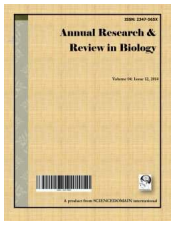

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Taxonomic Relationships and Biochemical Genetic Characterization of *Brassica* Resources: Towards a Recent Platform for Germplasm Improvement and Utilization

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

The genus *Brassica* L. is one of the most economically important genera in the family Brassicaceae. It has an essential role in agriculture and horticulture, as well as contributing to the economy and populations health. This genus includes numerous species comprising major vegetable and oilseed crops with various agronomic traits that need to be further characterized. The present paper highlights the current knowledge of taxonomy, chromosome number, genomic relationships, geographical distribution, origin, domestication, and breeding technologies of the six economically important *Brassica* species grown in Egypt, as well as describing their genetic diversity and relationships at the level of biochemical markers, including storage proteins and isozymes. This information would help developing new and more productive crops of disease resistant and highly agronomic traits, resulting in a recent platform for crop improvement and conservation.

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Keywords: Brassica species; taxonomy; genomic relationships; geographical distribution; origin; biochemical markers.

1. INTRODUCTION

The genus *Brassica* L. belonging to the family Brassicaceae, plays an important role in agriculture and horticulture, as well as contributing to the economy and populations health [1,2,3]. It includes numerous species comprising major vegetable and oilseed crops with various agronomic traits [4,5]. *Brassica* species are important sources of vegetables, vegetable oil, and condiments [6]. *Brassica napus, B. juncea, B. rapa*, and *B. carinata* provide approximately 12% of the worldwide edible vegetable oil supply [7]. The oil is either used for human consumption or further processed as a biofuel or renewable resource in the petrochemical industry. *Brassica oleracea* has a large storage capacity for nutrients and provides a large range of unique cole and cabbage crops used for human consumption [1]. The seed of *Brassica nigra* is used as a condiment mustard. Moreover, *Brassica* species are valuable sources of dietary fiber, potassium, phenolics, vitamins A, C and E, and other health-enhancing factors such as anticancer compounds [6,8]. Brassicaceae produces a class of biochemicals (glucosinolates) which are broken down to compounds (isothiocyanates) known to decrease tumour development and provide protection against a range of human cancers and heart diseases [2,9]. The plants containing high amount of glucosinolate may be further used as a potential genetic source for breeding [10]. *Brassica* vegetables prevent major diseases such as Alzheimers, and some of the functional declines associated with ageing [9]. *Brassica* secondary products have antioxidant, antibacterial and antiviral effects as well as stimulating the immune system and modulating steroid metabolism [2,9].

Various bacterial, fungal, viral, pest and insect pathogens, including *Peronospora parasitica* (downy mildew), *Plasmodiophora brassicae* (clubroot), *Ophiosphaerlla korrae* (ring spot), *Fusarium oxysporum* (yellows or fusarium wilt), *Leptosphaeria maculans* (blackleg), *Xanthomonas campestris* (black rot), *Prodenia* spp. (cut worms), *Brevicoryne brassicae* (aphids), *Pieris rapae* (cabbage worms) and *Delia radicum* (cabbage root fly) infect *Brassica* and crucifers causing harmful diseases and severe damage [11]. The use of pesticides to control these devastated diseases is harmful for human and environment. The issue that has led to searching for alternative resources to control these diseases. To close this gap, disease resistant *Brassica* varieties would be develped in future breeding programs in order to improve their conservation and agricultural production. Hence, attention has been paid to wild *Brassica* genetic resources (repositories of resistance genes) to identify the genes conferring resistance and good agronomic traits including oil content [2,12-14]. Better methods for characterizing those germplasm collections such as biochemical traits, have also been developed to improve strategies for their biodiversity conservation and utilization in varietal improvement. Therefore, the main aim of this review is to summarize current knowledge of the application of those biochemical genetic traits (storage proteins and isozymes) in the genus *Brassica* L. in order to understand its genetic diversity, conservation and breeding as a basis for further research to develop disease-resistant and more productive crops. Taxonomy, chromosome number, genomic relationships, geographical distribution, origin, domestication, breeding systems and technologies of *Brassica* species are also discussed.

2. TAXONOMY

The genus *Brassica* L. belongs to the tribe Brassiceae, which in turn belongs to the family Brassicaceae (Cruciferae) [1,2]. Scientists recorded different numbers of the genera and species of the Brassicaceae. Heywood [15] found 380 genera and 3000 species in this family, whereas Mabberley [16] recorded 365 genera and 3250 species. Judd et al*.* [17] also recorded 419 genera and 4130 species, while Warwick et al*.* [18] recorded 338 genera and 3709 species belonging to this family.

The taxonomic status of Brassicaceae and its relationships to other families have been the subject of controversy among taxonomists for many decades. Some taxonomists placed Brassicaceae close to the Resedaceae and Capparidaceae in the order Cruciales [19,20]. Others proposed a relationship between the Papaveraceae and Brassicaceae and place these families in the same order [21-24]. More recently, on the basis of the presence of glucosinolates and myrosincells [25] as well as on the DNA sequences of the genes [26], close

relationships among the Brassicaceae, Capparidaceae and Resedaceae have been confirmed, while chemical and molecular evidences have revealed that the Papaveraceae are unrelated to Brassicaceae [27]. Brassicaceae has been subdivided by many systematists [21,28,29], based on the nature of the hairs, fruits, nectar glands and myrosin cells, characters of corolla and calyx and position of the cotyledons relative to the radicle [27].

