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Meet the editor

Dr. Sarwan Kumar is a senior entomologist at Punjab Agricultural University (PAU), India, where he has been working for the past 16 years. He has extensive experience in host plant resistance in *Brassica* crops and has published numerous research papers in this area. Dr. Sarwan has a Ph.D. in Entomology from Chaudhary Charan Singh Haryana Agricultural University, India, and an MSc in Entomology from PAU. He has worked on various research projects related to insect pest management in *Brassica* crops. He has also served as a reviewer for several scientific journals and has presented his research at numerous national and international conferences. Dr. Kumar is a member of several professional societies including the Entomological Society of Iran and Society of Chemical Industry, UK. He has received awards for his outstanding contributions to the field of entomology, including the Australian Government's Prestigious Endeavour Research Fellowship. He is also a fellow of the Indian Society of Oilseeds Research.

Contents

Preface

Brassica species are of great importance in agriculture, horticulture, and human nutrition. The *Brassica* genus includes a wide range of economically important crops such as broccoli, cauliflower, cabbage, rapeseed, mustard, radish, and many others. These crops are not only a source of food (including oil) for human beings but also play a vital role in animal feed and industrial uses. The health-promoting and disease-preventing properties of *Brassica* vegetables are well documented, and their nutritional value makes them an indispensable part of a balanced diet.

This book provides a comprehensive overview of the recent advances in the research on *Brassica* crops. It covers a broad range of topics, from the plant breeding of *Brassica* crops to their agronomic practices, their role in human health and pest management, and host plant resistance. The book is a useful resource for researchers, students, and professionals who are interested in *Brassica* crops and their applications.

The book begins with a chapter presenting important information about the green synthesis of nanoparticles mediated by *Brassica* species and their applications. The subsequent chapters cover topics such as breeding and genetics, *Brassica* in human health and pest management, and plant secondary metabolites of *Brassica*.

I would like to acknowledge the contributing authors for their valuable insights and their efforts in bringing this book to fruition.

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Chapter 1

Perspective Chapter: Knowledge and Different Perceptions on Some Aspects in the Genus, *Brassica*

Rishan Singh

Abstract

Many years ago, the first *Brassica* species were propagated. There are several methods that can be used to grow *Brassica* plants, such as intergeneric hybridization, microscope cultivation, anther cultivation, CRISPR/Cas4 Technology and the phylogenetic analysis of *Brassica* genomes. The plants that have evolved from *Brassica* species are many, and these include Savoy cabbage, broccoli, mustard greens, Japanese mustard, horseradish, as well as kale. Although the main supplier of *Brassica* vegetables is China, these species have diverged and emerged to several other countries like Cyprus, Europe, Levant, Greece and the British Isles. Ogura cytoplasm introgression is a technique that has highlighted the differences in floral traits in species of *Brassica* plants. In cauliflower plants, pre-floral meristem division is a factor that's often investigated, as divisions of this plant part demonstrates plant growth and mobility. This perspective chapter will address all aspects pertaining to the genus *Brassica*, and it will provide an account of key characteristics and functions ascribed to *Brassica* plants.

Keywords: China, Japanese radish, curd, embryogenesis, chlorosis, chloroplasts compatibility, Triticum aestivum, Oryza sativa, *B. napus*, floral genes, health, *B. nigra*, genotoxic carcinogen, biotransformation, flower development

1. Introduction

It's been many years since the first *Brassica* plants have been propagated. In successful attempts to grow these plants, it's now apparent that there are many methods that can be used to grow them. This further implies that the *Brassica* genus of plants have become widespread, and this obvious statement is provided by fact that the plants of *Brassica* species are consumed everywhere, throughout the world [1]. In general, China is the main supplier of *Brassica* vegetables throughout the world, with approximately half of the produce being exported to other countries [2]. The genus, *Brassica* is made up of 37 different species, and of this, 6 are interrelated species, viz. *Brassica nigra, B. oleracea* and *Brassica rapa*, which are diploid, and 3 amphidiploids, viz. *Brassica carinata, B. juncea* and *Brassica napus* [3]. In terms of the common household vegetables, i.e. cabbage, cauliflower and broccoli, it has been reported by the Food and Agricultural Organisation in 2014, that the world's total production of these vegetables is 105.7 million tons (cabbage), and 33.5 million tons (cauliflower

and broccoli), respectively [2, 3]. The spicy flavour and pungent smell emitted from *Brassica* plants is ascribed due to the sulphur-containing compounds [4]. These compounds have many health benefits [2, 4].

2. Diversity and evolution

If one has to delve, or divulge, into the diversification and evolution of various *Brassica* species, it would be interesting to find that most of the world's 'cole' vegetables - e.g. kale, cabbage, Brussel sprouts, kohlrabi, broccoli, collards, savoy cabbage and chinese kale, are derivates of the ancestral species, *B. oleracea* [5]. Therefore, the dispersion of *Brassica* plants over a period of time is said to be visualised through evolved characteristics, such as buds, inflorescence, leaves, roots and seeds [6]. The wild cabbage, or *B. oleracea*, is home to coastal cliffs of western Europe and the northern Meditteranean, and they have found a suitable habitat in the British Isles from Greece [5, 6]. Prior to the 15th century, i.e. in the early Middle Ages, not much was known about cauliflower plants, however, until recently, it has been documented that cauliflower plants probably were introduced into Europe from Cyprus or Levant [7]. In today's world where a lot of molecular studies are being performed in plants, it has also been well emphasised that broccoli, an edible crop that's widely sold, is the closest cultivated relative of cauliflower. This deduction is made with possiblities that the phenotype of cauliflower is more likely caused by a defective CAL gene, as tested and confirmed in a study performed on *Arabidopsis* [6, 8]. Therefore, the abnormal flowering, or flowers, of cauliflower are likely to have arisen from the floral stem primordia of cauliflower, and as a result of this mutant CAL gene [8], the flowers of cauliflower have lost their identity [5]. However, apart from this dissimilarity, cauliflowers and brocolli have a shared phenotype, since their qualities are much similar, for example, they both are short with reduced auxillary shoorts on multibranced flowering stems [5, 6]. The only difference is that the flowering stems of broccoli are much shorter, and that the buds that are produced are packaged densely [5, 6]. Furthermore, in cauliflower, although the primordia at the apex of each elongating shoot, the meristem, grow and develop into leaves, the later primordia fails to produce flower buds. It is, therefore, the meristem of the stem that continuously replicates itself in a spiral fashion, and the continuation of this up to the 10th order of branching, or more, causes the phenotype of cauliflower to be made of undifferentiated inflorescence meristems that are closely packed and clustered geometrically [5, 6, 8]. This texture of plants found in the family Brassicaceae is novel, and it's absent in wildtype plants [9, 10].

3. Hybridization and embryo development

3.1 Techniques

Although, thus far, some species of plants from the genus *Brassica* have been reported, there are several other plants that belong to this diverse genus. Other *Brassica* plants include, among others, radish, arugula, watercress, horseradish, wasabi, daikon, gai-lohn, mustard greens, Japanese mustard and chinese broccoli [11–13]. We have now seen the manner in which the ancestral species of Brassicacaea spread to different regions of the world, and lead to so many methods of propagating

these plants. Across the world, many attempts have been made to cultivate hybrids of *Brassica oleracea var. botrytis*, or the cauliflower. A particular reason for wanting to conserve this plant species is because of its health promoting properties [3, 12–14]. These properties are due to several chemicals present within them, such as anthocyanins, vitamins A, C, E and K, folic acid, phenolics, carotenoids, glucosinolates (as mentioned previously) [15] and selenium [16]. Currently, there are two main techiques used to generate introgression into *Brassica* crops [14]. In the first method, an inbred line that is double haploid is produced, since it is reported that homozygosity using both methods is pivotal. This statement is backed by findings that there are several problems associated with being able to identify and maintain S-allele homozygosity in cole plants, for example, in some instances, although an S-allele homozygote may be present, it may be weak, while in other cases, no zygote may be available, thereby questioning the reliability of these methods [15, 16]. However, nonetheless successes are reported with these methods, and successes are dependent on seed/microspore recalcitrance and bud size. *B. oleracea var botrytis* and *B. napus* are the most recalcitrant among the *Brassica* crops, and this seed behaviour is known to have a huge impact on embryogenic responses to culture conditions, such as media conditions and culture incubation conditions [17]. It has also been observed, in some situations, that although bud size has a considerable impact on microspore production in *B. oleracea var botrytis*, the nucleation of microspores has a considerable effect on the ability of the plants to complete embryogenesis. This means that seed/microspore viability is dependent on bud size, as reported in 2015 by Bhatia *et al* [18]. These authors have suggested that in order to obtain microspore of the highest viability, the buds produced need to be 4–4.5 mm in size [19]. However, this was deduced for plants that were in early and mid-maturation. In addition, they suggested that for cauliflower plants in the late maturation phase, the bud size should be between 4.5 and 5 mm for the microspore to have a high viability [14, 19]. However, although viability is required for embryogenesis, it is worthwhile to report that since microspores are totipotent, the ones that are binucleate may be antagonistic for embryogenesis in comparison to those that are uninucleate or in the early binucleate stages of development [20]. In cauliflowers with small flower bud size genotypes, it was found that a higher percentage of microspores developed, particularly when the microspores were in the mid to late uninucleated stage of development [20, 21]. This means that efficient seed embryogensis was ideal for microspores that were at the late uninucleate to early binucleate phases of growth. Using this method, about 50% of the plants produced were double haploids, and this was attributed to many exogenous and endogenous factors like culture and microspore density [17, 21, 22]. When the bud size was optimum for embryogenesis, about 60–65% of the microspore produced were reported as being viable. When the microspore density was $>8 \times 10^4$ per mL of culture, it was found that less embryos were produced. This could be due to nutrient depletion in the culture media, or competition for nutrients among the embryos [23]. Furthermore, toxic compounds present in the media may have had a drastic effect on some embryos, leading to death [24–26].

3.2 Sugar concentration vs. androgenic responses

Sugar concentration also has a huge impact on callus formation, and therefore embryonic development and androgenic responses. Just like with embryogenesis using seeds and/or microspores, in anther culture, media conditions also have a tremendous impact on embryo development, and thus, in this case, androgenic response

(or androgenic capacity) [27, 28]. Roy *et al*. [27], reported that medium containing BS Salt with 100 mg / lt sucrose, 1 mg /lt 2, 4-D and 1 mg/lt NAA with 1 mg/lt BAP was sufficient to obtain successful androgenic callus development in tropical cauliflower. However, in general, it is recommended that a sucrose concentration of 140 g/l is sufficient to induce androgenic callus, with maltose and glucose being less effective in inducing callus formation. Yang *et al*. [17] highlighted that the genotype of a plant, as well as petal length, anther length and bud size has an influence on embryogenesis. Additionally, seasonally, the androgenic response among genotypes vary [28]. It was found that androgenic response to embryogenic growth was found to occur between 1 and 1.3 P/A lengths [14, 27, 28]. Further, Yang [29] also found that when the temperature was too high (25°C) or too low (e.g. below 10°C), the number of non-viable androgenic callus was high. This highlights the implications and necessity for having anther culture being performed during winter and spring seasons, rather than during autumn and summer season, because the latter seasons discourage the development of inbred lines, like in cauliflower and other crops [30].

3.3 Hybridisation and the case of the Japanese radish

Hybrid production in cauliflower is possible through a process called intergeneric hybridisation, or Ogura cytoplasm introgression [14]. This process involves a male, female and restorer line [1], with the restorer gene not necessarily required for hybrid seed production [31]. Inspite of this, this process is found effective to main homozyosity of the embryo, because submating has been found to disturb the uniformity of all 3 lines after 2 or 3 generations, and as a result a lower qualify hybrid is produced in comparison to the SI lineage zygote/curd [32]. However, during introgression, only the male floral traits appear to be reduced. Among others, these floral traits are flower size, length of style, and length of stamens. In contrast, the female traits remain intact, and include: petal colour, style shape, ovary type, and the presence of nectaries [1]. Since the curd, also referred to as the pre-floral meristem, is the edible part of the cauliflower, there is not a need to restore fertility, since the seeds are only used in propagatory practices [28]. Hence, the need for male sterility using genes from other crops belonging to the genus, *Brassica*. This procedure is necessary in order to prevent the formation of functional pollen grains, and in the process of doing so, keeping the female line functional and maintaining the female traits [33]. In *R. sativa*, or radish of Japanese origin, Ogura hybrid cytoplasm can be located [34]. This cytoplasm has been shown to be effective during intergenetic hybridization, particularly in plants that contain the Rf gene. An example of a radish cultiva possessing the *rf* gene is the European radish, in which Ogura introgression F1 hybrid breeding has been found to occur unlimited [35]. This unlimited hybridisation, due to the *rf* gene, has been found to produce rapid plant vigour, larger sized crops and nicely formed radishes. Similarly, in *B. oleracea*, cytoplasm male sterility is a method to prevent self-pollination so that the crops produced, like cauliflower, do not have reduced plant vigour, a small curd size, or deformed curd, as a result of suppressed inbreeding. However, despite the many advantages of male sterility cytoplasm, there are side-effects using this technique. This includes delayed chlorophyll development in the male sterile parts of *B. oleracea var. botrytis*, since introgression caused discoloration of the tissues, or chlorosis, since the Ogura hybrid cytoplasm from Japanese radish had chloroplast that was incompatible with the of *B. oleraceae* [36, 37]. In addition, there were also deformities found in the flowers, particularly as they were small in size, and it has been suggested that backcrossing may solve the problem. In order to solve the choroplast problem, it

is suggested that interbreeding with protoplast between sterile male cytoplasm and an ordinary breeding line, may enable chloroplast compatibility. Moreover, there are also other problems associated with cytoplasm introgression, viz. unopened and partially opened flowers, rudimentary ovaries, low female fertility, poorly developed nectaries, and yellowing at temperatures below 15°C [38].

