Iqbal et al., 2008), rice (Rashid et al., 2008; Bibi et al., 2009), Lactuca (El-Esawi, 2008) and Brassica (Rabbani et al., 1999; Kop et al., 2003; Balkaya et al., 2005).

Rabbani et al. (1999) assessed the morphological variation in oilseed mustard. The results showed a considerable level of variation among all accessions for various traits. Seedling characteristics exhibited less variation, while the largest variation was recorded for flowering and maturity stage characters. Some of the related traits were significantly correlated with each other. Furthermore, Balkaya et al. (2005) evaluated the morphological variation of white head cabbage (B. oleracea var. capitata subvar. alba). Cluster analysis based on 12 quantitative and ten qualitative
variables identified ten groups. Morphological variability was high among the genotypes studied.

**Cytological markers and karyotyping**

Karyotype study is a beneficial tool in taxonomy either to characterize taxa or to reconstruct their phylogeny (Koopman and De Jong, 1996; El-Esawi, 2008). Its value for phylogeny reconstruction in *Brassica* species has been confirmed and demonstrated (Allam et al., 1985). The cytological markers have been used by taxonomists to assess the relationships and genetic variability in plants, for example, barley (Jahan and Vahidy, 2008), *Lactuca* (Sammour et al., 2013; El-Esawi and Sammour, 2014) and *Brassica* (Allam et al., 1985; Hasterok and Maluszynska, 2000; Kulak et al., 2002).

Allam et al. (1985) studied the karyotype and meiotic behaviour of *B. oleracea*. The results revealed that the two karyotypes appeared to be similar in their gross morphology though they varied in minute details. In meiosis, the two varieties were almost normal though they varied in their chiasma frequency. Kulak et al. (2002) also studied the karyotypes of three amphidiploid species (*B. napus, B. juncea* and *B. carinata*). They identified eight out of 19 pairs of chromosomes in *B. napus*, ten out of 18 pairs in *B. juncea* and six out of 16 pairs in *B. carinata*. *Brassica* species have small, morphologically similar chromosomes.

The chromosomes of *Brassica* species are relatively small, morphologically similar and numerous in allotetraploids. However, their analysis based on morphometric and karyological features only, is extremely difficult and requires additional markers (Olin-Fatih and Heneen, 1992; Kulak et al., 2002). Therefore, isozyme and molecular markers have been established and used.

**Biochemical markers**

Biochemical markers including storage proteins and isozymes have been used to assess the genetic diversity and phylogenetic relationships of *Brassica* and several plant species.

**Storage proteins**

Proteins are the translational products of DNA molecules, and can form structural and enzymatic components of plant cells. The transcription and translation of the nucleotide sequences of genes result in the formation of amino acids (El-Esawi, 2008; Kephart, 1990). Therefore, the variation detected in proteins is as a mirror for genetic variations. Proteins have been extracted and assessed using different methodologies including, but not limited to, chromatography, ultracentrifugation and electrophoresis. Electrophoresis proved to be the most suitable method for separation and comparison of proteins (Gordon et al., 1988; El-Esawi, 2008), and could be used to characterize different plant genotypes (Sammour, 1990, 1999; Sammour et al., 1994; DellaGatta et al., 2002; Liang et al. 2006; Toosi et al., 2011). Electrophoretic analysis of seed storage proteins was used to assess the genetic variation, which provide a useful information for evaluating the taxonomic relationships at the plant species and subspecies levels (Vries, 1996; Rabbani et al., 2001; Sihag et al., 2004; Sammour et al., 2007; Kakaei and Kahrizi, 2011; Toosi et al., 2011; Khurshid and Rabbani, 2012; Khan et al., 2014; Choudhary et al., 2015; Mir et al., 2015). Toosi et al. (2011) used sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) to characterize the protein profiles of *B. juncea* var. Ensabi at different growth stages. Out of 11 protein bands detected in seed proteins, five bands matched the seed protein profiles of other *B. juncea* varieties. A comparison of the protein profiles at different growth stages revealed a steady expression of numerous genes encoding different proteins in *B. juncea*. Furthermore, SDS–PAGE was used to characterize the seed storage protein of 52 accessions of oilseed mustard germplasm from Pakistan (Rabbani et al., 2001). The results indicated that the generated protein markers could not distinguish the closely related oilseed cultivars from each other. However, these polypeptides proved to be efficient to distinguish *B. juncea* and *B. campestris*. Khurshid and Rabbani (2012) also studied the genetic diversity of *Brassica* species based on protein markers using SDS–PAGE technique. The results revealed considerable degree of polymorphism and distinguished among the genotypes analysed.