Brassica includes numerous species comprising major vegetable and oilseed crops with various agronomic traits [2,4,5]. It contains 6 economically important species, with much genetic and morphological diversity, and which are cultivated worldwide [30]. Three of these 6 species are diploid (*B. oleracea*, 2n = 18; *B. rapa*, 2n = 20; *B. nigra*, 2n = 16), and 3 are amphidiploid (*Brassica napus*, 2n = 38; *Brassica juncea*, 2n = 36; *B. carinata*, 2n = 34). *Brassica* species are characterised by a wide range of adaptations to various habitats [9,31].

Brassica oleracea includes many vegetable cultivars called cole crops [32]. These cole crops comprise cabbage (*B. oleracea* subspecies *capitata*), cauliflower (*B. oleracea* subspecies *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 characters. Examples of such morphologies include the enlarged infloresences of cauliflower and broccoli; the enlarged stems of kohlrabi and marrowstem kale; the enlarged apical bud of cabbage; and the numerous axillary buds of brussels sprout [31,33].

Brassica rapa L. (synonymous with *Brassica campestris* L.), commonly known as field mustard or turnip mustard, is a crop species widely grown as a leaf and root vegetable, and an oilseed. *Brassica rapa* and *B. campestris* were first described as 2 species (turnip and wild weeds forms) by Linnaeus [2]. Toxeopus et al. [34] and CFIA [35] reported that these were the same species, and merged the taxa under the name *B. rapa*. There are 3 well defined groups of *Brassica rapa*, based on their morphological characters [35]); (1) the oil-type rape or oleiferous, often referred to summer turnip rape or Polish 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 *Brassica rapa*, comprising the chinensis group (pak-choi, celery mustard), the pekinensis group (Chinese cabbage), and the perviridis group (tendergreen); and (3) the rapiferous type *Brassica rapa*, including the rapifera group (turnip, rapini), and the ruvo group (turnip broccoli, Italian turnip) [35,36]. Rakow [1] also reported that 7 varieties of vegetable *Brassica rapa* types are known, and these are: var. *campestris*, var. *pekinensis*, var. *chinensis*, var. *para-chinensis*, var. *narinosa*, var. *japonica* and var. *rapa*. Until recently, these varieties were considered as separate species due to the wide range of their variability and evolution in isolation from each other.

Brassica nigra, known as black mustard, is an annual weedy plant grown for its seeds. It is growing wild as a weed in the cultivated fields in the Mediterranean region [1]. *Brassica napus* L., commonly known as canola or oilseed rape, is the amphidiploid (allotetraploid) of *Brassica rapa* and *B. oleracea* [1]. The term "canola" was introduced in 1978 by the Canola Council of Canada, and often refers to *Brassica napus*, *B. juncea* and *B. rapa* species whose seed oil contains less than 2% erucic acid [33]. Both winter and summer forms of *B. napus* are cultivated as oilseeds in various countries. Additionally, root-forming *B. napus* types, known as tuber-bearing swede or rutabaga, are cultivated as vegetables [1].

Brassica juncea L. is an amphidiploid species originated from crosses between *Brassica rapa* and *Brassica nigra* [1]. It has a great seed yield potential for semi-arid conditions, and is known to be more drought tolerant than rapeseed species [37]. It is grown as an oilseed and leafy vegetable. *Brassica carinata*, or Ethiopian mustard, is an amphidiploid species originated from crosses between *Brassica nigra* and *Brassica oleracea*, and contains mustard oil [1].

3. CHROMOSOME NUMBER AND GENOMIC RELATIONSHIPS

The cytogenetic research in *Brassica* started in Japan with the identification of the chromosome number of *Brassica rapa* [38]. The genomic relationships among *Brassica* species have been determined. The genomes have been characterized as the A, B and C genomes, with 3 monogenomic diploid species, namely *Brassica rapa* (AA, 2n=20), *Brassica nigra* (BB, 2n=16) and *Brassica oleracea* (CC, 2n=18) [39]. Based on the studies of chromosome pairing in

interspecific hybrids, Morinaga [40] hypothesized that *Brassica* species with high chromosome numbers like *Brassica napus* (2n=38, AACC), *Brassica juncea* (2n=36, AABB) and *Brassica carinata* (2n=34, BBCC) originated as amphidiploids from combinations of pairs of species with lower chromosome numbers like *Brassica nigra* (2n=16, BB), *Brassica oleracea* (2n=18, CC) and *Brassica rapa* (2n=20, AA) [2,33,41]. U [42] verified this hypothesis by successfully resynthesizing *Brassica napus* from interspecific crosses between the diploid species *Brassica oleracea* and *Brassica rapa* and proposed the genomic relationship among *Brassica* species which is known as *Brassica* triangle or U's triangle (Fig. 1). These findings were later verified by resynthesizing *Brassica juncea*, *Brassica napus* and *Brassica carinata* through interspecific hybridisation between diploid species followed by chromosome doubling [33].