4. Genomes and leafing

The data from genetic studies and studies dealing with the phylogeny of *B. oleracea* are evidence enough that there is a relationship among cultivas and wildtype relatives belonging to the family, Brassicaceae. A study performed by Mabry *et al.* [39], suggests that *Brassica incana* shares its lineage with *Brassica cretica and Brassica montana,* and that these 3 species fall broadly under the *B. oleracea* species. The characteristics found in cauliflower plants, for example, are not entirely that of wildtype plant species, and, as previously described, much of the findings in *B. oleracea* are based on those found in cabbage crop varieties [40]. Therefore, given that the qualities exhibited in cauliflower plants are novel, and that experiments with Arabidopsis thaliana and *B. oleracea* show that there are similarities in both genomes [41], highlight that feral samples exist, and that these type of plants are not defined through cytoplasm introgression, hybridisation or culturing of the microspore or anthers of *B. oleracea* varieties. This also means that, in nature, one may encounter many domesticated crops that belong to Brassicacea, implying that species that belong to the genus, *Brassica*, may be that of a divergent population, another wild species, a wild conspecific, or even another domesticated species. Along the wildC genome, a newly identified wildC genome has been found [42], and this sequence is said to correlate to one cultivar and mixed wild ancestor of Tronchuda Kale [43] (when the wildC genome is compared to that of *B. oleracea*). However, in comparison to sample of *B. incana*, *B. cretica, B. montana* and *B. oleracea*, the wildC-2 gene wasn't expressed. Instead, this gene was found to cluster in 6 other *B. oleracea* crop types, namely *Brassica bourgeaui, Brassica hilarionis, Brassica insularis, Brassica macrocarpa, Brassica rapestris, Brassica villosa*, and *B. oleracea*. In Chinese white kale, Bussel sprouts and curly kale, distinct clades that correspond to the growth habitat of these plants have been found. Since the clades are distinct, particularly because RNA collection is performed early in the development of these plants (i.e. approximately the 7th leaf stage), each one of them can be distinguished on the basis of their phylogeny [40, 44]. For example, Brussel sprouts are identified based on their oblong to circular leaves, while curly kale have leaf margins that are undulate or frill-like during early development [40]. Another factor to be considered is that season growth plays and important role in distinguishing characteristics among crops that belong to Brassicaceae. For instance, Chinese white kale is found to have lanceolate leaves, while those in curly kale are curly-like, as well as, the period for growth, i.e. Chinese white kale is an annual, instead of a biennial season plant [40, 45]. However, further genetic analysis of clustering, and distinct clades, among the 10 *Brassica* crop types have a substantial overlap in genes (17–98.3%) in both, *B. oleracea* and *A. thaliana*, and this means that some biological processes are overexpressed in *B. oleracea*, whereas others are reserved. For instance, the glaucous leaves of *B.oleracea* is because of modules of genes responsible for wax formation [46]. Some other processes are those producing herbivory defence compounds, via. Secondary metabolite biosynthetic processes, phenylpropanoid biosynthetic processes and metabolic processes, in addition to suberin biosynthetic processes (wound formation) [47].

5. *Brassica* origins: Feral or not

There has been much debate on the origin of *Brassica* plant species, particularly, because there is a bit of confusion about whether *B. oleracea* is a progenitor species or not [6]. Archaeological and environmental data suggest that *B. cretica* and *B. hilarionis* are sister species, that these two species are a likely progenitor species of *B. oleracea* [40]. This deduction is made with the knowledge that *B. hilarionis* is homozygous, and it is this trait that makes it ideal for domestication purposes through either gene editing or inbreeding practices [10, 48]. Although *B. incana* is found to be a wildtype crop, admixture inference data has suggested that some populations may be feral [49]. Similarly, *B. cretica* has also been found to be feral, however, some populations have been located as being domesticated. Using *B. oleracea* as a model for introgression, it is concluded that the genetic composition of particular cropts are a result of introgression from wild to feral populations. To add to problems associated with postdomestication of *Brassica* plants, it must be said that due to frequent introgression and difficulties in identifying seeds of individual crop types that it's an extremely tedious task to obtain purely domesticated species of *Brassica* crops [48, 49]. It is, therefore, because of this gene flow between wild and cultivated populations that the evolutionary history of B. oleracea has become complicated. Just like *B. incana* and *B. cretica*, *B. montana* has also been identified as being partially feral in origin, however, there has been a dilemma regarding the relationship *B. montana* shares with *B. oleracea* [50]. Lanner et al. [50] has found, using chloroplast data, that *B. montana* and *B. oleracea* cluster together, whereas Panda *et al*. [51] went against this, and has suggested that B. montana is a subspecies of *B. oleracea*.

Although cabbage has its origins in the Meditteranean civilisations, *B. cretica* also has the same origins. This is said to have occurred due to germplasm transfer between wild and domesticated cultivats of *Brassica* crops [9]. However, today, *B. cretica* also occurs in Lebanon and it has been found to resemble *B. cretica* subsp. nivea, and it is this migration from Ionia, in the Western coast of present day Turkey ca. 2,102,050 BP, that suggests widespread trade of this crop by the early Mediterannean inhabitants [9]. However, it is because of he presence of Austrian archaeological evidence that we know that *B. oleracea* did not diversify itself from England [52]. Instead, because *B. nigra* and *B. rapa* are the major crops in Austria, and since there is evidence that *B. oleracea* existed in Greece during this period, we know that *B. oleracea* was absent in the Eastern Mediterannean, Europe, Britain and Czech Republic, due to the lack of data from these regions during this time [53]. Furthermore, there is no archaeological records on the cultivation of cabbages prior to the Late Iron Age (2350–2000) in Europe and the Roman periods (1950 1650 BP), however, there is documentation for the appearance of other *Brassica* species [54]. It is only around ca. 1850 BP that *B. oleracea* spread through seed dispersal to the Roman Empire [54]. Thereafter, many populations belonging to the Brassiciaceae family emerged in the British Isles [55], the Atlantic coast of Western Europe [56], South West England [57], and the Atlantic Coast of France [53]. However, in spite of this widespread dispersion of *B. oleracea* crops, these plants do not havehigh levels of genetic diversity, and some of them are isolated from other populations. Therefore, although they are wildtype plants, they are greatly feral in origin.

6. Genetic variation and introgression of the *B. rapa genome*

B. napus, B. juncea and *B. carinata* are associated with polyploidy, owing to the fact that resynthesis of diploid species hybridization, as well as, chromosome doubling

influences the ability of the DNA to remain intact [58, 59]. As mentioned, it is due to the homozygous polyploid lines that make species in the genus, *Brassica*, ideal for scientific evaluation, specifically since self-pollination promotes the synthesis of DNA restriction fragments that vary, as well as phenotypes that differ among crops in Brassicacea [60]. Furthermore, inter-fertility is common among vegetables like cabbage, cauliflower, brocolli, brussel sprouts, and kohlrabi [61]. In a genetic study involving the genome of *A. thaliana* and *Brassica* species, it was a definite observation that some genes in *Arabidopsis* are related to *Brassica* quantitative trait loci (QTLs) [62]. In the *Arabidopsis* genome, chromosome 5 was found to be similar to a region in the chromosome of *B. rapa*, and this sequence is said to be a flowering-time gene, which was present as *flc* in *Arabidopsis* upon backcrossing. This finding, together with other sequences, viz. tfl1, flc, tfc2, co, fy, art1, emf1, efs, fha, gi, hy2, and vrn1, are all flower-associated gene, which participate in the genetic control of complete traits in the genus, *Brassica*. CAULI-FLOWER and APETALA1, are responsible for the curd-like inflorescence in *Arabidopsis*, however, the architecture of the curd produced, is far more complex, and, thus, it is under much complex genetic control mechanisms [8]. Therefore, the genome of *Brassica* is able to provide further clarity and insight about the size and shape of plants. In order to add to the concept of genetic variation, in 2022, Zhang *et al.* [63, 64], a study on introgression of the *B. rapa* genome into *Brassica juncea* was conducted. In this study, the researchers attempted to track segments of the *B. rapa* genome in the *B. juncea* germplasm. Currently, a lot of effort is being placed on conserving plant germplasm, particularly so that plants that are about to become extinct, or are at the verge of extinction, are able to be preserved and regrown, when the need arises. In *Brassica*, this is required because the seeds of all the crops belonging to this genus are unable to be separated based on morphological descriptions [8]. Therefore, by introgressing the *B. rapa* genome, it was observed that 59.2% of the A genome of *B. juncea* was covered by the donor segments. By using 132 single-nucleotide polymorphisms markers, it was suggested, that due to wide genetic diversity, that the recipient genotypes had a strong selection for the donor genetic sequence. The parental resequencing data in relation to the marker genotyping results show that there were morphological differences among the formation of leaf blades among the 3 categories of B. juncea parent plants [8]. In one case, the leafy head was maintained in the introgression lines, while in the remaining cases, the head was seen being more compact (due to the leaving heading around the shoot apexes and then folding upward and inward) and eventually, even, changing shape completely. This could have been due to the large gaps (>20 cm) in the 132 SNP marker, including gaps located on both ends of A01, A9 and A10 [8]. However, nevertheless, the expected results and that of which was obtained varied significantly in that the progeny retained the *B. rapa* genome in the process of distant hybridisation of the both studied species, i.e. *B. juncea* and *B. rapa* [8]. Therefore, the progeny existed in the heterozygous form, which is not ideal for selective breeding or gene transfer. Furthermore, the progeny inherited 57.31%, 59.57%, and 60.34% of the A genotype, and these percentages are that of heading, semi-heading and semi-heading II of the leaves of the progenies [63–65]. In terms of the lead regiments, the *B. rapa* and *B. juncea* parental lines showed no difference in retainment upon retrogression. This could have been due to certain regions in the *B. juncea* genome being not readily replaced by that of *B. rapa* segments.

Phylogenetic analysis of *Brassica* genomes with that of B. juncea introgression lines and representatives of *B. juncea* and *B. rapa* accessions, have revealed that the genetic diversity of *B. juncea var multiceps* (potherb mustard) and *B. juncea var. megarrhize* (root mustard) were much narrower that in comparison with heading mustard (which

showed no diversification) [66, 67]. Furthermore, the dendrogram of 1642 SNPs of 154 investigated lines showed that the *B. juncea* introgression lines exhibited rich genetic diversity, and that the heading *B. juncea* accessions were measured independently of this genetic diversity. Studies found that the genetic distance of the heading *B. juncea* was increased to 0.33 from 0.03, and this relates to definite phenotypical variations among the introgression lines [63, 64]. Zhang *et al*. further, states that this introgression strategy could be extended to allotetraploid species of *Brassica*.

7. Biotransformation

7.1 The use of germplasm conservation and preservation

Aside from introgression, another technique used in the breeding of *B. napus* has to do with CRISP/Cas9 technology. This technology has been used in the creation of germplasm resources, as well as the genetic improvement of rapeseed [68]. In addition, many molecular mechanisms regarding this oil-bearing crop has been understood using this technology. Some of the methods involved with using this technology are the gene gun method, protoplast transformation method, pollen channel method, and, electric stimulation [69]. However, mutation detection methods have been found less effective than if it had to be applied to *T. aestivum* and *O. sativa*. Furthermore, gene knockout studies in *B. napus* are only possible if a specific promoter is able to be designed for CRISP/Cas9 system in *B. napus* [68]. This is probable, especially since both *Arabidopsis* and *B. napus* are related through their genomes, and since they belong to the same family - Cruciferae. With CRISP/Cas9 technology, *B. napus* genomes can now be targetted such that multiple DNA sequences can be edited, single clades (i.e. gene linkages can be avoided) obtained, and multiple mutations created through gene penetration [68]. This means that gene editing is advantageous in *B. napus*, since both the qualitative and quantitative traits can be analysed. In *Gao et al*. [70], the knocking out of BnaFAD2 and BnaFAE1, which controls the metabolism of oleic acid, was found to generate mutant materials with exogenous genes. If such can happen in *B. napus* using this technology, then, further products would be possible for this *Brassica* plant. In terms of progeny screening, by analysing gene editing materials, one is able to improve editing efficiency. This improvement can occur once mutations are detected in progenies and parent plants, and in which organ or propagule (type) the mutation has occurred in, because sometimes not all genetic mutations are transferred between generation. For example, in some cases, mutations may occur in the leaves and absent in the progenies of seeds, and vice versa [71]. Given that there are vast differences in the editing efficiencies between somatics and germ cells, it implies that the same technique/approach can be used in the transformation of *B. napus*, and that mutations can be eliminated by backcrossing and self-pollination of the *B. napus* species [68].

It has been found that the most important yield components of *B. napus* are: pods per plant, seeds per pod, seed weight, plant height and top branch angel [72]. Currently, CRISP/Cas9 technology is successful with removing gene resistance and creating multichamber pods. It is believed that this technology can help improve oil productivity in *B. napus*, but due to the complexity of this process, one may only be able to match pod length in relation to the shattering gene resistance, as independent factors [72]. In addition, studying BnaCLV3, CLV1, CLV2 and the signalling pathway (CLV pathway) responsible for producing different phenotypes, one could

approximate - through experimentation - the type of chambered pods that B. napus could produce, i.e. dual chamber, multi-chambers, or single chambers. Furthermore, the seed weight of single *B. napus* plants of mutants could be deduced, as well as, the leaf numbers in relation to yield potential in *B. napus* [73]. BnaMAX1 genes are present in *B. napus*, which accounts for yield and petal character, while it is reported that apetalous plants are better suited to investigate photosynthetic capacity and disease resistance in *B. napus* [74]. Yang *et al*. (2018) described that the flowering times by regulating the growth genes, BnaSDG and BnaRGA, were more than 40 days sooner than in ordinary plants, and this was due to editing of their genes. These genes also participate in growth and chlorophyll synthesis of *B. napus*, and with editing of the BnaHemd gene, the photosynthetic rate, as well as the growth of *B. napus* may be studied [75]. In addition to this function, when BnaWRKKY11 and BnaWRKKY70 genes were expressed in *Sclerotinia sclerotiorum* (Lib.) de Bary, it was found that edited BnaWRKKT11 had no effect on different *S. sclerotiorum* in comparison to the wild type, whereas BnaWRKKY70 mutant exhibited as higher resistance to *S. sclerotiorum*, which Sun *et al.* [76] described as a negative regulatory factor in *S. sclerotiorum*. To add to this, it is known that the content of unsaturated fatty acids from *B. napus* can be increased by gene editing, however, the composition of fatty acids can also be changed with the editing genes [76]. Editing of the TT8 gene by CRISP/Cas9 technology, on the A09 and C09 chromosomes, produce a yellow seed phenotype. Futhermore, it was noted that the oil and protein contents of the mutant seeds were increased. This highlights that the seed coat-related genes in B. napus are responsible for oil content, seed coat thickness, and protein content, and that unlike black seeds, in yellow seed rape, the quality of the oil and protein produced are much better [72].