Khan et al. (2014) used SDS–PAGE to analyse the seed storage proteins of a collection of 136 accessions of *B. napus* L. A total of 21 protein sub-units were detected and used to distinguish among the accessions. Out of these 21 bands, 16 (76.19%) were polymorphic and five (23.81%) were monomorphic. The similarity coefficient among these accessions varied between 0.83 and 0.98. The cluster analysis divided the accessions into five major clusters. The results also showed a low level of genetic variation. Further studies are recommended using two-dimensional (2D) gel electrophoresis along with molecular markers in order to reveal high levels of genetic variation among these accessions. Mir et al. (2015) evaluated the genetic variation and phylogenetic relationships among *B. juncea* accessions using SDS–PAGE. The cluster analysis divided the accessions into two main clusters and distinguished among the accessions analysed. SDS–PAGE of seed storage proteins proved to be an efficient method for distinguishing among plant populations.
Isozyme markers

Isozymes are molecular forms of an enzyme, having a different structure and a similar catalytic function (El-Esawi, 2008). Allozymes are allelic variants of enzymes encoded by structural genes of the same locus. Isozymes originate through the changes of amino acids, that change the net charge, the spatial structure of the enzyme molecules and their electrophoretic mobility. After specific staining, the isozyme profile of individuals can be detected (Dziechciarková et al., 2004; Kumar et al., 2009). Isozyme traits have been successfully utilized to study the genetic variation, phylogenetic relationships, population genetics, taxonomy and developmental biology as well as to direct utilization in plant genetic resources management and plant breeding (Dziechciarková et al., 2004; Kumar et al., 2009; El-Esawi, 2015a, b), for example, red clover (Mosjidis et al., 2004), blue pine (Bakshi and Konnert, 2011), Lactuca (El-Esawi, 2008) and Brassica (Lázaro and Auginagalde, 1998a). Lázaro and Auginagalde (1998a) assessed the genetic diversity of B. oleracea based on five enzyme systems. The average values for the expected homozygosity and percentage of polymorphic loci were 0.224 and 54%, respectively. The intra- and interpopulational variations were 67 and 53%, respectively. Raybould et al. (1999) also evaluated the genetic diversity in natural populations of B. oleracea using four isozymes and seven microsatellite loci. All loci were polymorphic, and the diversity index of microsatellite loci was similar to that of isozymes. Genetic differentiation among accessions (FST) was significant for all loci. The above studies have definitely proven that isozyme traits are efficient for characterizing genetic variability, taxonomic relationships and species identity.

Molecular markers

Molecular markers are regions in the genome that are heritable as simple Mendelian traits (Schulman et al., 2004) and can be used to assess the genetic variation and phylogenetic relationships in plants. Several criteria should be considered in choosing molecular techniques for genetic diversity studies including the following: whether the techniques are highly reproducible between laboratories and whether the data that are generated can be reliably transferred; whether markers are dominant or codominant, allowing heterozygotes and homozygotes to be distinguished; the amount of genomic sequence information required; and whether the markers detect highly polymorphic loci (Osman et al., 2003; El-Esawi, 2008, 2012). At present, various dominant and codominant molecular markers are available for assessing genetic diversity in plants, such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), restricted fragment length polymorphism (RFLP), microsatellites (simple sequence repeats (SSRs)) and single nucleotide polymorphisms (SNPs).