The close relationship between the A- and C-genome has clearly been demonstrated by the observation that significantly higher amounts of chromosome pairing occur in the AC amphihaploids, compared to AB and BC amphihaploids [33,43,44]. Formation of multivalents in AC amphihaploids (haploids that contain one copy of the 2 diploid genomes present in the polyploid) suggests that there are structural similarities within and among chromosomes of these 2 genomes [2]. On the other hand, the lack of pairing of the B-genome chromosomes with the AC chromosomes suggests that this genome is more distantly related to the other 2 genomes [33]. It was first believed that *Brassica nigra* may carry a genetic factor to suppress homologous pairing, but no genetic or cytoplasmic factor has been reported that controls pairing [33].

The diploid *Brassica* genomes vary from 1.1 pg/2C (529 Mbp/1C, where C is haploid DNA per nucleus) for *Brassica rapa* to 1.4 pg/2C (696 Mbp/1C) for *Brassica oleracea* [31,45]. The genomes of the allotetraploids (amphidiploids) range from 2.2 pg/2C (1,068 Mbp/1C) for *Brassica juncea* to 2.6 pg/2C (1,284 Mbp/1C) for *Brassica carinata* (Fig. 1).

4. GEOGRAPHICAL DISTRIBUTION, ORIGIN AND DOMESTICATION

Species of the family Brassicaceae are believed to have originated in the Himalayan region [33,46]. The tribe Brassiceae is distributed throughout the Mediterranean, the Irano-Turanian and the Saharo-Sindian phytogeographic regions which are different in altitude, climate and ecological diversity [33,46]. Most of the diversity in the *Brassica* species occurs in the southwest Mediterranean area including Algeria, Morocco, Spain, and some of the Atlantic islands [47]. It is believed that the tribe Brassiceae originated first in the southwest Mediterranean region [33,46].

The wild form of *Brassica oleracea* grows perennially along the coast of the Mediterranean region from Greece through to the Atlantic coasts of France and Spain, around the coast of England, and to a certian extent in Helgoland [48]. The wild type is found on the limestone and chalk cliffs in places protected from grazing. In North America and Europe, domesticated types have been identified as escapes [48]. *Brassica oleracea* is a recent introduction into North America. It is commonly accepted that the cabbage origin is the north European countries and the Baltic Sea coast [49,50], and Mediterranean region [51].

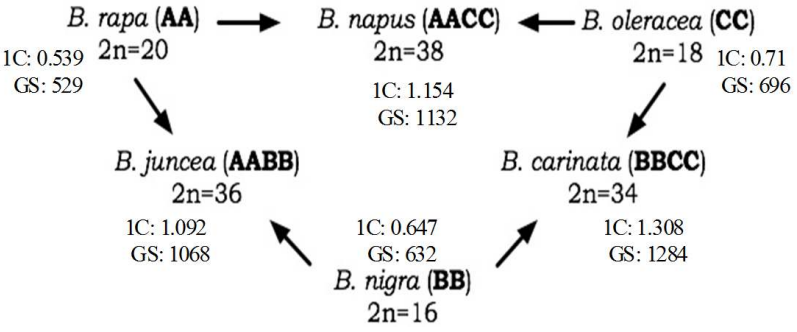

Fig. 1. Genomic relationships of the different diploid and amphidiploid *Brassica* species, known as U's triangle. 1C, 1C nuclear DNA content (pg); GS, genome size (Mbp) [31,33,42,45]

Brassica rapa originated from the highlands near the Mediterranean Sea rather than from the Mediterranean coastal areas [1]. The climate in these mountainous regions is very cold, and *Brassica rapa* shows rapid vegetative growth under low-temperature conditions. From here, it spreads northward into Scandinavia and westward to Eastern Europe and Germany [1,52]. It is believed that *Brassica rapa* was introduced into China through Mongolia or western Asia as an agricultural species [1]. The introduction into Japan could have occurred via China or Siberia. Hence, South India, central Asia and China are considered to be secondary centers of origin, where the three distinct ecotypes of *Brassica rapa* (brown sarson, yellow sarson and toria) have evolved [33,38,53].

Brassica nigra grows in the Mediterranean region, extending into central Asia and the Middle East [1,33]. It has been found on road sides and fields near Tangiers, Morocco, Egypt, and under semi-cultivated conditions in Rhodes, Sicily, Turkey and Ethiopia. In the climatic conditions of the Mediterranean regions, *Brassica nigra* grows relatively quickly [33].

Wild forms of *Brassica napus* occur on the beaches of the northern parts of Europe, including Sweden, the Netherlands and Britain [1]. Naturalized forms of *Brassica napus*, which are very distinct from cultivated forms are found on coastal cliffs of New Zealand, where *Brassica oleracea* and *Brassica rapa* grow wild [1]. It is believed that *Brassica napus* originated in the Mediterranean regions or western regions of Europe.

Brassica juncea has a long history of cultivation in temperate and humid parts of southern China [33,38]. It is believed that the Middle East regions are the primary centers of origin of *Brassica juncea*, where the oilseed form evolved [38,54]. Central and western China, the arid plateaus of Asia and southern Iran were considered as secondary centers of origin, where its wild relatives are found [55]. Recent cytological, biochemical and molecular evidence suggest a polyphyletic origin for *Brassica juncea*, in areas where the parental species have a sympatric distribution [33,56].

The cultivation of *Brassica carinata* is restricted to the Ethiopian plateau and parts of Kenya for centuries [1,38]. It might have originated from hybrids between kale, which has been cultivated in the Ethiopian plateau since ancient times, and wild or cultivated *Brassica nigra* [1]. No wild forms of *Brassica carinata* has been found on the Ethiopian plateau [1,33,54].