7.2 The use of structural variants vs. morphotype growth

The two *B. oleracea* morphotypes, namely cabbage and cauliflower, have been genetically studied using high-quality chromosome-scale genome assemblies. According to studies that use large structural variants, or SVs, the different morphotypes are a result of the different relations between the plants found in the genus, *Brassica* [77]. Furthermore, the intraspecific divergence, found to be exhibited by various variants of *B. oleracea*, are to be accounted for by the SVs. When 271 *B. oleracea* accessions were tested with these structural variants, it was found that various functions in cabbage and cauliflower were enhanced [78]. These functions are associated with plant responses to various cascades, as a result of stress factors, or stimuli. Also, flower development as well as development at the level of the premordia (as mentioned) - or meristem - were observed. As there is a lot of research performed on the development of curd in cauliflower, it is noted that this process can be mediated by many structural variants [78]. Studies have reinterated that there is a profound difference in vegetative and generative growth, and that both these processes are regulated by these structural variants. The switch from vegetative growth to generative growth is what drives inflorescence meristem proliferation, and this, in turn, results in curd development [79]. Therefore, the initiation, maintenance and enlargement of curd, is a result of SVs that have a considerable impact on development of curd, and it is those genes and SVs that form part of the regulatory network for curd development. The orange curd in cauliflower, for example, occurs as a result of a 4.7 kb insertion in the third exon of the *Or* gene, whereas, in rapeseed cultivars, an insertion of the 621 bp sequence in the promotor region of BnaFCCA10 is responsible for the adaptation of rapeseed plantations to winter climates [79]. With regard

to comparative genomics, a study performed using full-length long terminal repeat retrotransposons (LTR-RTs)from Korso, Ox-heart and *B. rapa* revealed that centromeres were able to be identified on the genomes of cauliflower Korso and cabbage Ox-heart [77]. This as ascertained using fluorescent *in situ* hybridization analysis, and it was declared that there was genome and sub-genome divergence occuring in *Brassica* species, and this was achieved when synteny analysis was performed on Korso, Ox-heart, *B. rapa* and *A. thaliana* genomes and sub-genomes. Another remarkable finding was that some duplicated genes were retained during diploidisation, and this represented a biased retention pattern [80–82].

7.3 Genome sequencing and curd formation

The main purpose, or underlying basis, of performing genome resequencing is to be able to understand and investigate the dynamics of SVs (e.g. from Korso and Ox-heart) so that it's possible to obtain morphologically different, or divergent, *B. oleracea* accessions. In Korso and Ox-heart, it is found that various biological processes are affected by at least one structural variant in the promoter region of genes [78]. These biological processes are: flower and meristem development, gene expression and epigenetic regulation, embryo development, cellular component organisation, response to stress and stimulus, signal transduction and cell differentiation [78]. In *B. oleracea*, the two indels, namely BoFLC3 and BoFRIu play two very differentiated roles. The first one helps broccoli to adapt to subtropical climates, while the second gene is involved in seasonal adaptation of cauliflower and cabbage - particularly the winter annual or biennial habit of these two *Brassica* species [83]. In addition to the above, *B. oleracea* contains homologues that assist in plants undergoing transition from the vegetative to the generative stages. For instance, BoFES1.1 and BoSUF4.2 have SVs in cauliflowers, and only when they are down-regulated that cauliflower plants adapt to the mentioned transitions. These homologues are, however, not found in cabbage [78, 83].

Although there are homologues that aid in the transition from vegetative to generative growth, there are others, such as PRC1 and PRC2, which assists in epigenetic modification, and thus assists in regulating the process of flowering. This means that it is because of the FLC-related autonomous and vernalisation pathways that a generative stage is reached at different timing intervals in cabbage and cauliflower. Introns and exons are particularly important during inflorescence meristem proliferation. An example where this is evident is in cabbage, where it has been found that Korso alleles are rare, whereas in cauliflower and broccoli, homozygous alleles for both structural variants are present [78]. This indicates exon deletions and intron insertion events in the Korso genotypes of all 3 crops, namely cabbage, broccoli and cauliflower. It is also worth noting that an upregulation of the BoWUS2 genes in all 3 crops result in both structural variants playing a vital role in curd formation [78]. In contrast, floral arrest and curd maintenance are processes that are essential for meristem development/arrest. In cabbage, it has been found that about 79.2% of accessions contained the OX-heart allele, while at the same locus point in broccoli and cabbage, the selection was for BoCAL Korso allele, and this suggested the role of this process in curd formation.

Some other genes that participate in the vegetative, transition and curd stages are the BoAP 1.2, BoFUL 1, BoFUL 3 and BoSEP 3, which are affected by structural variants [78]. There has been evidence that BoSVP 1 has an inverse relationship with curd development and flowering in that, in *Arabidopsis*, it was found that when this variant

was upregulated for curd formation that its role in flower bud development suppression was more prominent, thereby highlighting its repressor role in the latter process. In addition, BoCCE participate in flower arrest, and it is found covering the entire genotype of Korso [84]. Through genotype experiments it has been found that it occurs in 97.1% pf cauliflower accessions, and that it is absent in cabbage and broccoli accession. This genetically emphasises that this gene arrests buds in the latter stages of development in broccoli, and much earlier in cauliflower buds [78].

There are several genes that participate in different phases of curd development. The below paragraph will mention a few, as well as their roles in this complex process. The first is the BoARL 2 gene that occurs to promote cauliflower curd size. The second is the BODRNL 1, which has a potential role in determining curd architecture, and which has deletions in its promoter region and determine curd development in cauliflower and broccoli. The helical growth is as a result of the BoTUA 2 and four BoTUA 3 genes found in *Arabidopsis* species [78]. During the initiation of curd, the transition from vegetative to generative growth is a result of the flowering-time regulation (FLC and FRI), while floral meristem arrest is a result of several matching floral genes, viz. CAL, API, SEP3 for organ size control, CYP78A5 and ARL are involved, which during curd spiral organisation, DRNL and TUA, are the genes that play and active role [78].

7.4 The TOC1 gene

In a study that attempted to unleash the genome of *B. rapa*, a study on finding duplicated orthologues in *B.rapa* was conducted [31]. This was done by analysing the TOC 1 genes found in the circadian rhythms pathways of *A. thaliana*. When this was conducted in order to assess non-coding conserved sequences, it was found that B. rapa retained copies of TOC 1 [85]. The authors questioned the relevance of this in the functionality of the genome of *B. rapa*, particularly, because they were concerns on the effect of this gene in circadian rhythmicity [85]. Their concerns had to deal with the cis-acting elements, as well as the promoter sequence participating in this process. This is because over a period of time, which fractionation, duplication was not possible, however, in *B. rapa*, the duplicate gene should not have existed since the gene families in *B. rapa* are not resistant to fractionation, and therefore it should not have been able to provide a signal to detect syntenic regions [86]. In addition, diploidization would have degraded the collinear signal. This degradation would have caused genes and genomic region transportation, chromosomal inversion, chromosomal fission and fusion, and, polyploidy events. This should have happened since *Brassica* is characterised by its paleohexaploidicity [86]. It is due to this that authors questioned whether there was something special about the truncation of intron 1 in Bra012964, and whether the interplay between this gene and TOC 1, are interrupting/stimulating the circadian pathway via sub- /neo-fractionisation of the homeologous genes involved in genome divergence in the family, Brassicaceae [31].

Thus far, you may have gathered that accessions relate to the utilisation of germplasm. However, much more needs to be done in order to make accessions more feasible. In the past, the 1950s, there were a variety of studies performed on cabbage germplasm. The studies performed are on ascorbic acid, dry matter, sugars, fibre, mineral elements, carotene, and proteins [87]. Furthermore, the accumulation and consumption of nutrients were also studied. Moreover, vitamins, pigments, and mustard oils in cabbage, turnips, and rutabaga and radishes were studied [87, 88]. Later on, by the 1970s, efforts were made to deepen studies on the diversity and biochemical composition of plants belonging to *Brassica* collections. And, in recent years,

biologically active substances and biochemical components were being attempted to be studied for their health benefits. It is well known that in *B. rapa*, *B. oleracea* and *R. sativus* that accessions have been studied using SIRs, particularly since these plants have strong morphology and agronomy traits [89]. These traits are approximated to qualify and productivity, as well as biochemical traits, viz. dry matter content, proteins, sugars, ascorbic acid, chlorophyll and carotenoids [90]. The sections below would now focus on the health properties of plants of *Brassica* origin.

8. Biological effects on human health

Brassica plants have been found to possess many minerals and vitamins, and therefore, the crops from Brassicaceae have many benefits to human health. It has been found that the high folate content of the crops belonging to this species are able to reduce cancer, neural tube defects, as well as, vascular diseases [3]. The malignant and degenerative diseases, in contrast, are treated efficiently by the vitamin C, vitamin E and carotenoids found in these crops. In kale plants, for instance, a very high concentration of elements, namely: P, S, Cl, Ca, Fe, Sr. and K have been found [91–93]. While cabbage accumulates a considerable amount of copper, zinc and other trace elements, in broccoli, selenium is the main element that promotes health properties. Since *Brassica* can be grown hydroponically, independent of the other propagation methods, Cr, Fe, Mn, Se and Zn are elements that can, possibly, also, be extracted [15]. In contrast to cabbage, where trace elements can be found, in Radish, heavy metals are present, because this is a cruciferous species. Since the plants of *Brassica* are leafy, a fairly large amount of potassium are also found in them [11].

In addition to the above, *Brassica* also contains elements that account for pigmentation. For example, the anthocyanins are responsible for the red colour in red cabbage and broccoli species [15]. In terms of phenol compounds, the most common polyphenols occurring in *Brassica* species are flavonoids and hydroxcinnamic acid [15]. In Brussels cabbage, cabbage and broccoli, a fairly high concentration – say 1.500–2.000 ug/g – of glucosinolates are present. These compounds (β-thioglycoside-N-hydroxysulfates) are also prominent in horseradish, mustard, and the root and seed regions of *Brassica* vegetables [12]. In vegetable plants, approximately 75,000 μg/g of the fresh weight is what comprises the body of the mentioned plants. Although glucosinolates are hydrolysed in the human intestinal tract, in plant tissues they are biologically inactive [12]. Therefore, they are able to be utilised in cooked vegetables, more so, because the hydrolysed products are biologically active, whereas on the chemical and thermal front, they are stable. The glucosinolates have an essential role to plant in the treatment of cancers, particularly because the hydrolysis of these compounds in plant tissues are said to produce particularly useful products. During hydrolysis, in plant tissues, the enzyme β-thioglucosidase catalyse the breakage of the thioglucosidic bond, and this reaction causes the products; glucose and thiohydrosimate-o-sulphonate (or unstable aglycone) to be released [94]. Since glucosinolate produces products that are dependent on pH and the structure of glucsinolate ar the time of hydrolysis, there are a variety of products that are produced. Indoyl, ozazolidin-2-thiones, epithiitriles, thiocyanates, sulphides, isothiocyanates and nitrile are among the products produced. However, the compounds, glucosinolates, glucoraphanin, gluconasturtin and glucobrassicin, are anticarcinogenic, while, indol-3-carbinol have been found to inhibit breast and ovarian cancer. In addition to the anticarcinogenic compounds, isothiocyanates are phytochemicals present in some *Brassica* plants. Since they are a

result of glucosinolate metabolism, it is expected that they have widespread functions [3, 95]. Some roles include their ability to reduce oxidate stress, alter cytokine activity (inflammatory response), induce apoptosis, inhibit angiogenesis, and inhibit cell cycle progession [96]. In addition, the isothiocyanates also possess anti-bacterial and anti-fungal properties, and this is related to its chemopreventative effect. There are 2 mechanisms involved in the chemopreventative properties of this phytochemical. The first one is that isothiocyanates inhibit cell cycle progression, initiating cell death, while the second involves the inactivation of phase I enzymes, and the activation of phase II enzymes [97]. The latter mechanism is responsible for stimulating the production of this phytochemical in *Brassica* vegetables.

The most natural phytochemical found in *Brassica* vegetables is called sulphoraphane. Another name of this substance is 1-isothiocyanate-(4R)-(methylsulfinyl) butane. This phytochemical is the most promising among the 4 chemopreventative agents found in *Brassica* crops. Just like isothiocyanate, sulphoraphane also inhibits tumour development by inducing cell-protective phase II enzymes [3]. Since vegetables of *Brassica* are consumed by humans, and mature broccoli is said to contain 10 times more sulphoraphane than juvenile broccoli cultivars, consumption of sulphoraphane is possible by humans [3]. It has been found that 10 mg of purified sulphoraphane can be tolerated by humans per day, while 100 mg of glucoaphanin is tolerable by humans per day. In order to prevent cancer development, it is recommended that humans consume 3–5 servings of cauliflower or broccoli per week [98].

9. Enzymes and biotransformation

It is because of the components found in *Brassica* crops that the enzymes involved in cancer prevention undergo biotransformation. These enzymes regulate the toxic, mutagenic and neoplastic effects of chemical messengers [99]. There are 2 type of enzymes, and these are the Phase I enzymes, and Phase II enzymes, which participate in DNA damage. The Phase I enzymes are the activators (cytochrome P-450, and Flavin-dependent monooxygenase), while the Phase II enzymes are the detoxifiers (GSTs, UDP-glucuronosyltransferase, sulfotransferase and N-acetyltransferase). These enzymes assist each other in the detoxification process. Phase I enzymes catalyse oxidation, reduction and hydrolytic reactions, and these reactions make compounds hydrophilic and accessible for detoxification. In contrast, the Phase II enzymes readily remove stable metabolites by catalysing conjugation, as well as other metabolic pathways that protect cell systems from electophiles and oxidants. Since the biotransformation of enzyme expression alters steroid hormone exposure, it is found that the progression of malignant and premalignant tissues are indirectly affected, and hence, carcinogenesis is affected [4, 12, 99].

There are many type of cancers that are affected by the substances present in *Brassica* plants. The paragraphs that follow would demonstrate this widely researched area.

In *Brassica nigra* seeds, sinigrin, a major product of glucosinolate hydrolysis, has been found to participate in liver tumour cell progression. It is said to achieve this through p53-dependent apoptosis. However, in rocket plant species, in a controlled experiment, apoptosis and necrosis was not induced by a p53-independent mode of cell death when glucoerucin was hydrolysed to 4-methylthiobutyl isothicyanate [100]. Instead, this compound was found to be selectively toxic to tumour-initiating cells. In another study it was found that cabbage and kale extracts caused DNA damage to

be inhibited in rats, and this was said to be a result of the hepatocarcinogenic properties exhibited by cabbage extracts. This heptaoprotective effect is also present, and applicable, by the antioxidant properties of the volatile fatty acids found in cabbage plants [101, 102].