Random amplified polymorphic DNA

RAPD is a PCR-based technology based on enzymatic amplification of target or random DNA fragments with arbitrary primers (El-Esawi, 2008; Kumar et al., 2009). Each product is derived from a genome region, which contains two short segments in inverted orientation, on opposite strands which are complementary to the primer. Amplified products are generally separated on agarose gels in the presence of ethidium bromide and visualized under ultraviolet light (El-Esawi, 2008; Kumar et al., 2009). The RAPD system has been used in characterization of different resistance genes, hybrid origin identification (Friesen et al., 1997) and breeding utilization (Baril et al., 1997). The RAPD technique has also been used for assessing the genetic variation in plant species and to establish differences among lines of apparently closely related populations in germplasm collections, for example, rice (Bibi et al., 2009), Lactuca (El-Esawi, 2008) and Brassica (Lázaro and Auginagalde, 1998b; Crockett et al., 2000; Ali et al., 2007; Saha et al., 2008).

Lázaro and Auginagalde (1998b) used RAPD markers to evaluate the genetic diversity in B. oleracea group. The average value of Nei genetic distance among taxa was 1.825. Analysis of molecular variance (AMOVA) detected the highest amount of genetic variation in B. oleracea subsp. cretica (variance = 0.519), followed by B. oleracea subsp. rapesistis (variance = 0.420). Saha et al. (2008) also studied the genetic diversity and relationship among Brassica species based on RAPD markers. The highest percentage of polymorphic loci (37.29%) was found in the accession of BARI sarisha-12 (B. rapa). In conclusion, genetic analysis with dominant RAPD markers is often quick, less technical and less expensive than other molecular markers (Kumar et al., 2009).

Restricted fragment length polymorphism

RFLP is a technique in which plants and organisms may be differentiated by analysis of patterns derived from cleavages of their DNA (Kumar et al., 2009). RFLP markers have been used in studying genetic variability and relationships in plant species, for example, Lathyrus (Chitourou-Ghorbel et al., 2001) and Pleurotus eryngii (Urbanelli et al., 2007). RFLP markers have also been used in assessing genetic variation and developing detailed genetic maps of Brassica (Diers and Osborn, 1994; Diers et al., 1996; Pradhan et al., 2003) and lettuce (Landry et al., 1987;
Kesseli et al. (1994), assessing polymorphisms in Brassica lines resistant and susceptible to Xcc (Malvas et al., 2003; El-Esawi, 2012), and identification of origin of cultivated lettuce (Kesseli et al., 1991). Malvas et al. (2003) identified two DNA fragments of RPS2 gene homologs in two Brassica lines resistant and susceptible to Xcc. The digestion of these fragments with restriction enzymes showed polymorphisms at the XbaI restriction sites. In addition to their high genomic abundance and their moderate polymorphism, RFLP markers are codominant and having high reproducibility (Kumar et al., 2009).

Amplified fragment length polymorphism

AFLP is a DNA fingerprinting method that detects restricted DNA fragments by PCR (Kumar et al., 2009; El-ESawi, 2012). The method is based on selectively amplifying a subset of restriction fragments from a mixture of DNA fragments obtained after genomic DNA digestion with restriction endonucleases (Kumar et al., 2009). Polymorphisms can be detected from the length differences of the amplified fragments by PAGE or by capillary electrophoresis. The technique involves four steps: (1) restriction of DNA and ligation of oligonucleotide adapters, (2) preselective amplification, (3) selective amplification and (4) gel analysis of amplified fragments. The major advantages of AFLPs scored as dominant markers, include the short time required to assay large numbers of DNA loci, the effectively unlimited number of loci, and the greatly enhanced performance in terms of reproducibility, sensitivity, resolution and time efficiency (Dziechciarková et al., 2004; Kumar et al., 2009).