5. BREEDING SYSTEMS AND TECHNOLOGIES

Due to the strong self-incompatibility system, most *Brassica* crops are outbreeders with a high degree of heterozygosity in natural populations and open-pollinated crops [9]. *Brassica oleracea* is insect cross-pollinated with self-pollination prevented by a sporophytic self-incompatibility system [32]. Doubled Haploid (DH) technology has been widely applied in *Brassica* crops to generate inbred lines, and self-incompatibility (SI) has been successfully used to produce F1 hybrids. Cytoplasmic male sterility (CMS) is used in hybrid production, and provides an excellent tool to study genetic interactions between nucleus and mitochondria during flower development [6,32].

The cross between *B. napus* and *B. oleracea* is normally infertile, however the use of embryo culture techniques can produce viable hybrids [57]. Hybrids have been produced between forage rape (*B. napus* ssp. *biennis*) and kale (*B. oleracea* ssp. *acephala*) and also between rape and cauliflower (*B. oleracea* ssp. *botrytis*). The main program has involved doubling the the hybrids chromosomes with colchicine to produce the possible new species hybrid *B. napoleracea*. The hybrids are highly fertile when back-crossed to rape but produce only occasional seed when back-crossed to kale. Aphid resistance and self-incompatibility have been transferred to rape, and new combinations of glucosinolates in the plant tissues have been obtained [57].

Breeding of *Brassica* aims to increase yield and improve agronomic characteristics and quality. In oil types, an essential aim in breeding programs is to increase seed oil content and seed yield, although it is difficult to achieve these two simultaneously. Disease resistance is also an important breeding aim. In *Brassica* vegetables, breeding programmes have different objectives and priorities since each vegetable type is characterised by its own characteristics [6]. *Brassica* crops were among the first to be targeted for commercial transgenic genetic modification, for traits such as modification of male sterility and herbicide resistance [9,32]. Recently, improved nutritional quality of *Brassica* products has become an important selection criterion to globally improve the living standard.

Improving fatty acid composition and increasing tocopherol content of oilseeds has been a target for breeding [6].

6. BIOCHEMICAL CHARACTERIZATION OF *Brassica* GENETIC DIVERSITY

Genetic diversity is defined as the variation of individual genotypes within and among species, and is the raw material permitting species to adjust to a changing world [2,58]. The genetic profile of whole populations varies from place to place across a species range. These differences may arise as the result of chance occurrences, such as the genetic composition of dispersing individuals that create a new population (founder effect), or changes in allele frequencies that result from chance matings in very small populations (genetic drift) [2,59]. Differences among populations may also arise systematically, especially if the environment in different places exposes the individuals to different optima for survival and reproduction (fitness). For these and other reasons, populations can diverge from one to another in their genetic composition. This divergence is especially strong and rapid when there is a little gene flow among populations (e.g., limited dispersal of seeds or pollen, or limited movement of animals across physiographic barriers) [59]. Over evolutionary time, such among-population genetic differences can accumulate and result in the development of a new species (allopatric speciation). Knowledge of the amount and distribution of genetic variability within a species is vital for establishing efficient conservation and breeding practices [3,58,60,61], whereas it provides plant breeders with options to develop, through selection and breeding, new and more productive crops that are resistant to diseases and pests and adapted to changing environments. It also provides information for domestication and designing sampling protocols [62]. Therefore, assessing genetic diversity is essential for providing information for domestication, propagation and breeding programs as well as conservation of plant genetic resources. The biochemical markers (storage proteins and isozymes) have been developed and used to evaluate the genetic diversity and relationships of *Brassica* and various plant species.

6.1 Storage Proteins

Proteins are the post-transcriptional and translational products of an organism's DNA and form structural and enzymatic components of cells. Their size and amino acids sequences are the direct results of transcription and translation of the nucleotide sequences of the genes [63,64]. Hence, any observed variation in protein systems is considered as a mirror for genetic variations, specifically seed proteins which reflect the genetic history of the species and are not influenced by the environmental fluctuations.

Proteins have been separated and characterized by different methods such as ultracentrifugation, chromatography, serology and electrophoresis. Of these methods, only electrophoresis provided data for gene-ecological studies. This method is the most appropriate for the separation and equivocal comparison of proteins [64,65]. Electrophoretic techniques have been widely used as a rapid and accurate test to identify and characterize different cultivars and genotypes of plants. Genotype identification by electrophoretic protein fingerprinting was used to assess the uniformity, purity and agronomic merits [66-68].

Electrophoretic analysis of native or denatured seed storage proteins was used to provide information concerning the genetic variability, which represent a source of information for assessing genetic and taxonomic relationships at the species level and below, for example, *Lathyrus sativus* [69], *Lactuca* [70] and *Brassica* [68,71-74].

Toosi et al. [68] analyzed protein profiles of *B. juncea* var. Ensabi at different growth stages. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of seed, shoot and root samples of seedling, before-flowering and after-flowering stages of the plant were performed on 10% polyacrylamide gel. Out of 11 bands noticed in seed proteins of Ensabi, five polypeptides matched closely with seed protein profiles of other *B. juncea* varieties. A comparison of the protein profiles at different growth stages suggested steady expression as well as up- and down-regulation of numerous genes encoding different proteins in *B. juncea* var. Ensabi.