Unlike 4–methylthiobutyl isothiocyanate, 1-methoxy-3-indolylmethyl alcohol, another hydrolysis product of glucosinolate, forms DNA adjuncts in the liver, and this metabolite of neoglucobrassin, is a genotoxic carcinogen [94]. According to the World Cancer Research Fund, and the American Institute for Cancer Research, the risk of humans acquiring gastric cancer is inversely proportional to the high amount of *Brassica* crop intake. Since the level of 2-amino-1-methyl-6-phenylimidazole pyridine, and other dietary-related heterocyclic amine carcinogens, are increasingly eliminated through the consumption of *Brassica* vegetables, the risk of colorectal cancer appears to be reduced. This was found to be true when broccoli and Brussel sprouts consumed in the presence of well-cooked meat. This is a good finding, because, in the USA for example, colon cancer-related deaths are the third most common [103]. This statement if confirmed by 5 out of 8 controlled studies showing a reduction in cancer risk with high *Brassica* vegetable intake, with 3 studies showing a negative relationship. It is due to the reporting of negative relationship with *Brassica* vegetable consumption that it's impossible for one to conclude that the consumption of *Brassica* crops are related to the risk of colorectal cancer development or inhibition [104].

Even through the affect of *Brassica* vegetable consumption on the risk of lung cancer is well-known, it's impact is not as great as if one was to leave smoking. Lung cancer, in general, is caused by genetic lesions caused by exposure to smoking or ROS (reactive oxygen species), oestrogens, bacterial and viral infections [105]. Unlike colon cancer, lung cancer is the leading cause of death globally. When 1 μM of glucosinolate was isolated with cut rat liver slices for 24 hours, it was found that the glucosinolates were able to modulate the cytochrome P450 and Phase II enzymes, and this lead to the belief that glucosinolates have a profound effect on pulmonary carcinogen metabolism, and therefore, *Brassica* vegetables have the ability to exhibit chemopreventative activity in the lung of rats [106]. To date, no relationships between isothiocyanate urine levels and lung cancer risk in non-smokers have been reported. Also, the p53 status of lung cancer cells (A549; lung adenocarcinoma, H1299; larger lung carcinoma) have been reported as being affected in a dose-dependent manner upon isothiocyanate adenocarcinoma, and this was found through its cytotoxic effect. There is also evidence that the antioxidant effect of *Brassica* crops also play a role in protecting the cellular integrity and homeostasis of the benzo (a) pyrene [B (a) P]. This was found when 9 μmol/day of sulphoraphane were orally administered approximately 6 mice, and results on the basis of oxidative damage were noted. Unlike with lung cancer and smoking, breast cancer is age dependent, but not entirely [105]. In a study that consisted of 2832 women, aged between 50 and 74 years, when the deaths of 2650 women were compared, it was found that approximately 20–40% of the risk of breast cancer was induced [107]. This result was retrieved from women who consumed between 1 and 2 portions of *Brassica* vegetables. This result also assumed that the eating of these vegetables also altered the oestrogen metabolism pathway of these women. Furthermore, there was no relationship between cancer risk and the total vegetable and fruit consumption in the studied women. However, in a study that evaluated the effect of eating cauliflower on breast cancer, it was found that the substances contained in cauliflower had an inhibitory effect on breast cancer cells, in both oestrogen receptor-positive and oestrogen receptor-negative individuals [107]. In Chinese women, consuming *Brassica* crops reduced breast cancer when urinary

isothiocyanate biomarkers were studied. In Caucasian women, on the other hand, the eating of broccoli was found to be negatively associated with breast cancer risk in premenopausal women, and this suggests that *Brassica* vegetables may be a curative agent in treating breast cancer in premenopausal women [108]. In men, on the other hand, prostate cancer is found to occur due to many reasons, but of those, the main issues arise due to nutritional status, particularly the consumption of fat and high fat foods [3]. The glutathionine S-transferase (GST)-$\prod$ gene is said to play a role in the progression of prostate cancer, and this gene is said to disappear in prostate cancer, prostate cancer precursor lesions and prostate intra epithelial neoplasm. In a study where animals were fed with broccoli, it was found that the upregulation of this gene altered biotransformation enzyme levels in the peripheral tissues, thereby protecting against prostate cancer growth. In patients under 65, *Brassica* vegetables have been found to reduce the risk of prostate cancer, however, with the consumption of high amount of these vegetables, advanced and metastatic prostate cancer can also be managed [109].

Pancreatic cancer is also an illness that requires treatment using plants, particularly since in the USA it is the fourth cause of cancer-related deaths, whereas in Japan, it is the fifth. Cabbage has been shown to be most effective in treating pancreatic cancer among patients who consume 1 or more portions per week [110]. This observation was made with comparisons of subgroups of crops and fruits. Also, benzyl isothiocyanate, a member of the isothiocyanate family, was found to be an effective supplement alternative to X-ray therapy for pancreatic cancer. Bladder cancer, alternatively, occurs from the bladder epithelium, and is it this isothiocyanate present in *Brassica* vegetables that protect the epithelium cells from cancer. This was found in mice that were fed on broccoli sprouts. There, it was found that GST and quinone oxidoreductase in the bladder tissues and cells were induced, thereby preventing bladder carcinogensis [16]. Studies have reported that broccoli and cabbage, as well as other vegetables of the family, Brassicaceae, reduce the risk of bladder cancer. However, other type of vegetables and fruits may not necessarily be beneficial to reduce bladder cancer development. In a biological study, involving rats being fed on freeze-dried broccoli extracts, an alteration was found on the bladder, and bladder cancer was inhibited in a dose-dependent manner [111].

As already mentioned, many plants from the family, Brassicaceae, are involved in neurological diseases, and it had been noted that his has been due to oxidative stress. Furthermore, isothocyanates play an essential role in chronic diseases like cancer and neurodegenerative diseases [3]. This degradation product reduces the activation of cell death, and thereby also modulates inflammatory pathways, such as apoptosis. It does this by activating proinflammatory cytokine production, as well as, the production of oxidative species and the initiation of neuronal apoptosis death pathways, through NF-kβ translocation [112]. In a study where the Nrf2-ARE signalling pathways were activated, through mitochondrion function modulation, HSP70 gene transcription and expression, it was found that broccoli sprouts juice has a protective effect against β-amyloid peptide-induced cytotoxicity and apoptosis [113, 114]. In addition, due to Nrf2 activation, the broccoli juice was said to have also increased the activity of antioxidant enzymes, like HO-1, thioredoxin, thioredoxin reductase, NQ01, mRNA levels, as well as, intracellular glutathionine [112]. As a result of these roles of broccoli juice, it is said that plants of *Brassica* are effective to treat Alzheimer diseases [112].

There are various diabetic complications associated with diabetes. These complications include cardiomyopathy, nephropathy, neuropathy and retinopathy through Nrf2 activation [115–117]. Since *Brassica* crops form a part of functional foods, and

sulphoraphane is an essential element of *Brassica*, type 2 diabetes mellitus can be controlled, instead of it reaching long-term complications. Since red cabbage has been found to decrease the catalase activity in diabetic kidney, it can be deduced that the functional foods belonging to this group are potential sources to treat diabetes [3]. Cholesterol is another factor which can be controlled by eating foods composed of *Brassica* vegetables. For example, broccoli sprouts has been found, over a course of a week eating 100 g fresh broccoli, to have decreased total, LDL and HDL cholesterol levels. Furthermore, this eating strategy has been found to improve cholesterol metabolism, and reduce oxidation stress markers [118]. In hepatoma-carry rats, on the other hand, cabbage extracts were found to decrease serum cholesterol levels. In addition, bile excretion and 7-alpha hydrolase activity in the faeces of these rats were increased. This suggested that cabbage determines cholesterol levels by increasing its metabolism in hepatoma developing rats. Also, in hypercholesterolemia patients, red cabbage and Brussels polyphenols affected the concentration of cholesterol in red blood cells membranes, and this is directly associated with the concentration of anthocyanins [119]. Thus far, it has been highlighted that the role of broccoli, and cauliflower, have profound effects on controlling neuro-degenerative diseases. However, in addition to the diseases mentioned, broccoli sprouts are also effective in the treatment of gastrointestinal diseases, via. Stomach adenocarcinoma, gastric ulcer, duodenal ulcer, chronic superficial gastritis, non-Hodgkins lymphoma, and gastric infection. Since *Heliobacter pylori* causes oxidative stress, a daily intake of 70 g/d glucoraphanin-rich broccoli sprouts for 2 months, they provide a protective role by presenting gastritis in humans and animals. Alternatively, sulphoraphane plays a cytoprotective role in the gastric mucosa by not inhibiting the severity of infections by *Helicobacter pylori* [120, 121].

The detoxification properties of *Brassica* vegetables contribute toward their anti-inflammatory properties by clearing free radicals and inducing immune functions. And, apart from the gastrointestinal disorders which *Brassica* vegetable can suppress, the compounds found in these plants can also be used to treat small incisions, wounds and mastitis. As it is known, glutathionine S-transferase (GST), plays a remarkable detoxification role in detoxifying carcinogens, environmental toxins and oxidative stress products. In addition, the vitamin c properties of *Brassica* plants have been found to be an effective antioxidant, which protects against various degenerative diseases [3, 104]. In a study that assayed the ascorbic acid content in *B. oleracea*, Singh [122] found that the cabbage (10 g) contained more ascorbic acid compared to cooked cabbage. This was ascertained using a large amount of 2,dichlorophenol indicator with raw cabbage, during the titration. It was concluded that there was a direct proportionality in the amount of 2,6-dichlorophenol used to obtain a pink colour [122]. The results showed that approximately 40–55 mg of ascorbate could be found in raw cabbage, and that this was in keeping with the recommended daily allowance (60 mg) that a person is ought to eat. The 15–29 mg ascorbate found in cooked cabbage highlights that the raw cabbage is a better source of antioxidants. Since cabbage contains ascorbate, it is recommended as a functional group, as it maintains healthy gums, teeth and bones, as well as participates in immunoprotection, like scurvy and rickets [122]. *Brassica* vegetables also promote an increase in the secretion of THS and thyroid cells as thiocyanate (metabolised from thioglycosides) inhibits iodine transport and the incorporation of iodine into thytoglobulin. The thiocyanate ions and oxazolidin-2-thiones are goitrogenic, because they contain a β-hydroxyl group. The goitrogenic activity can be counteracted by increasing the amount of iodine consumed in the diet. In a group of 293 Malaysian women, it was found that a high consumption of *Brassica*

vegetables and mild iodine deficiency was enough to explain the high incidence of thyroid cancer against them. Therefore, a positive relation was established between cancer of the thyroid and the consumption of *Brassica* functional foods [3, 123].

10. Conclusion

The family, Brassicaceae encompasses many plant species that have many important biological properties, such as antioxidant, antibacterial and anticancer properties, among others. In this paper, various studies about the growth and propagation of *Brassica* plants have been discussed, particularly with reference to anther culture, microspore culture, and male sterility cytoplasm introgression. This paper also looked into the many features of *Brassica* plants, and perceptions, upon introgression of cytoplasm from on *Brassica* plant species to another. This was discussed quite intricately in relation to *Brassica* divergence, and the various traits such as the pattern of leaf growth in cauliflower and the origin of *Brassica* plants in general. It was reinterated, in this paper, that cauliflower and broccoli have many nutraceutical properties as functional foods, and that this stemmed from an original ancestor, the cabbage. In addition to the pattern of development in the inflorescence of cauliflower, introgression was also found to have an effect on the colouring of plants, and in cases it was occuringly prominent that chlorosis occurs during introgression. With regard to microspore culturing, an essential requirement was to maintain seed viability; so embryogenesis was possible. It was also undesirable to have self-pollination occur, as it is known to produce small curd size in cauliflower. *A. thaliana* was the ideal model for experiments with *Brassica* genomes. With Chinese white kale, for example, distinct clades were found as a result of biennial growth was favoured in this plant, over annualism. It was also established that more effort was required in germplasm experiments, and that germplasm was present for hybridization experiments, as well as experiments involving curd development. Furthermore, genome phylogenic analysis between heading mustard, *B. rapa* and *B. juncea* showed a narrow genetic diversity among each other. Also, CRISP/Cas9 technology may be successful in future to optimise oil production from B. napus. Furthermore, like with all plants, even in *Brassica* plants – like *B. napus* – the type of chambered pots produced are regulated by signal pathways, and gene coding may inhibit, or stimulate, the production of unsaturated fatty acids from *B. napus*. Structural variation have also been used to study meristem and flower development in cauliflower. In addition, in rapeseed cultivars, CVs are used to assess acclimatisation patterns to winter climates. Also, we have learnt about the different minerals and vitamins occurring in *Brassica*. A key component of glucosinolate hydrolysis, namely the sulphurphanes, is a key substance that inhibits turnover growth, and, as a result, it is a chemopreventative agent. This paper also detailed the various cancers which *Brassica* phytochemicals are able to treat, and in all cases these were either via the mitochondria, oe p-53-independent cell death. In addition, neurological defects, as well as, assaying of the vitamin C content of raw and cooked cabbage has also been discussed. In conclusion, this paper reports on the knowledge and different perceptions on some aspects in the genus, *Brassica*.

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Chapter 2

Perspective Chapter: Creation and Evolution of Intergeneric Hybrids between *Brassica rapa* and *Raphanus sativus*

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Abstract

Although research has been conducted on intergeneric hybridization between *Brassica* and *Raphanus*, much of it remains unpublished. We have acquired numerous *Brassica rapa* ssp. *pekinensis* (kimchi cabbage) and *R. sativus var. major* ("big root radish") hybrids, originally classified as intergeneric hybrids and named "baechumu" in 1995. A cultivar was identified BB#12, (renamed BB#1 for registration) in baemoochae following stabilization via a microspore mutation in 2006. Numerous hybrids were created for various purposes; some were sterile when self-pollinated but fertile in crosses with other cultivars. Microspore mutation also produced, BB#12x is a novel intergeneric hybrid. A new stable plant variety, BB#5, was selected from numerous inbred lines and produced via microspore culture; it has a very late bolting time and is cultivated in spring. The cultivar purple BB#10 was developed by adding radish chromosomes to turnip, including one providing the purple color, and double-crossing with BB#12, CMS BB#12, and normal BB#12. Now that the hybrid between ssp. *pekinensis* and radish has produced mature seeds as a dominant property, intergeneric hybrid cultivars can be bred in the future.

Keywords: intergeneric hybrids, *Brassica rapa*, *Raphanus sativus*, self-sterile, cross-fertile, mature seeds

1. Introduction

There are two main kinds of intergeneric hybrids involving *Raphanus*: *Raphanus* and *Brassica*, and *Brassica* and *Raphanus*. Hybrids between *Raphanus* (radish) and *Brassica oleracea* var. (cabbage) were bred by Sageret [1] (cited from Prakash et al. [2]), Karpechenko [3], and McNaughton [4] and stabilized by Chen and Wu [5].