AFLP is a widely valued technology for accelerating plant improvement and gene mapping studies (Vos et al., 1995). AFLP markers have been successfully used for evaluating the genetic diversity and relationships in plant species, for example, sesame (Laurentin and Karlovsky, 2006), common bean (Kumar et al., 2008), potato (Wang et al., 2011) and Brassica (Lombard et al., 2000; Warwick et al., 2000, 2008; Genet et al., 2005; Ren-Hu and Jin-Ling, 2006; Watson-Jones et al., 2006; van Hintum et al., 2007; Christensen et al., 2011; Faltusová et al., 2011; El-ESawi, 2012). The studies on Brassica populations showed considerable levels of genetic variation.

Genet et al. (2005) demonstrated that AFLP is a reliable tool for assessing the genetic diversity of B. carinata. Polymorphic rates varied from 50 to 80%. Cluster analysis divided these genotypes into seven distinct clusters. Watson-Jones et al. (2006) used AFLP markers to evaluate the genetic variation within three species of Brassica (B. nigra, B. oleracea and B. rapa). The results revealed higher diversity within B. oleracea populations than the other two species and it had the highest range of diversity among populations. van Hintum et al. (2007) also studied the genetic diversity in B. oleracea using AFLP markers. The average genetic diversity within single accessions was 0.13 and the total diversity (Ht) was 0.24.

Warwick et al. (2008) assessed the genetic variation and relationships among taxa of B. rapa using seven AFLP primer pairs which displayed similar amounts of polymorphisms (84–97%) among accessions. Christensen et al. (2011) also studied the diversity and genetic structure among 17 Brassica accessions using AFLP markers. Several landraces showed higher levels of diversity than the wild populations. An AMOVA showed that 62% of the total variation was found within accessions. Furthermore, Faltusová et al. (2011) evaluated the genetic diversity of B. oleracea using AFLP markers. A total of 806 polymorphic fragments were found across the accessions. The accessions were clustered into two main groups. Special subgroups, reflecting origin, were observed within these groups.

Microsatellites (SSRs)

Microsatellites, alternatively known as SSRs or short tandem repeats, are sections of DNA consisting of tandemly repeating mono-, di-, tri-, tetra-or penta-nucleotide units that are arranged throughout genomes of most eukaryotic species (Powell et al., 1996; Kumar et al., 2009; El-ESawi, 2012). The advantages of microsatellites include the codominance of alleles, their high abundance in eukaryotes and their random distribution throughout the genome, with an association in low-copy regions (Morgante et al., 2002). Due to using long PCR primers, the microsatellites reproducibility is high, and their analyses do not require a high quality or quantity of DNA. Although microsatellites are codominant markers, mutations in the primer annealing sites may result in the appearance of null alleles (no amplification of the intended PCR product) (Kumar et al., 2009). Microsatellites are ideal markers in gene mapping studies (Jinek and Lagoda, 1996), and assessing genetic variation and relationships in germplasm collections, for example, Oryza sativa (Chakravarthi and Naravaneni, 2006), wheat (Iqbal et al., 2009), Prunus avium (Ercisi et al., 2011) and Brassica (Flannery et al., 2006; Hasan et al., 2006; Louarn et al., 2007; Ofri et al., 2008; Moghaddam et al., 2009; Wang et al., 2009a, b; El-Esawi, 2012; El-ESawi et al., 2012b; Wu et al., 2014). The above studies on Brassica populations helped in understanding their genetic variability and taxonomic relationships.