Rabbani et al. [71] used SDS-PAGE to analyse the total seed storage protein of oilseed mustard (*Brassica juncea*) germplasm from Pakistan. Eight types of protein were revealed based on the banding patterns of 52 accessions. The results indicated that SDS-PAGE markers applied to seed proteins could not distinguish the closely related oilseed collections and cultivars

from each other as they were characterized by the same banding pattern and formed a common gene-pool. However, seed proteins were useful to separate *B. juncea* and *B. campestris*. It also distinguished the oilseed mustard from the vegetable form. Khurshid and Rabbani [72] also assessed genetic divergence of *Brassica* species based on protein polymorphism using SDS-PAGE. The study revealed considerable degree of peptide polymorphism and distinguished among genotypes studied.

Khan et al. [73] analyzed the total seed strorage proteins of 136 accessions of *Brassica napus* L. based on SDS-PAGE technique. A total of 21 protein sub-units were revealed among the accessions. Out of these 21 bands, 16 (76.19%) were polymorphic and 5 (23.81%) were monomorphic. The similarity coefficient among these accessions ranged from 0.83 to 0.98. The cluster analysis divided the accessions into five major clusters. A low level of genetic variation was found. So, to reveal high level of genetic diversity among these accessions 2-D gel electrophoresis along with other molecular techniques should be used in future. Mir et al. [74] also assessed patterns of genetic diversity and relationships among different accessions of *Brassica juncea* using sodium dodecyl sulphate. The dendrogram clustered the the accessions into two main clusters and distinguished among the accessions studied. Therefore, SDS-PAGE of seed storage proteins has proven to be a simple and effective method for distinguishing among plant accessions.

6.2 Isozyme Markers

Isozymes (isoenzymes) are structurally different molecular forms of an enzyme having, qualitatively, the same catalytic function. Allozymes are allelic variants of enzymes encoded by structural genes of the same locus. Isozymes emerge through amino acid alterations, which may cause changes in net charge, or the spatial structure (conformation) of the enzyme molecules as well as their electrophoretic mobility. After specific staining, the isozyme profile of individuals could be observed [64,75, 76]. Isozyme analysis has been used for various purposes in biology, e.g., to assess phylogenetic relationships, estimate genetic variability and taxonomy, study population genetics and developmental biology and to direct utilisation in plant genetic resources management and plant breeding [75,76], for example, red clover [77], *Ballota* [78], *Lespedeza* [79], blue pine [80], *Lactuca* [64] and *Brassica* [2,12,81]. Lázaro and Aguinagalde [12] studied the genetic variation in 36 populations of wild taxa of *Brassica oleracea* and 2 cultivated forms using isozyme variation at 11 loci for 5 enzyme systems. The mean values for the percentage of polymorphic loci and expected heterozygosity were 54% and 0.224, respectively. The intrapopulational genetic diversity was 67%, while interpopulational genetic diversity was only 33%. The cluster analysis divided the accessions into 3 different groups. Raybould et al. [81] also examined the genetic variation at 4 isozyme and 7 microsatellite loci in natural populations of *Brassica oleracea* on the coast of Dorset. All loci were polymorphic, and the diversity index of isozyme loci was similar to that of the microsatellites. Genetic differentiation among accessions (F_{ST}) was significant for all loci and there was evidence of isolation by distance at both microsatellite and isozyme loci. The above studies showed a variation in the data displayed by different isozyme markers and proved to be successful for assessing the genetic variation, taxonomic relationships and species identity. In conclusion, these studies data could be used to improve *Brassica* crops through future breeding programs and further research studies. Biochemical and molecular markers have an essential role in crops improvement [12,82-84].