Our laboratory began conducting studies on intergeneric hybridization between kimchi (Chinese) cabbage (*B. rapa* ssp. *pekinensis*) and radish (*R. sativus* var. *major*) in 1986 [6], and research has been ongoing ever since (i.e., for 36 years). Originally, this was classified as an intergeneric hybrid and was named "baechumu" after a successful microspore culture in 1995 [7]. In 1997, the crossbreed was renamed again

as "baemoochae" (where "bae" is from "baechu", [kimchi cabbage], "moo" is from the Korean for radish [i.e., "mu"], and chae is from "chaeso" [vegetable in Korean]) [8]. Baemoochae was stabilized in 2006 [9]. In total, 25 papers on baemoochae have been published or accepted, 17 of which are either from our laboratory or list me as an author (four of these are in Korean). However, these papers do not represent all of the work that we have done, and some are available only in Korean.

Terasawa [10] was the first to report intergeneric hybridization between *Brassica* and *Raphanus*, followed by Takeshita et al. [11], Dolstra [12], Lange et al. [13], Lee et al. (1986–2022), and members of the laboratories of Professor Hyo Guen Park [14, 15], Professor Jongkee Kim [16–18], Professor Il-Sup Noh [19], Professor Jun Gu Lee [20], Professor Hyun Hee Kim [21, 22], and Professor Jin Hue Huh [23, 24]. Tonosaki et al. [25] in Japan, and Lou et al. [26], Zhang et al. [27], and Jin et al. [28] in China, have published papers on hybridization between *Brassica* and *Raphanus*. These studies written in English are briefly introduced and the Korean papers are discussed in detail; previously unpublished photographs and tables are also provided.

2. Development and application of an ovule culture system

To obtain a hybrid between *Brassica* and *Raphanus, Brassica* should be the maternal parent [14, 29]. The first successful *Brassica-Raphanus* hybrid seeds were acquired by Terasawa [10]. However, the hybrid seeds were between *B. rapa* ssp. *chinensis* and *R. sativus*, not ssp. *pekinensis*. Dolstra collected a wide range of varieties and used *B. rapa*, ssp. *rapifera* (turnip), ssp. *oleifera* (turnip-rape), ssp. *chinensis* (Chinese mustard), and ssp. *pekinensis* (Chinese cabbage) as female parents [12]. However, he could not obtain seeds from ssp. *Pekinensis*, but did obtain them from the other three subspecies. *B. rapa* ssp. *pekinensis* seemed to have a characteristic preventing the development of mature seeds. Lange et al. published a paper on *Brassica-Raphanus* hybrids in 1989. Most of Dolstra's research focused on the creation of *Brassica-Raphanus* hybrids, with little discussion of future directions.

Takeshita et al. [11] attempted to germinate a hybrid seed between ssp. *pekinensis* and *R. sativus* using a culture of young ovules, but was not successful. Successful germination was reported by Been and Park [14], but the mature plant did not produce any seeds. Subsequently, a student of Professor Hyo G. Park studied an ovule culture to increase the number of germinating ovules [15]. Several sprouts were observed from one ovule, and the addition of 0.1 mg each of benzyl adenine (BA) and naphthalene acetic acid (NAA) to 1 L B_5 medium increased the number of plants (**Table 1**).

On the basis of these results, the Horticultural Experiment Station (HES) cultured ovules of intergeneric hybrids between ssp. *pekinensis* and *Raphanus* on the modified B5

Amount (mg/L) of NAA and BA add to 1 L B_5 medium	Cultured ovules	Calluses	Shoots	Roots and shoots	Plantlets established
0.1	93	5	24	4	27
0.5	92	0	2	0	0
1.0	98	5	0	0	0

Table 1.
*Effects of NAA and BA on excised intergeneric ovules in hybridization between Kenshin (*Brassica*) and Jinju daepyung (Raphanus) performed in 1985.*

Crop	Cultured ovules	Germinated ovules	Plants (including multi-shoot ovules	Pollenating plants	seeded
Kimchi cabbage	1.893	391 (20.7%)	Total 591 (31.1%) Tested 466 (24.6%)	98 (5.2%)	65 (3.4%)

Medium: B5 + NAA 0.1 ppm + 24-D or BA 0.1 ppm.
(125.6%) ovules or plants died.

Table 2.
Data from the experiments of the HES conducted in 1987–1990.

medium in 1986 and 1987 [30]. All three cultivars of kimchi cabbage and radish produced intergeneric hybrids, and BA, NAA, and 2, 4-dichlorophenoxy acetic acid (24-D) combined with 8- or 24-h dark treatment per day did not greatly influence the ovule culture. Of the 676 ovules cultured, 102 (15.1%) produced plants, including 22 multi-shoot embryos. Of the successfully germinated plants, 22 were harvested and 439 seeds were collected. This study was the first to harvest F_2 seeds from *Brassica-Raphnus* hybrids [6].

Two F_1 cultivars, Jeonsueng (*Brassica*) and Taeback (*Raphanus*), which were included in the above-described study, were used for subsequent experiments conducted in 1987–1990 [8]. In total, 1893 ovules were cultured: 1250 for 2n × 2n and 643 for combinations of 2n × 4n, 4n × 2n and 4n × 4n (**Table 2**). Once the seeds had been harvested, 4–5 seeds were sown according to their ploidy level. They were germinated evenly with 3–5 plants; 2n × 2n resulted in plants with a normal appearance, while 2n × 4n, 4n × 2n and 4n × 4n plants were mostly albino, for reasons that remain unclear. Notably, subsequent intergeneric hybrids of *Brassica* and *Raphanus* were all obtained by ovule culture.

3. General characteristics of intergeneric hybrids

The morphology of the hybrid plants is intermediate between the two parents. The seed pod has a narrow septum at the center dividing the top and bottom parts (**Figure 1**). The lower part that attaches to the stem (bottom) is entirely kimchi cabbage, and the upper part (top) is entirely radish. This also applies inside the pod [8]. However, the seed appearance is indistinguishable between the kimchi cabbage and radish portions [13, 31]. The siliqua morphology seems to be a distinctive characteristic of the intergeneric hybrid between *Brassica* and *Raphanus*.

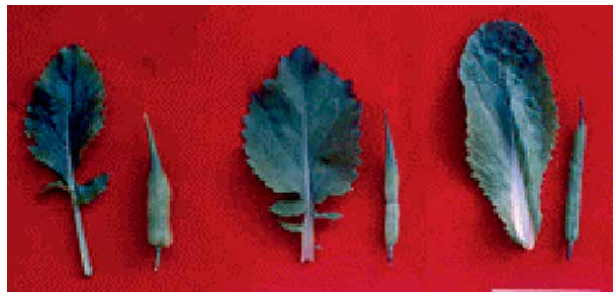

Figure 1.
Leaf and pod morphology of an intergeneric hybrid (shown between its parents).

Line code [z]	Head (g)	Plant weight (kg)	Leaf length (cm)	No. of leaves (each)	No. of lobules (each)	Petiole circum-ference (cm)	Root length (cm)	Root width (cm)	Root (g)
BB#1	900 loose	4.7	45	30	22	10.0	18.0	3.5	210

[1]*Direct sowing on August 14, observed on November 7.*

Table 3.
Characteristics of baemoochae grown in the fall.[1]

Seeds were harvested from plants cultivated in the fall. The general characteristics of baemoochae plants are as follows ([31], **Table 3**). The leaves resemble those of a radish and have many lobules and a robust appearance. Baemoochae plants have only a few leaves (~30), with very large petioles (~10 cm in circumference). The petiole is white and round, differing from the parent cultivar; kimchi cabbage has a broad white petiole, and radish has a thin, green, circular petiole. The heading ability is very low. The roots are small, but the midportion bulges (similar to radish) before stabilization [31].

The flower of intergeneric hybrids is typical of Cruciferous plants, i.e., it is generally white [31]. The plants with yellow flowers presented in the cultivation of BB#6 (a novel unstable line). The yellow stock has not been registered.

Progeny (F_2) of the ssp. *pekinensis* and *R. sativus* hybrids were attained first. The average number of seeds produced was less than one per pod, similar to the results of Dolstra [12]. However, every pod reached maturity despite the empty husk [31]. The seed size differed even within the same year. The random weight of 1000 seeds of kimchi cabbage is approximately 3.5 g, compared with 7.0 g for the intergeneric hybrid and 14.0 g for radish. The number of seeds per milliliter is ~200 for kimchi cabbage, ~120 for the intergeneric hybrid, and ~50 for radish; seed vigor also differs [31].

The total number of ovules produced by the hybrid is 10–12, which is less than the average of 25 produced by kimchi cabbage and more than the 5–7 produced by radish. Of the seeds produced, 7–8 form the bottom kimchi cabbage part; the remaining 3–4 form the top radish part [31].

4. Development of a dihaploid production system

Eleven plants were randomly chosen to produce pollen, including OV115C, which was obtained by ovule culture and colchicine treatment; it was exposed to anther culture in 1986 and embryos appeared in six plants [6]. Eighty-four embryos from 20 anthers germinated. Fifty anther-derived plants flowered and 14 produced self-fertilized seeds. The number of chromosomes in the pollen of the mother cells of these 50 individuals indicated that there were 5, 30, 15 allodiploid, (n = 2x = 19), allotetraploid, (2n = 4x = 38), and allooctaploid (4n = 8x = 76) plants, respectively.

When individuals with different ploidy levels were cultured, more embryos appeared in allooctaploid (38II, 2n = 8x = 76) than allotetraploid (2n = 4x = 38) [pp. 56–67 of a 1998 report from the HES [32] (**Table 4**)]. These results can serve as a useful reference for future anther or microspore cultures.

Microspores were cultured from the OA-20 line, which originated from the OV115C anther culture [7]. Of the 114 embryos, only 14 plants germinated, although a lot of calluses were present on these plants. The microspore culture successfully produced an intergeneric hybrid between kimchi cabbage and radish. A washing

Line code	Ploidy	No. of anthers		No. of embryos
Code		Inoculation	Germination	
OA 15	19 I	300	0	0
OA 20	19 II	300	2	4
OA 31	19 IV	300	14	131

[1]*OV: ovule culture after hybridization. C: colchicine treatment. OA: anther culture.*

Table 4.
Numbers of embryos formed from anther cultures derived from OV115C with different ploidy levels.[1]

solution composed of B5 and NLN medium was more effective than a reduced half-concentration mixture, as was a density of 100,000 microspores over 72 hours than 50,000 over 24 hours or 200,000 over 120 hours (at 32.5°C).

To determine the correlation between ploidy and germination, the chromosomes at the root tip of plants and chloroplasts within the guard cell were counted. Haploid plants have about 10 chloroplasts, while diploids have about 14, tetraploids about 22, and octoploids about 36. Diploids were the most commonly germinated embryo, accounting for nine of the 14 plants.

Results from unpublished work showed that adding a liter of NLN medium containing 0.1 mg of BA to the microspore culture reduced the incidence of callus formation and increased embryo yields by an average of 6.8 per Petri dish. To acclimate the derived hybrid, B_5M_2-II medium, which comprises 400 mg/L of KCl and 600 mg/L of $CaCl_22H_2O$, was added to the B_5 basal medium, resulting in an increase in the plant survival rate from 24% in the MS2 medium to 75% (**Table 5**). Comparing the stable and unstable lines, the **BB#12**-stabilized line generated many more embryos, demonstrating that stabilization is an important factor in the production of embryos in microspore cultures (**Table 6**). In another unpublished experiment, acclimation using a microponic system was performed repeatedly, and a plant survival rate of almost 100% was obtained when plants were acclimated for 20–30 days. This represents an important finding with respect to the microspore culture process (**Figure 2**). These improved microspore culture techniques have been providing excellent results for the baemoochae experiments conducted in our laboratory.

Medium	Numbers of embryos		Regenerated plants	
	Transplanted	Died	Abnormal	Transplantable
MS2	102	40 (40%)	38 (37%)	24 (24%)
MS4N	90	53 (59%)	21 (23%)	16 (18%)
MS4K1	93	37 (40%)	12 (13%)	45 (48%)
B5M2-II	186	4 (2%)	43 (23%)	140 (75%)

[1]*Cultivar: BB#12 (stabilized).*
MS2: 2% sucrose in MS medium, as recommended by Keller and Armstrong (1979).
MS_4N: NH_4NO_3 was reduced to 550 mg/L from 1900 mg/L in the MS medium, with 2% sucrose.
MS_4K_1: BA (0.1 mg/L) and NAA (0.2 mg/L) were added to the NH_4NO_3-free MS medium, with 2% sucrose.
B_5M_2-II: 400 mg/L of KCl and 600 mg/L of $CaCl_22H_2O$ were added to the B5 basal medium, with 2% sucrose.

Table 5.
Effects of plant medium on the regeneration of microspore-derived embryos of baemoochae (xBrassicoraphanus koranhort).[1]

Line code	Genetical status	Embryos/Petri dish	
		Average of 30	Maximum of 10
BB#4	Unstable	0.8	2.6
BB#12	Stable	27.0	56.8

[1]Each culture was conducted three times in April, in 10 Falcon Petri dishes each time. Medium: NLN13 (13% sucrose in NLN medium) and BA (0.1 mg/L).

Table 6.
Genotype specificity for embryo induction in microspore culture for unstable and stable baemoochae lines (xBrassicoraphanus koranhort).[1]

Figure 2.
Baemoochae nurseries acclimated in a microponic system (Dr. Yoon presented). The box of the microponic system was 60 cm long, 37.6 cm wide, and 18.2 cm high, and the top was covered with cellophane with 12 small holes. Thin tubes were connected to a machine to create air bubbles in the bottom of the medium.

5. Taste and nutrition of baemoochae

The nutritional composition of baemoochae derived from microspore culture was analyzed at the Dietary Life Improvement Research Institute, Rural Development Administration, by request of Mr. Moo Kyoung Yoon based on standards for kimchi cabbage and radish (1996, **Table 7**). Baemoochae showed high nutritional value in both the fresh top-part and underground roots. In total, 14 of 20 elements were overrepresented in the fresh parts and roots [energy, moisture, protein, fat, sugar, fiber (cellulose), phosphorus, natrium (sodium), kalium (potassium), zinc, magnesium, vitamin B1, vitamin B2, and vitamin C]; the remaining six components (ash, calcium, iron, vitamin A and its precursors, β-carotene, and niacin) were genetically dominant [31].