Flannery et al. (2006) used ten plastid SSR primer sets to detect polymorphism in Brassica, Arabidopsis, Camelina, Raphanus and Sinapis. Eight loci were polymorphic. SSR data separated the individuals of Brassicaceae into taxon-specific groups (Arabidopsis, Camelina, Sinapis and Brassica genera). Within Brassica, B. oleracea is separated
from *B. napus* and *B. rapa*. Louarn et al. (2007) also evaluated 59 *B. oleracea* cultivars for microsatellites polymorphisms. All SSR markers, except one, reported a polymorphic information content (PIC) value of 0.5 or above. Ofori et al. (2008) used 16 microsatellite markers to evaluate the genetic diversity in European winter *B. rapa*. The results showed that the majority of genetic variation (83%) resided within cultivars. Furthermore, Moghaddam et al. (2009) used microsatellite and RAPD markers to assess the genetic variability among 32 rapeseed cultivars. The PIC of microsatellite markers varied from 0.60 to 0.91. Na12-C01, a microsatellite marker amplifying two different genomic regions in the *Brassica* genome was observed in the spring cultivars in one of the regions.

**Genome sequencing and SNPs**

Genomics technologies apply recombinant DNA, DNA sequencing methods, and bioinformatics to sequence, assemble, and analyse the structure and function of genomes (NHGRI, 2014). Sequencing aims to determine the exact order of the bases in a DNA strand. Next-generation sequencing (NGS), known as high-throughput sequencing, is a term used to describe a number of different novel sequencing technologies including Illumina (Solexa), Roche 454, Ion torrent Proton/PGM and SOLiD (NHGRI, 2014). These recent technologies conduct sequencing DNA and RNA quicker and cheaper than the previously used Sanger sequencing, and as such have revolutionized the studies of genomics and molecular biology. DNA sequencing can be used to assess mutations that may play a role in developing diseases (NHGRI, 2014). The mutations may be substitution (SNPs) or insertion and deletion (INDELs) of a single base pair or a deletion of thousands of bases. Besides their abundance in genomes, SNP markers have the advantages of being codominant and amenable to high-throughput automation (Tsuchihashi and Dracopoli, 2002). Whole-genome sequencing and SNP markers have been recently used in the genetic analyses of *Brassica* and other plants, such as phylogenetic analysis, taxonomy, estimation of genetic variation and population structure, genome-wide association studies and construction of genetic linkage maps (Trick et al., 2009; Bancroft et al., 2011; Lai et al., 2012; Huang et al., 2013).

Lai et al. (2012) sequenced leaf transcriptomes across a mapping population of *B. napus*. Analysis of sequence variation and transcript abundance helped constructing single nucleotide polymorphism linkage maps of *B. napus*, comprising 23,037 markers. They also used these analyses to align the *B. napus* genome with that of *Arabidopsis thaliana*, and to genome sequence assemblies of *B. rapa* and *B. oleracea*. Huang et al. (2015) identified a total of 892,536 bi-allelic SNPs throughout the *B. napus* genome. Using the GoldenGate genotyping platform, 94 of 96 SNPs sampled could effectively differentiate genotypes of 130 lines from two mapping populations, with an average call rate of 92%. SNPs identified in this could also be used to directly identify causal genes in association studies. In conclusion, it is expected that current advances in *Brassica* genomics will stimulate researchers to use these commercially available genechips based on SNPs markers for fast and cost-efficient breeding and population genetic studies in the near future. Sequencing platforms will also continue to improve the output length and quality, and that the complementary algorithms and bioinformatic software required to handle large genomes, will be enhanced.

**Conclusions**

In this paper, the issues and recent knowledge of the genetic diversity and phylogenetic studies of *Brassica* genetic resources were elucidated at different levels extending from morphological traits to advanced molecular markers passing with cytological and biochemical traits which have proven to be efficient for assessing the genetic variability, relationships and species identity. This information could be potentially used for enhancing future *Brassica* breeding programmes of highly agronomic *Brassica* species as well as improving their phylogeny, propagation and conservation strategies. Furthermore, it may be utilized for marker-aided selection and quantitative trait loci analyses.

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**References**


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