7. CONCLUSION

This review summarized the biology, distribution, origin, and breeding systems of *Brassica* species as well as their genetic diversity and relationships at the level of biochemical markers, including storage proteins and isozymes which have proven to be effective for evaluating the genetic variation, taxonomic relationships and species identity. Undoubtedly, this current knowledge would be potentially used for enhancing future breeding programs of highly agronomic *Brassica* species as well as improving their propagation, sampling protocols, and conservation strategies.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Rakow G. Species origin and economic importance of *Brassica*. In: Pua EC, Douglas CJ, editors. Biotechnology in agriculture and forestry. Vol. 54. New York: Springer-Verlag Berlin Heidelberg; 2004; 3–11.
2. El-Esawi MA. Assessing the genetic diversity, phylogenetic relationships, and disease resistance genes in Irish *Brassica oleracea* species. Ph.D. Thesis, Dublin Institute of Technology, Ireland; 2012.
3. El-Esawi M, Bourke P, Germaine K, Malone R. Assessment of morphological variation in Irish *Brassica oleracea* species. JAS. 2012a;4:20-34.
4. Rich TCG. Crucifers of Great Britain and Ireland. Botanical Society of the British Isles, London. 1991;336.
5. Christopher GL, Andrew JR, Geraldine ACL, Clare JH, Jacqueline B, Gary B, German CS, David E. *Brassica* ASTRA: an integrated database for *Brassica* genomic research. Nucleic Acids Research. 2005; 1(33):D656-59.
6. Zhao J. The genetics of phytate content and morphological traits in *Brassica rapa*. PhD thesis, Wageningen University, The Netherlands; 2007.
7. Labana KS, Gupta ML. Importance and origin. In: Breeding oilseed *Brassicas*. Edited by Labana KS, Banga SS, Banga SK. Springer, Verlage Press; 1-20, Berlin, Germany; 1993.
8. Fahey J, Talalay P. The role of crucifers in cancer chemoprotection. In: Phyto-chemicals and health. Edited by Gustine DL, Florens HE. American Society of Plant Physiologists; Rockvill, USA. 1995;87–93.
9. King GJ. A white paper for the multinational *Brassica* genome project; 2005. Available:http://www.*brassica*.info/info/publications/white_paper.php
10. Faltusová Z, Kučera L, Ovesná J. Genetic diversity of *Brassica oleracea* var. *capitata* Gene Bank accessions assessed by AFLP. Elect J Biotech. 2011;14(3):1-10. Available:http://dx.doi.org/10.2225/vol14-issue3-fulltext-4
11. Relf D, McDaniel A. Cole Crops or *Brassica*s. Virginia Cooperative Extension, Publication number 426-403, Virginia State University, Petersburg, USA; 2009.
12. Lázaro A, Auginagalde I. Genetic diversity in *Brassica oleracea* L. (Cruciferae) and wild relatives (2n = 18) using isozymes. Ann Bot. 1998;82:821-828.
13. Warwick SI, Francis A, La Fleche J. Guide to wild germplasm of *Brassica* and allied crops (tribe Brassiceae, Brassicaceae). agriculture and agri-food Canada, Eastern Cereal and Oilseeds Research Centre, Ottawa, Canada; 2000.
14. Watson-Jones SJ, Maxted N, Ford-Lloyd BV. Population baseline data for monitoring genetic diversity loss for 2010: A case study for *Brassica* species in the UK. Biological Conseravtion. 2006;132: 490-99.
15. Heywood V. Flowering plants of the world. B.T. Batsford Ltd., London, UK; 1993.
16. Mabberley DJ. The plant-book, A Protable Dictionary of the Vascular Plants, 2nd edn. Cambridge: Cambridge University Press, London, UK; 1997.
17. Judd WS, Campbell CS, Kellogg EA, Stevens PF. Plant Systematics, A Phylogenetic Approach. Sinauer Associates, Inc: USA; 1999.
18. Warwick SI, Francis A, Al-Shehbaz IA. Brassicaceae: Species checklist and database on CD-Rom. Plant Sys Evol. 2006;259:249–58.
19. Puri V. The role of floral anatomy in the solution of morphological problems. Bot Rev. 1951;17:472–553.
20. Eames AJ. The vascular anatomy of the flower, with refutation of the theory of carpel polymorphism. AM J Bot. 1961;18: 147–188.
21. Rendle AB. Classification of flowering plants. In: Dicotyledons, vol. II. Cambridge University Press, UK; 1952.
22. Melchior H. Engler's Syllabun Pflanzenfamilien, second ed., vol. 2. Wilhelm Engelmann, Leipzig; 1964.
23. Benson L. Brassicaceae. In: Plant Classification, seconded. Heath, D.C., Co., Lexington, MA, USA. 1979;152–160.
24. Lawrence GHM. Taxonomy of vascular plants. Macmillian Company, New York; 1989.
25. Rodman JE. A taxonomic analysis of glucosinolate producing plants. Syst Botany. 1991;16:598–629.