Baemoochae has a pleasant texture (crisp and juicy) and a unique flavor similar to wasabi. The component responsible for the spicy and sweet taste is sulforaphene, which has a very similar chemical structure to sulforaphane; however, sulforaphene has an additional double bond. Jongkee Kim [34] found that sulforaphene had the same anticancer effects as sulforaphane. Additionally, baemoochae juice had the same ability to eradicate *Staphylococcus aureus* as sulforaphene. These results have encouraged other scientists to investigate the baemoochae glucosinolate to sulforaphene via saliva.

Analysis of contents by part showed that baemoochae BB#6 had 294 μg sulforaphene per g of fresh root when harvested in November ([31], **Table 8**).

The location and cause of the high sulforaphene content in baemoochae were investigated. A small amount was found in the kimchi cabbage portion, but the

Crop & part		E	M %	Pg	F g	S g	C g	A g	c mg	P mg	I mg	n mg	k mg	z mg	m mg	A	β-c µg	B1 mg	B2 mg	Ni mg	VC mg
BR	R1	**46**	**85**	**3.7**	**0.2**	**8.9**	**1.2**	1.0	33	**106**	0.2	**44**	**504**	**0.4**	**28**	0	0	**.12**	**.05**	0.6	**51**
	L	**37**	**88**	**3.2**	**0.5**	**6.1**	**1.1**	1.2	55	**59**	1.6	**52**	**504**	**0.1**	**15**	60	360	**.14**	**.13**	0.5	**99**
CC		13	94	1.3	0.2	2.4	0.7	1.5	51	29	0.3	5	230	—	—	9	56	.05	.06	0.3	46
RS	R1	18	94	0.8	0.1	3.8	0.6	—	26	23	0.7	13	213	—	—	8	46	.03	0.02	0.4	15
	L	19	92	2.0	0.2	3.2	1.0	—	249	35	3.0	36	273	—	—	368	2210	.05	.10	0.6	75

BR: baemoochae, CC: kimchi cabbage, RS: radish, R1: root, L: leaf, E: energy (Kcal), M: moiture, P: protein, F: fat, S: sugar, C: cellulose, A: ash, c: calcium, p: phosphate I: ion, n: natrium, k: kalium, z: zinc, m: magnesium, R.E: vitamins A, ß-c, B1, and B2, ni: niacin, VC: vitamin C.

Table 7.
General nutritional information (provided by Moo K. Yoon [33]).

Outer leaf		Middle leaf		Inner leaf		Root
Midrib	**Fresh**	**Midrib**	**Fresh**	**Midrib**	**Fresh**	
30.8	36.0	191.4	150.3	137.3	268.2	294.3

[1]*Mean of three replicates.*

Table 8.
Content of sulforaphene in various parts of baemoochae BB#4 grown for 80 days in the fall (μg/g FW).[1]

majority was in the Taebaek radish portion (the male parent of baemoochae cross) [17]. The dry weight (DW) of baemoochae is about 55 μmol/g, which is less than that of Taebaek radish (75 μmol/g DW) but more than that of kimchi cabbage (28 μmol/g DW) [16, 18]. In another experiment that examined nine different crops (cauliflower, cabbage, broccoli, radish, baemuchae, pakchoi, Chinese cabbage, leaf mustard, and kale), the glucosinolate concentrations were lowest in radish tissues and differed widely among varieties [20].

In addition to the high concentrations of anticancer and antibacterial glucosinolate, baemoochae also has a high content of flavonoids, which have antiviral, antihistamine, and antioxidant effects [27].

6. Stabilization and evolution of baemoochae

When broccoli microspores were cultured with 0.01 μmole of n-nitroso n-methyl urethane (NMU), the embryo yield fell to 53%. However, the treatment increased the proportion of pollen-producing individuals to 73% (129 plants) and induced sterility in male plants [35]. Thus, whether NMU treatment increased the number of pollen-producing and sterile male intergeneric hybrid plants was investigated. In an experiment in which the microspore culture was treated with 0.01 μmole NMU, only nine plants produced pollen, with a seed yield of less than 0.5 seeds per pollination in 2005. When every strain was sown again for seed production, all seeds from the pods of four strains of nine lines matured into a greenish color, with the exception of one or two degenerates and the **BB#1, BB#4,** and **OV115C** cultivars, which turned brown; only one or two seeds matured in 2006. Unfortunately, after root harvest in the fall of 2007, the tap roots did not have swollen parts like a radish and had rotted inside (**Figure 3**). Seeds were produced again in 2008 and cultivated in autumn, like

Figure 3.
The appearance of Mi2-generation plants from stabilized seeds of baemoochae in 2007.

the previous year. The tap root was retained, but the root was not rigid and the inside did not rot, as it had the previous year. All lines were the same as before. The root rotting was probably a physiological disorder in the earlier generation of the mutation, although the cause was not clear.

Seeds were obtained by hybridization of a reciprocal cross between **BB#12** and a new intergeneric hybrid that had not produced any self-seeds, i.e., **Jombaechu × Jeku Gaetmoo (**04-33-**81** × 04-80-**8, 9)** [36]. Among them, **BB#12** was used as a mother line and applied for microspore mutation. One plant was selected and stabilized as a new late-bolting variety, i.e., **BB#5** ([21]: [37]).

According to international regulations, the genus name should be x*Brassicoraphanus*, irrespective of the female and male parents, to commemorate **Saqeret**, who was the first to announce successful hybridization between *Raphanus* and *Brassica*. Species can be named according to future needs [38]. For example, the species name for baemoochae **BB#5** was announced as *koranhort* [37], since this crop originated at the HES in Korea in 1986 and was developed as a stable cultivar at Chung-Ang University in Anseong in 2006. The scientific name of baemoochae is x*Brassicoraphanus koranhort* Saqeret & Lee (Soo-Seong).

To develop a new baemoochae line having a swollen root like a radish, the cultivar nidomi turnip (07-80-166. *Brassica*) was first hybridized with coastal south Gaetmoo radish (05-80-45. *Raphanus*) and subsequently crossed with baemoochae BB#12 to create this hybrid (166 × 45). Although the coastal south Gaetmoo radish had a purple vein, it was ignored since the leaf was green, and it was not clear whether the purple vein would become a purple leaf after several generations. Two plants used for multiplying seeds of turnip × radish were crossed to achieve cytoplasmic male sterility (CMS) of BB#12, i.e., to breed a CMS hybrid. This resulted in a CMS turnip × radish combination hybridized to a normal BB#12 to induce another CMS line of BB#12. Two plants crossed with normal **BB#12** of **CMSBB#12-11 × (166 × 45**) produced only one or two seeds. The other two plants, crossed with **BB#12** of **CMSBB#12-7 × (166 × 45**), produced seeds in amounts of 21.7% (81 seeds) and 20.0% (72 seeds) relative to that produced by the standard **BB#12**. Some purple plants (16 of 22 cultured) were produced from the 81 seeds mentioned above [39].

Since the seed production of **BB#12 ×** {**CMSBB#12-11 × (166x 45**)} was unusual, a "marker test" was performed by Seoul National University, along with chromosome detection by Sahmyook University, which showed that the two lines were not genetically crossed. Radish chromosomes were intact before hybridization with the turnip, even though the radish chromosomes had "sandwiched" turnip chromosomes after hybridization. Turnip chromosomes are already intercalated with B genome chromosomes. Therefore, it was inferred that the turnip was intercalated with the radish chromosomes, including those responsible for the purple color. Therefore, the resulting purple cultivar was produced by chance [39].

7. Lack of a commercial F_1 hybrid in baemoochae

As the F_1 hybrid cannot be copied by other companies and plant breeders, growers must purchase these excellent seed varieties every year. To guarantee profit, the company sells a finite amount of seeds per year. Before the baemoochae was stable, the F_1 hybrid was developed for breeding. A male sterile line of mustard (Ogura) was received from Professor Il-Seop Noh of Sunchon National University and used (2004); an attempt was made to induce a baemoochae CMS line. An F_1 plant of

Generation	Numbers of		
	Plants investigated	Male sterile plants	Fertile male plants
BC1F1	43	7	36
BC2F1	46	38	8
BC3F1	30	18	12
BC4F1	40	22	18
Total	159	85	74

Table 9.
Results of backcrossing experiments with Brassica *juncea to induce CMS in xBrassicoraphanus koranhort.*

baemoochae hybridized with "CMS mustard." Seven out of 43 BC_1F_1 hybrids were fertile and produced pollen during the next year (**Table 9**). In total, 38 of 46 plants were fertile, with pollen at BC_2F_1. It was thought that the CMS of mustard would be recovered in baemoochae. Therefore, two generations advanced more, it was stopped (unpublished data). We then attempted to breed a line of baemoochae from a kimchi cabbage with CMS, in a further attempt to induce CMS. However, some plants remained fertile, similar to *B. juncea*.

Among crucifers, *Brassica napus*, *B. juncea*, and *B. carinata* are self-compatible amphidiploids. The above pollination results show that this is also the case for baemoochae. Professor Il-Seop Noh, an expert in molecular biology specializing in self-incompatibility, investigated the reason for the self-compatibility of baemoochae. The self-incompatibility factors of both kimchi cabbage (*BrRsSRK-1*) and radish (*BrRsSRK-2*) pistils, as well as the pollen from kimchi cabbage (*BrRsSP11-1*), were functioning in baemoochae. However, radish pollen (*BrRsSP11-2*) did not function in baemoochae [19]. Therefore, the self-compatibility in baemoochae is due to the pollen from the radish portion of the hybrid. Since CMS is recovered and self-incompatibility does not function in baemoochae, there is no way to produce the F_1 hybrid seed.

8. Chromosome configuration of baemoochae

Investigations of the correlation between low seed yields and multivalent chromosomes in the original intergeneric hybrid from 1986 were conducted; OV115C plants produced abundant pollen and pods via self-pollination, but seed yields remained very low (< 1.0 per pollinated pod). Observations of chromosomes in the pachytene stage from the OV115C plant showed that four units formed a diamond shape, and five chromosome pieces were stacked with the three chromosomes in the cells. Thus, the meiosis itself was abnormal, resulting in lower seed productivity.

A report was published on the breeding of an unstable heading rapeseed, called "Hakuran," at the Japanese Vegetable and Tea Industry Station in 1968. Our laboratory requested seeds and received a line in 1972. When pollinated for multiplication, the seed yield was low. The low fertility of this interspecific hybrid could be due to multivalent chromosomes, although McNaughton [40] and Dolstra [12] theorized that the low seed yield is due to a "genic imbalance" and "breeding barrier" between radish and cabbage, and Chinese cabbage and radish, respectively.

In 1999, Seon-Jung Lim used genomic in situ hybridization (GISH) to observe homologous chromosomes in baemoochae and seek a crossover. Since the homologous chromosomes were tetravalent (composed of two genera), crossovers in chromosomes were anticipated, but not discovered. However, it was demonstrated that baemoochae is a true hybrid of kimchi cabbage and radish in terms of chromosome constitution. GISH revealed 20 kimchi cabbage and 18 radish chromosomes. The results of this master's thesis, including the first photograph of baemoochae, were presented at the first Chromosomal Conference, which was held in Shanghai, China, in 2000, and had attendees from Korea, Japan, and China. The paper was subsequently published in a journal [41].

Crossovers occur more frequently in tetraploids (4n = 2n = 38) than diploids (2n = n = 19) of x*Brassicoraphanus*, but less frequently than in stabilized lines such as **BB#12**. It is possible that the chromosomal dissimilarity of *B. rapa* and *R. sativus* prevents nonhomologous interactions between the parental chromosomes in allotetraploid x*Brassicoraphanus*, allowing normal diploid-like meiosis when homologous pairing partners are present [24].

Professor Hyun Hee Kim, a cytologist at Sahmyook University, was asked to analyze the chromosome composition of a stable **BB#5** in meiosis. Miss Hadassah Roa Belandres, working in Professor Dr. Kim's laboratory, performed fluorescence *in situ* hybridization (FISH) using 5S and 45S rDNA, along with GISH using a *B. rapa* genomic DNA probe [21]. According to the somatic chromosome complement revealed by FISH, baemoochae has 2n = 38, consisting of 17 metacentric and 2 submetacentric chromosome pairs. According to the GISH analysis, 19 bivalents were present in 42% of 100 meiotic cells, while a commination of 1 tetravalent and 17 bivalents was present in 28%, a commination of two tetravalents and 15 bivalents in 24%, and a commination of three tetravalents and 13 bivalents in 6%. She concluded that the 19 bivalents present in 42% of the meiotic cells were the main cause of the stability.

Professor **Kim** extended the study to investigate abnormal meiosis of pollen within a mother cell from the unstable line **BB#4**. In total, 500 pollen mother cells of the unstable line **BB#4** and stable lines **BB#12** and **BB#5** were assessed in all five stages of meiosis, from diakinesis to anaphase. In the unstable line **BB#4,** 57.4% (n = 287) of the dividing cells were abnormal, compared with only 10–12% (n = 50–60) of the dividing cells of two stable hybrids, **BB#12** and **BB#5** (**Table 10**).

Line code	**Diakinesis**			**Metaphase**		**Anaphase**		**Total (%) (500 cells)**
				I	II	I	II	
	Sticky	**Rod and ring**	**Laggard**	**Laggard**		**Bridge and laggard**		
BB#4 (unstable)	35	18	22	154	8	49	1	287 (57.4%)
BB#12 (stable)	4	8	12	15	6	5	0	50 (10%)
BB#5 (stable)	6	0	5	17	5	27	0	60 (12%)

Table 10.
Numbers of abnormal meiotic cells in various baemoochae strains.

9. Properties of self-sterile but cross-fertile intergeneric hybrids

Five different hybrids of the intergeneric cross between kimchi cabbage (*Brassica*) and radish (*Raphanus*), **Jombaechu × Jeju Gaetmoo (04-80-8 or 9), CR291M-64 × Shogoin, Taiwan Baiyu × Shogoin, Taiwan Baiyu × 40 days, and Chibu × Woenkyo#39**, were developed for various purposes and none produced self-seeds. However, cross-seeds were produced in four combinations with existing baemoochae or mooyangchae lines. Combinations with **Taiwan Baiyu × 40 days**, which has no hair on its leaves, are expected; all other existing cultivars have hair on their leaves [36]. Pollen samples from a selection of 30 plants that did not produce self-seeds were stained and observed. Pollen from one plant did not stain at all, while <10% of the pollen of 18 plants stained; > 30% of the pollen of seven other plants was stained, while for two plants each >70% and 90% of the pollen was stained. There appears to be no correlation between pollen dyeing and self-seed production.