26. Rodman JE, Price RA, Karol KG, Corti E, Systma KJ, Plamer JD. Nucleotide sequences of the rbcl gene indicate morphology of mustard oil plants. Ann Mo Bot Gard. 1993;80:686–99.
27. Marzouk MM, Al-Nowaihi AM, Kawashty SA, Saleh NAM. Chemosystematic studies on certain species of the family Brassicaceae (Cruciferae) in Egypt. Biochem Syst and Ecol; 2010.
DOI: 10.1016/j.bse.2010.04.004
28. Clemente M, Hernandez-Bermejo JE. El caliz en la tribu Brassiceae (Cruciferae). Anales del Jardín Botánico de Madrid. 1980;36:71–96.
29. Al-Shehbaz IA. The tribes of Cruciferae (Brassicaceae) in the southeastern United States. J Arnold Arboretum. 1984;65:343–73.
30. Saha S, Molla MR, Chandra D, Rahman L. Assessment of genetic variation and relationships within the varieties of four *Brassica* species by RAPD markers. Aust J Crop Sci. 2008;2:105-14.
31. Hong CP, Kwon SJ, Kim JS, Yang TJ, Park BS, Lim YP. Progress in understanding and sequencing the genome of *Brassica rapa*. Int J Plant Genomics; 2008.
DOI: 10.1155/582837
32. Katz SH. Cabbage and Crucifer plants. Encyclopedia of Food & Culture, vol. 1. Gale Cengage; 2003. eNotes.com. 2006. Cited on 5th August; 2010.
Available:http://www.enotes.com/food-encyclopedia/cabbage-crucifer-plants
33. Navabi Z. Genetic analysis of the B-genome chromosomes in the *Brassica* species. Ph.D. Thesis, Department of Agricultural, Food and Nutritional Science, Faculty of Graduate Studies and Research, University of Alberta, Edmonton, Alberta, Canada; 2009.
34. Toxeopus H, Oost EH, Reuling G. Current aspects of the taxonomy of cultivated *Brassica* species. The use of *B. rapa* L. versus *B. campestris* L. and a proposal for a new intraspecific classification of *B. rapa* L. Crucifer Newsletter. 1984;9:55–57.
35. Canadian Food Inspection Agency (CFIA). The Biology of *Brassica rapa* L. A comparison document to *the Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits*. Regulatory Directive Dir1999-02, CFIA; 1999.
Available:http://www.maltawildplants.com/CRUC/ Docs/ BRSRA/*Brassica*Rapa.pdf
36. Prakash S, Hinata K. Taxonomy, cytogenetics and origin of crop *Brassica*s, a review. Opera Bot. 1980;55:3-57.
37. Rabbani MA, IwabuchiA, MurakamiY, Suzuki T, Takayanagi K. Collection, evaluation and utilization of oilseed mustard (*Brassica juncea* L.) in Pakistan. Pakistan J Biol Sci. 1999;2:88-94.
38. Gomez-Campo C, Prakash S. Origin and domestication. In *Biology of Brassica coenospecies*. Edited by Gomez-Campo C. Elsevier Publishers, Amsterdam, Netherlands. 1999;33-58.
39. Redden R, Vardy M, Edwards D, Raman H, Batley J. Genetic and morphological diversity in the *Brassicas* and wild relatives. 16th Australian Research Assembly on *Brassica*s. Ballarat Victoria; 2009.
40. Morinaga T. Interspecific hybridization in *Brassica*. The cytology of F1 hybrids of *B. juncea* and *B. nigra*. Cytologia. 1934;6:62-67.
41. Abbas SJ, Farhatullah, Marwat KB, Khan IA, Munir I. Molecular analysis of genetic diversity in *Brassica* species. Pakistan J Bot. 2009;41:167-76.
42. UN. Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilisation. J Jpn Bot.1935;7:389-452.
43. Attia T, Busso C, Röbbelen G. Digenomic triploids for an assessment of chromosome relationships in the cultivated diploid *Brassica* species. Genome. 1987;29:326-30.
44. Attia T, Röbbelen G. Cytogenetic relationship within cultivated *Brassica* analyzed in amphihaploids from the 3 diploid ancestors. Can J Genet Cytol. 1986;28:323-29.
45. Johnston JS, Pepper AE, Hall AE. Evolution of genome size in Brassicaceae. Ann Bot. 2005;95:229–235.
46. Hedge IC. In The biology and chemistry of the Crucifereae. Edited by Vaughan J.G. Acadaemic Press, London, UK; 1976.
47. Gomez-Campo C. Morphology and morpho-taxonomy in the tribe *Brassiceae*. In *Brassica* crops and wild allies. Edited by Tsunoda S, Hinata K, Gomez-Campo C. Japan Scientific Societies Press, Tokyo, Japan. 1980;3-31.

48. Organisation for Economic Co-operation and Development (OECD). Consensus Document on the Biology of *Brassica napus* L. (Oilseed Rape). *Series on Harmonization of Regulatory Oversight of Biotechnology,* No. 7. OCDE/GD(1997)63, Paris, France.
Available:http://www.oecd.org/dataoecd/28/22/27531440.pdf
49. Monteiro A, Lunn T. Trends and perspectives of vegetable *Brassica* breeding. World Conference on Horticultural Research, 17-20 June 1998, Italy.
50. Balkaya A, Yanmaz R, Kar AA. Morphological characterisation of white head cabbage *(Brassica oleracea* var. *capitata* subvar. *alba)* genotypes in Turkey. New Zeal J Crop Hort. 2005;33:333-41.
51. Vural H, Eşiyok D, Duman I. The culture vegetables (vegetable growing). 440 p. Izmir, Turkey, (In. Turkish); 2000.
52. Nishi S. Differentiation of *Brassica* crops in Asia and breeding of 'Hakuran' a newly synthesized leafy vegetable, In: Tsunoda S, Hinata K, Gomez-Campo C (eds) *Brassica* Crops and Wild Allies, The Japan Science Society Press. 1980;133-150.
53. Quijada P, Cao J, Wang X, Hirai M, Kole C. *Brassica rapa*. In Genome mapping and molecular breeding in plants. Edited by Kole C. Springer, Verlag, Berlin Heidelberg, Germany; 2007.
54. Mizushima U, Tsunoda S. A plant exploration in *Brassica* and allied genera. Tohoku J Agric Res. 1967;17:249-77.
55. Vavilov N. The origin, immunity and breeding of cultivated plants. Chron Botany. 1949;13:1-364.
56. Vaughan JG. Multidisciplinary study of taxonomy and origin of *Brassica* crops. Bioscience. 1977;27:35-40.
57. Gowers S, Christey MC. Intercrossing *Brassica napus* and *B. oleracea* to introgress characters from kale to rape. In: Wratten N, Salisbury PA (eds.) Proceeding of the 10th International Oilseed Congress, Canberra, Australia; 1999.
Available:http://www.regional.org.au/au/gcirc/4/586.htm
58. Chaveerach A, Sudmoon R, Tanee T, Mokkamul P, Tanomtong A. Genetic relationships in a population of *Nelumbo nucifera* Gaertn (Nelumbonaceae). J Biol Sci. 2007;7:1388-93.
59. Falk DA, Knapp E, Guerrant EO. Introduction to restoration genetics. Society for Ecological Restoration, USA; 2001.
Available:http://www.nps.gov/plants/restore/pubs/restgene/restgene.pdf
60. Sammour R, Badr S, Mustafa A, El-Esawi M. Genetic variation within and among some *Lactuca spp*. based on karyotype analysis. Applied Cell Biology (ACB). 2013;2:136-143.
61. El-Esawi MA, Sammour R. Karyological and phylogenetic studies in the genus *Lactuca* L. (Asteraceae). Cytologia. 2014;79:269–75.
62. Yu, J, Mosjidis J, Klingler K, Woods F. Isozyme diversity in North American Cultivated Red Clover. Crop Sci. 2001;41: 1625-28.
63. Kephart S. Starch gel electrophoresis of plant isozymes: A comparative analysis of techniques. Am J Bot. 1990;77:693-712.
64. El-Esawi MA. Genomic characterization and genetic improvement of some *Lactuca* spp. M.Sc. Thesis, Faculty of Science, Tanta University, Egypt; 2008.
65. Gordon M, Huang X, Pentoney S, Zare R. Capillary electrophoresis. Science. 1988;242:224-228.
66. DellaGatta C, Polignano G, Bisignano V. Variation for protein content and seed weight in grass pea (*Lathyrus* spp.) germplasm. PGR Newsletter. 2002;132:30-34.
67. Liang X, Luo M, Holbrook C, Guo B. Storage protein profiles in Spanish and runner market type peanuts and potential markers. BMC Plant Biol. 2006;6-24.
68. Toosi AF, Arumugam B, Baki BB, Tayyab S. Protein Profiling of *Brassica juncea* (L.) Czern var. Ensabi at Different Developmental Stages. J Biol Sci. 2011; 11:165-72.
69. Sammour R, Mustafa A, Badr S, Tahr W. Genetic variations in accessions of *Lathyrus sativus* L. Acta Bot Croat. 2007;66:1-13.
70. Vries I. Characterization and identification of *Lactuca sativa* cultivars and wild relatives with SDS-electrophoresis (*Lactuca* sect. *Lactuca*, Compositae). Genet Resour Crop Ev. 1996;43:193-202.
71. Rabbani MA, Qureshi A, Afzal M, Anwar R, Komatsu S. Characterization of mustard (*Brassica juncea* L.) Czern. & coss.] germplasm by SDS-PAGE of total seed proteins. Pakistan J Bot. 2001;32:173-79.
72. Khurshid H, Rabbani MA. Comparison of electrophoretic protein profiles from seed

of different oilseed *Brassica* cultivars. J Pub Health Biol Sci. 2012;1:36-42.

73. Khan SA, Iqbal J, Khurshid H, Zia M, Shinwari ZK, Rabbani MA. Intra-specific genetic divergence in rapeseed (*Brassica* napus L.) genotypes estimated through SDS-PAGE of total seed proteins. I. J. Basic Appl. Sci. 2014;3:110-117.
74. Mir JI, Islam S, Kudesia R. Evaluation of genetic diversity in *Brassica juncea* (L.) using protein profiling and molecular marker (RFLP). I. J. Plant Breed. Genet. 2015;9:77-85.
75. Dziechciarková M, Lebeda A, Doležalová I, Astley D. Characterization of *Lactuca* spp. germplasm by protein and molecular markers - a review. PSE. 2004;50:47-58.
76. Kumar P, Gupta VK, Misra AK, Modi DR, Pandey BK. Potential of molecular markers in plant biotechnology. POJ. 2009;2:141-162.
77. Mosjidis J, Greene S, Klingler K, Afonin A. Isozyme diversity in wild red clover populations from the Caucasus. Crop Sci. 2004;44:665-70.
78. Zaghloul M, Hamrick J, Moustafa A, Kamel W, El-Ghareeb R. Genetic diversity within and among sinai populations of three *Ballota* species (Lamiaceae). J Hered. 2006;97:45-54.
79. Zhang J, Yuan Q, Meng Y, Li X, Nan Z, Wang Y, Zhang W. A genetic diversity analysis of wild *Lespedeza* populations based on morphological characters, allozyme and RAPD methods. Plant Breed. 2007;126:89–94.
80. Bakshi M, Konnert M. Genetic diversity and differentiation through isozymes in natural populations of *Pinus wallichiana* A.B. Jacks (Blue Pine) in India. Ann For Res. 2011;54:23-37.
81. Raybould AF, Mogg RJ, Clarke RT, Gliddon CJ, Gray AJ. Variation and population structure at microsatellite and isozyme loci in wild cabbage (*Brassica oleracea* L.) in Dorset (UK). Genet Resour Crop Ev. 1999;46:351-360.
82. Jourdan N, Martino C, El-Esawi M, Witczak J, Bouchet PE, d'Harlingue A, Ahmad M. Blue-light dependent ROS formation by *Arabidopsis* Cryptochrome-2 may contribute towards its signaling role. Plant Signal. Behav. 2015;10(8).

    DOI: 10.1080/15592324.2015.1042647
83. Consentino L, Lambert S, Martino C, Jourdan N, Bouchet PE, Witczak J, Castello P, El-Esawi M, Corbineau F, d'Harlingue A, Ahmad, M. Blue-light dependent reactive oxygen species formation by *Arabidopsis* cryptochrome may define a novel evolutionarily conserved signaling mechanism. New Phytol. 2015;206:1450-1462.
84. El-Esawi M, Glascoe A, Engle D, Ritz T, Link J, Ahmad M. Cellular metabolites modulate in vivo signaling of *Arabidopsis* cryptochrome-1. Plant Signal. Behav. 2015;10(9).

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