BioBreeding has four stable cultivars: **BB#12**, **BB#5**, **purple BB#10**, and mooyangchae. Despite a lack of understanding of the non-self-fertilization seen in new intergeneric hybrids, new baemoochae varieties can be bred by crossing with existing cultivars, such as **BB#5** [37]. This could lead to the development of intergeneric hybrids between kimchi cabbage and radish.

10. Production of mature seeds by baemoochae

Hybrids between kimchi cabbage *B. rapa* ssp. *pekinensis* and radish initially failed to produce mature seeds ([11]: [12]). Thus, an ovule culture was developed ([14]: [15]: [30]). Cultivars **BB#12** and **BB#5** were bred by applying this ovule culture technique [9, 37]. Ovule culture is a powerful means of acquiring intergeneric hybrids between kimchi cabbage and radish. Notably, an inbreed of kimchi cabbage did create mature seeds as a dominant property. Radish cultivars and lines have little effect on this property; therefore, the kimchi cabbage is mainly responsible for the production of the mature seeds [39].

The major subspecies of *B. rapa*, i.e., ssp. *rapifera* (turnip) [12, 28], ssp. *oleifera* (turnip-rape) [12], and ssp. *chinensis* (pakchoi) ([10]: [12]) have also produced mature seeds. It is important to note that the subspecies of *Brassica* can be dominant or recessive when crossed with *Raphanus* first. When they are dominant, all combinations can be acquired between and within subspecies, including ssp. *pekinensis*, and numerous types of intergeneric hybrids can be produced. Ovule cultures older than about 10 days after hybridization are difficult to grow; it is possible that mature seeds are produced in *Brassica* only when needed.

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Chapter 3

Perspective Chapter: *Brassica* Species Mediated Green Synthesis of Nanoparticles and Its Potential Biological Applications

Sufian Rasheed, Shan Arif, Amir Ullah, Wajid Rehman and Magda H. Abdellatif

Abstract

Nanotechnology is a recent technology which is developing rapidly and it has a wide range of potential applications. It is the atomic-level tailoring of materials to achieve unique features that may be controlled for the intended purposes. Nanomaterials can be prepared via several physico-chemical methods but bioreduction of bulk to nanomaterials via green synthesis has developed as a viable alternative to physico-chemical methods in order to overcome their limitations. Plant-mediated nanomaterial synthesis has been found to be environmentally friendly, less costly, and safe with no use of chemicals for medicinal and biological applications where the nanoparticles purity is of major concern. Plant extract is used for the reduction of materials from bulk into nano scale instead of other toxic reducing agents used in chemical methods. The phytochemicals present the extract of plant not only facilitate the synthesis of nanomaterials but act as stabilizing and capping agent, also the shape and size of nanoparticles can be tailored by changing the nature and concentration of plant extract. The present chapter focuses on the green synthesis of nanoparticles mediated by various *Brassica* species and their potential medicinal and biological applications.

Keywords: green synthesis, *brassica* mediated nanoparticles, biological activities, size, morphology

1. Introduction

The word nano comes from a Greek word which means "dwarf". The term nanotechnology is described as to synthesize measure and observe materials at nanometer range. A nanometer (nm) is one billionth part of a meter or 10^{-9} m. So, nanotechnology is the field that includes the synthesis, fabrication and application of nanomaterials or nanoparticles where nanomaterials or nanoparticles can be defined as the materials or particles having at least one dimension in size range of 1–100 nm.

The applications of nanoparticles in different fields submerged nanotechnology with numerous other fields such as chemistry, physics, biotechnology, materials sciences, medicine, and engineering as a result new fields such as nano-chemistry, nanophysics, nanobiotechnology, nanomedicine, and nanoengineering etc. has emerged recently [1]. Nanotechnology is gaining prominence rapidly because of its wide range application in a number of other areas such as information technology, energy, environmental science, food packing, cosmetics, medicine and many more. Among them, the most important use of nanotechnology, however, is in medicine and health care [2].

2. Nanoparticles

A "nanoparticle" is a particle having a size in at least one dimension ranging from 1 nm to 100 nm. Looking at it another way, nanoparticles have the similar description as the ultrafine or airborne particles and may be categorized as a subclass of colloidal particles depending on their size [3]. A lot of research work has been done in the development of novel and efficient methods to synthesize the nanoparticles of controlled and desired shape, size and morphology. Depending on the method of synthesis several shapes and sizes of nanoparticles have been observed including nanospheres, nano-cubes, nanorods, nanowires, nanotubes, nano-stars etc. which are often surface functionalized in order to be utilized in desired applications. Furthermore due to their unique size and shape they have been used prominent sensing materials, studying the biological processes and for the treatment of several diseases [4].

3. Classification of nanoparticles

The advancement of nanotechnology enables researchers and scientists to prepare nanoparticles of different sizes and shapes. Nanoparticles of different sizes and shapes exhibit unique chemical and physical properties depending upon the method of their synthesis. The broad classification of nanoparticles is based upon their nanometer range dimensions.

3.1 Zero dimensional particles

Zero dimensional particles are referred to the particles having all three dimensions within nanometer range. The size of zero dimensional ranges from few tens to few hundreds of nanometers. Due to such small size they have very high surface area to volume ratio as compared to bulk materials. It is for this reason that they have unique and improved physico-chemical properties [5].

3.2 One dimensional particles

One dimensional particles are those having two dimension within nanometer range and one dimension out of nanometer range. Nanorods, nanowires and nanotubes are the most common examples of one dimensional nanoparticles. The outer layer of one dimensional nanoparticles are composed of single of multiple atoms with the inner diameter of few nanometers. They are extremely light weighted and strong materials with enhanced thermal and electrical properties [6].

3.3 Two dimensional particles

Two dimensional particles referred to those having only one dimension in nanometer range while two of their dimensions are out of nanometer range. Nanosheets are the best examples of two dimensional particles. Two dimensional nanosheets are extremely thin with the highest surface area having excellent mechanical, chemical and optical properties allowing them to be utilized in a wide range of applications [7].

4. Synthesis of nanoparticles

As discussed earlier that the method of synthesis of nanoparticle is the key factor responsible for their unique size and shape. Nanoparticles can be synthesized by chemical, physical or mechanical methods. Generally the methods of synthesis of nanoparticles are classified into two broad categories i.e. Top down and bottom up methods (**Figure 1**).

4.1 Top-down methods

Top down method referred to the strategy of going from bulk to tiny. In top down methods nanoparticles come into their unique morphology via breakdown of bulk materials into smaller particles. Lithography and sputtering are the most widely used top down methods to synthesize nanoparticles. Other examples include

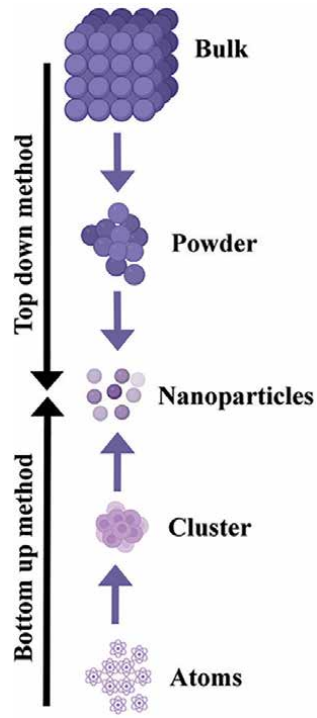

Figure 1.
General schematic representation of top down and bottom up methods.

electrochemical explosion, photoirradiation, ultrasonication, laser ablation, mechanical milling and chemical etching etc.

4.2 Bottom-up methods

The bottom up approach generally referred to going to large from small. In bottom up methods the nanoparticles take their ultimate structure via merging of small building blocks. Examples of bottom up methods for the synthesis of nanoparticles include sol-gel method, co-precipitation method, chemical reduction method, hydrothermal/solvothermal method, biological/green synthesis methods etc. [8].

5. Green synthesis of nanoparticles

A wide number of physico-chemical methods have been discovered over the past 50 years for the synthesis of nanoparticles having desired size and shapes. The need for nanoparticles is increased due to its wide range of applications in almost every area of modern time. This increasing demand of nanoparticles also poses threat to environment as the synthesis of nanoparticles by various physico-chemical methods utilizes several hazardous chemicals. As a result scientists developed a much safer method known as green synthesis of nanoparticles. Green synthesis of sometimes referred to biological or biogenic synthesis of nanoparticles is basically bottom up approach in which the precursor metal salts undergo reduction resulting in the synthesis of nanoparticles. The phenomenon of green synthesis of similar to chemical reduction method with the benefit of replacement of toxic reducing agent by the natural products extracts (**Figure 2**). The natural product extract is devoid of hazardous chemicals and has inherent stabilizing, capping properties, and growth inhibiting, making this technique eco-friendly, non-toxic, cost-effective, and ideally suited for biological and medicinal applications. Plants, algae, fungi, and bacteria etc. can be used for this purpose where the nature of biomolecules at various concentrations and in various combinations with precursor salts influences the size and structure of NPs [9].

5.1 Green synthesis of nanoparticles using *brassica* genus

Brassica is genus consist of 37 flowering plants in the mustard and cabbage family (*Brassicaceae*). Among them majority of species are agricultural corps. The genus *Brassica* is native to temperate Asia and Europe, and is particularly prevalent in the Mediterranean region; nonetheless, several species have been reported invasive in places beyond their natural range. Broccoli, sprouts, cabbage, brussels, rape, kale, kohlrabi, rutabaga, cauliflower, turnip and brown mustard are all economically significant members. It is among the most frequently produced plant genera in the world, with vegetables high in minerals and vitamins that are beneficial to the human diet. Plant extracts (flower, leaf, root, and seed) have recently been widely researched as a biological method for the production of nanoparticles (mostly silver and gold nanoparticles). Some plants have also been shown to have the ability of in vivo synthesis of nanoparticles. Plant-derived chemicals of different types exhibit potential biological activities. The phytochemistry of the genus *Brassica* has been researched, and the following substances have been identified: proteins, steroids, carbohydrates, phenols, terpenoids, flavanoids, quinones, coumarines, phlobatannins, tannins, saponins, vitamins and different minerals. These phytochemicals are responsible for the synthesis and stabilization of

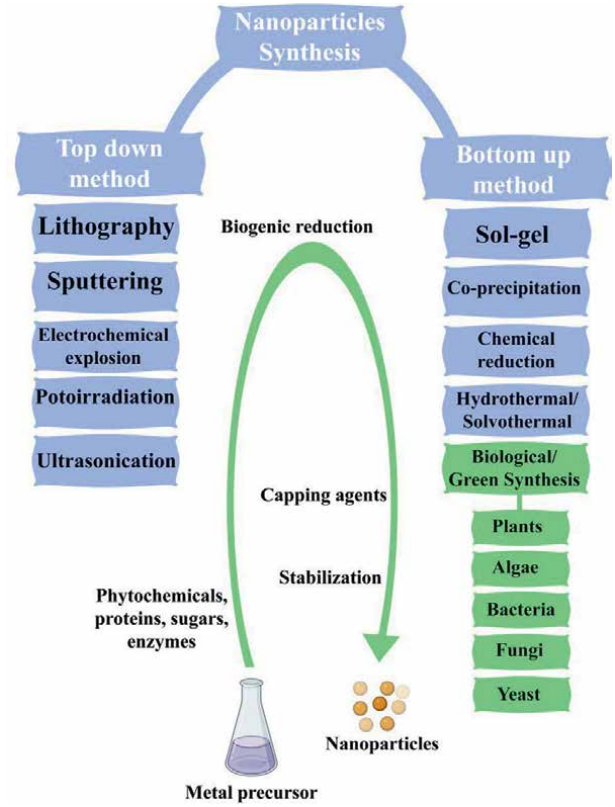

Figure 2.
Classification of methods of nanoparticles synthesis and schematic representation of biological/green synthesis.

metal nanoparticle [10]. Extensive study has recently been conducted on plant mediated reduction of metal nanoparticles and the function of phytochemicals in this technique. A great number of articles on the green synthesis of nanoparticles utilizing phytochemical enriched plant extracts have been published. The extract of broccoli has been utilized for the bio-reduction of various metal precursors to synthesize the corresponding metallic nanoparticles. Osuntokun J. et al., reported the synthesis of CuO nanoparticles using aqueous extract of broccoli having size of 30–40 nm [11]. Similarly the synthesis of gold and silver nanoparticles were reported having particle size of 24–38 nm and 38–45 nm respectively [12]. Recently selenium nanoparticles with anti-carcinogenic properties were synthesized having an average particles size of 10–28 nm [13]. The phytochemical screening of broccoli extract showed that the active compound responsible for the synthesis of nanoparticles were alkaloids, phenolic compounds, saponins, flavonoids and ascorbic acid. The extract of other species of *brassica* genus is extensively studied for the synthesis of various metallic nanoparticles in literature. Some of the species of genus *brassica* have the potential for in-vivo synthesis of nanoparticles. For this purpose the plant is grown on the surface or land having certain metal ions, the plant extracts metal from land through their hyper cumulating capacity and then through their reducing capacity they reduce metal ions into metal nanoparticles in various organs and tissues. This approach is known as phytominning and the accumulated metal is recovered after different steps of harvesting. Some of *brassica* species are known to be better hyper accumulators such as *Brassica juncea*. *B. juncea* accumulated about silver ions and synthesized

about 2–25 nm silver nanoparticles when grown silver containing land [14]. Furthermore the mechanism of reduction of metal ions into metal nanoparticles and organs responsible for the storage of reduced metal nanoparticles were also investigated in *B. juncea* plants. Gold nanoparticles were synthesized by in vivo method where *B. juncea* were grown on the solutions $AgNO_3$ and $HAuCl_4$. In case of gold the reduction Ag^{3+} ions to Au0 nanoparticles and in case of the silver the reduction Ag^{+} ions and Ag^{0} nanoparticles were investigated and the sites of their responsible for their reduction were investigated. The major amounts of nanoparticles were found in the leaf area which showed that the reduction of metal ions into metal nanoparticles mainly occurred in chloroplasts (the regions of high contents of glucose and fructose). Glucose and fructose were found to be the sugars with high reducing properties and the amount of these sugar had a directly relation to the amount of nanoparticles synthesized [15]. Having high reducing properties these glucose can be used for synthesis of nanoparticles in conventional chemical methods by replacing the toxic reducing agent with glucose. The synthesis of metal nanoparticles using other extract of organs of *Brassica genus* such as roots, seeds, flowers etc. having potential biological activities has also been reported in literature which will be discussed in the coming sections.

5.2 Potential biological activities of *brassica* genus mediated synthesized nanoparticles

Green synthesized nanoparticles being safe and economic having a wide range of applications in the area of health and medicine. The extract used for the reduction of metal ions into metal nanoparticles and also act as capping agent has an important role in their applications. The extract its-self have biological properties and depending on the extract used for synthesis and capping of nanoparticles the applications of prepared nanoparticles varies. Different species of genus *Brassica* has been reported in literature for the synthesis of metal nanoparticles and used for different biological activities. The detailed discussion of different biological applications of *Brassica* genus mediated nanoparticles is present in the coming sections.

5.2.1 Antifungal activity

Green synthesized nanoparticles have gained popularity in recent years because of their applications to control and treat several human and plants diseases, and due to their nanosized dependent unique chemical and physical properties they are found to be very effective materials in the area of medicine and biology. Initially *Brassica rapa L*. leaf extract was used for the synthesis of silver nanoparticles which were employed for the antifungal activity against a wide range of wood rotting pathogens such as *Gloeophyllum abietinum, G. trabeum, Chaetomium globosum, and Phanerochaete sordida*. This was the first study regarding the application of green synthesized nanoparticles for the treatment of plant disease. The results indicated that prepared nanoparticles were effective to protect soft and hand wood against pathogenic fungi [16]. Gold nanoparticles were synthesized using the extract of *Brassica oleracea* specie of genus *Brassica* which act as antifungal agent against human pathogenic fungi. The prepared gold nanoparticles were found to be effective antifungal agent against *Aspergillus niger, Aspergillus flavus, and Candida albicans*. The results showed the gold nanoparticles prepared using extract of *B. oleracea* exhibited enhanced antifungal activity as compared to the previously prepared using extract of other plants. The mechanism of such great antifungal activity of prepared gold nanoparticles was also investigated, as the

nanoparticles had a very small size, i.e. 12–22 nm, which was 275 times smaller than the size of fungi, making them more likely to adhere to the cell wall of fungi and resulting in death of human pathogenic fungi [17]. Copper nanoparticles being relatively economic than silver and gold nanoparticles but exhibits excellent antimicrobial, antibacterial, antifungal and anticancer activities. Copper nanoparticles prepared using the aqueous extract of *B. oleracea* var. italic were found to be very efficient against human pathogenic fungi such as *Aspergillus niger and Candida Albicans* by disc diffusion method. The phytochemical screening showed that polyphenols, glucosinolates, flavonoids, minerals and vitamins were present in the aqueous extract of *B. oleracea* var. *italic* which was responsible for the reduction of copper ions into copper nanoparticles [18]. Zinc oxide nanoparticles (ZnO NPs) have a broad range of applications and they have been extensively studied for antibacterial and antifungal properties. Zinc nanoparticles were prepared using the extract of *Brassica oleracea var. italica* and their antimicrobial and antifungal efficiency against *Staphylococcus aureus* and *Escherichia coli* and *Aspergillus niger* and *C. albicans*. ZnO-NPs synthesized *B. oleracea var. italica* extract were found to be effective against both the fungal stains [19]. Several studies have shown that nanoparticles prepared via *Brassica* genus species extracts have excellent antifungal characteristics and have the potential to be used as an antibacterial agent against a variety of microbiological species.

5.2.2 Antibacterial activity

Using various extraction solvents such as ethanol, methanol, acetone, chloroform, and water, the antibacterial activity of *Brassica* plants against Gram-positive and Gram-negative bacteria was examined. When compared to other extracts, methanolic extracts were found to be more effective at controlling the development of all bacteria [20]. Antibacterial properties of four *Brassica* species were investigated, specifically *Brassica cretica Lam.* (broccoli) and three *B. oleracea* variants (portuguese galega, Portuguese tronchuda and red cabbage). They applied different ratios of bioactive substances such as phenolic compounds and organic acids (2.5–25 mg/ml) to prevent common foodborne bacterial infections (*E. coli* O 157: H7, *Salmonella typhimurium, Listeria monocytogenes, Bacillus cereus,* and *Staphylococus aureus*). A solution of INT was used to estimate the minimal inhibitory concentration (MICINT) based on metabolic respiratory activity (2-p-iodophenyl-3-p-nitrophenyl- 5-phenyl tetrazolium chloride) [21]. Silver and gold nanoparticles synthesized from plant and vegetable extracts of the genus *Brassica* have become extremely popular because of their antibacterial qualities. The nanoparticles are even being considered as antibacterial agents of the future. Various researchers have thoroughly made consistent the production of antibacterial nanoparticles using green technology, however the induced generation of metal nanoparticles in living plants is poorly understood in this study. Tamileswari et al. (2015) described the production of silver nanoparticles using *B. oleracea* (cauliflower) and *B. oleracea capitata* (cabbage), as well as their antibacterial efficacy against pathogenic microorganisms. Sridhara et al. found that silver nanoparticles synthesized from cauliflower broth had full antibacterial action against two human pathogens, *E. coli* (*E. coli*) and *S. aureus* (*S. aureus*), at a concentration of 50 mg/liter (2013) [22]. Silver nanoparticles synthesized from broccoli were found to be effective in antibacterial activity when combined with silver nanoparticles and antibiotics in a prior study. The antibacterial efficacy of gold nanoparticles derived from *B. oleracea* (broccoli) flower bud aqueous extracts was investigated using the disc diffusion method and human pathogenic bacteria (*S. aureus* and *Klebsiella pneumonia*). When compared to

traditional antibiotics, gold nanoparticles were found to have the best antibacterial action (Gentamicin and Fluconazole). The sensitivity zone for all the studied microorganisms increased as the concentrations of gold nanoparticles (10, 25, and 50 g/ml) were raised [17]. These silver nanoparticles were tested for antibacterial efficacy against four human pathogens: *Klebsiella pneumoniae, E. coli, Staphylococcus saprophyticus,* and *B. cereus*. Gram negative (−ve) bacteria include *K. pneumonia* and *E. coli,* while gram positive (+ve) bacteria include *S. saprophyticus* and *B. cereus,* and biosynthesized silver nanoparticles demonstrated a distinct zone of inhibition. Antibacterial activity of the ethanolic crude extract and synthesized nanoparticles was studied in this work the Agar disc diffusion method was used, which measured the inhibition zone of the test microorganisms. The antibacterial potential of ethanolic crude extract and copper nanoparticles against *E.coli, S.aureus,* and *P.aeruginosa* was demonstrated by extract of *Brassica oleracea var. acephala*. It was also found to have antifungal properties against *C. albicans*. When comparing the two samples, copper nanoparticles demonstrated the greatest inhibition zone against the test organisms. This was because nanoparticles had higher antibacterial activity than the crude extract. As a result, this research suggests that copper nanoparticles generated from the leaf extract have wide antibacterial efficacy against these harmful species [23].

5.2.3 Antioxidant activity

Due to the presence of a variety of oxidants and varied ways to scavenge them, determining the antioxidant capacity of fruits and vegetables is a difficult task. There is no one assay that can evaluate the biological samples' overall antioxidant potential. As a result, various tests are used to obtain a conclusive picture of the antioxidant capacity of the materials. For the evaluation of antioxidant capacity in plant samples, the ferric reducing antioxidant potential (FRAP) and 1,1-diphenyl-2-28 picrylhydrazyl radical (DPPH) tests are the most used assays. During physiological homeostasis, organisms continuously produce large levels of molecules, many of which are reactive, known as reactive oxygen species (ROS). The oxidants that are produced can interact with proteins, lipids, and nucleic acids, among other biological components. Proteins are, indeed, oxidants' primary targets. Lipid peroxidation, on the other hand, is caused by free radicals such hydroxyl, alkoxyl, and peroxyl, particularly in polyunsaturated fatty acids. Antioxidant molecules are receiving a lot of attention these days to prevent diseases caused by oxidative stress. Polyphenols have been related to anticancer, antiaging, neuroprotective, antidiabetic, and cardioprotective properties because of their excellent structural chemistry for free radical scavenging activities. Furthermore, ascorbic acid and its oxidation product, dehydroascorbic acid, have been linked to a lower risk of cancer, cardiovascular disease, and diabetes in humans [24]. Copper nanoparticles, have a low redox potential and are more likely to oxidize when exposed to air. Microwave aided pylol, hydrothermal technique, thermal reduction, and other methods are commonly used to make them. However, these methods are not inexpensive and require the use of toxic chemical solvents. As a result, ecologically friendly synthetic methods are preferred. The leaf extract binds to the copper nanoparticles, *B. oleracea* has a high antioxidant capacity [23]. The metabolic & antioxidant properties of synthesized copper oxide nanoparticles using *Solanum lycopersicum* & *B. oleracea* were studied. The amount of copper oxide nanoparticles accumulated in the two species of plants was dosage dependent, and the results revealed that tomato plants accumulated nanoparticles more actively than cauliflower plants, probably due to differences in root architecture [25].

5.2.4 Anticancer activity

Pharmaceutical companies have periodically produced a significant number of commercial anticancer medicines. Because these treatments have such a high rate of side effects, natural effective drugs with the fewest negative effects are in demand. According to a study, the active component 2-pyrrolidinone found in the leaves of *B. oleracea* has anticancer properties. This chemical was projected to be useful in the creation of novel anticancer drugs that could stop cancer cells from growing in vitro. In HeLa (IC50 2.5 g/ml at 24 h and 1.5 g/ml at 48 h) and PC-3 cancer cells (IC50 3.0 g/ml at 24 h and 2.0 g/ml at 48 h), this compound inhibited the proliferator cells, resulting in a considerable decrease in cell proliferation, cell viability, and significant induction of apoptosis [26]. In vitro studies were conducted on HCT116 colorectal cancer and H1299 non-small-cell lung carcinoma cells using ethanolic extracts of mustard (*B. juncea*) leaves. The release of vascular endothelial cell growth factor & basic fibroblast growth factor was significantly suppressed in both cell lines after treatment with mustard leaves extract. This study discovered that mustard leaves extract had anticancer properties in vitro against colon (IC50 253 g/ml at 72 h and 153 g/ml at 96 h) & lung cancers (IC50 700 g/ml at 72 h and 130 g/ml at 96 h), but the findings need to be confirmed in vivo [27]. Nanoparticles could be a more active and lucrative source of novel cancer treatments in the current context. As a result, biologically synthesized nanoparticles have recently received a lot of attention as chemotherapeutic agents. Mayilsamy and Krishnaswamy (2016) looked at the anticancer properties of silver nanoparticles made from *B. rapa chinensis* L., as well as their in vivo experiments in mice. Treatment with a methanolic extract of *B. rapa chinensis* leaves resulted in a rise in hemoglobin content and RBC, as well as a decrease in WBC count. The histological evaluation of control animals revealed aberrant growth, but treatment with nanoparticles resulted in hepatocyte growth that was practically normal. Many scientists and researchers have recently worked on silver nanoparticles and examined their anticancer activity in lung and liver cancer cells as well as HEpG-2 cells and gold nanoparticles against HL-60 cells [28, 29]. Ethanolic crude extract and copper nanoparticles were tested in vitro against the HeLa cervical cancer cell line at various doses. The samples had a high level of cytotoxicity when tested on the HeLa cell line. The results showed that ethanolic crude extract and copper nanoparticles effectively inhibited HeLa cell proliferation, with an IC 50 value of 170.6622 (g/ml) for the crude extract and 119.0805 (g/ml) for the nanoparticles. The fact that the percent toxicity increased as the concentration of copper nanoparticles grew suggests that synthesized copper nanoparticles could be useful in medicine as an anticancer drug. The vitality of cancer cells decreases as the concentration of the samples increases, whereas cytotoxicity against HeLa cell lines increases as the concentration of the samples increases [23].

5.2.5 Cytotoxic activity

Cytotoxicity tests, which include plant extracts or physiologically active chemicals derived from plants, are a valuable first step in identifying the potential toxicity of a test drug. For the effective development of a pharmaceutical or cosmetic product, minimal to no toxicity is required, and cellular toxicity studies play a critical role in this regard [30]. Using AgNO3 solution as a precursor and *B. rapa* var. *japonica* leaf extract as a reducing and capping agent, silver nanoparticles (AgNPs) were successfully synthesized from Ag + reduction. The goal of this research was to synthesize AgNPs that were less toxic. On PC12 cells, crystalline phased *Brassica* AgNPs were

less toxic than Com AgNPs. *Brassica* AgNPs' lower cytotoxic activity could be related to their stability, which was attributed to the presence of a capping agent on AgNPs given by *Brassica rap* var. *japonica.* As a result, *Brassica* AgNPs could be a good choice for safe application in consumer products because they are simple and inexpensive [31]. The 70 percent aqueous ethanolic extract of *B. rapa* and silver nanoparticles were studied for chemical components and cytotoxic effects. The ethanolic extract included silver nanoparticles that were less than 20 nm in size. The two extracts (ethanolic extract and its nanoparticles) were tested for cytotoxicity against HELA cells (human cervical cancer cell line) and M-NFS-60 cells (human Mouse Myelogenous Leukemia carcinoma) using Doxorubicin as the control medication. *Brassica* extract showed to be less toxic to both cell lines [32]. First, Ag-NPs were successfully synthesized by reducing Ag + with AgNO3 solution and *B. rapa* var. *nipposinica/japonica* leaf extract as reducing and capping agents. There were no further chemical reductants or stabilizing agents used in the synthesis process. Following tests were done to conform the production of Ag-NPs to lower toxicity. The cytotoxicity of *Brassica* AgNPs was compared to that of commercial AgNPs by using PC12 cell system. Commercial Ag-NPs reduced cell viability to 23% (control: 97%) and increased lactate dehydrogenase activity in PC12 cells at three ppm, whereas *Brassica* Ag-NPs showed no cytotoxicity on both parameters up to a concentration level of 10 ppm. The lower cytotoxicity of green produced Ag-NPs will be beneficial for safely use of Ag-NP in consumer products [33]. Greenly synthesized silver nanoparticles (Ag-NPs) have shown promising effect on different cell lines including cytotoxicity and anticancer potential. As a result, Ag-NPs were synthesized from AgNO3 reduction using *B. rapa var.japonica (Bj)* leaf extract as a reducing and stabilizing agent in a prior study. The Ag-NPs were spherical in form and ranged in size from 15 to 30 nm. They had a phase-centered cubic structure and could effectively limit the growth of various bacteria. In this work, we wanted to see if Ag-NPs have an autophagy-regulated lethal effect on human epithelial colorectal adenocarcinoma cells, like we did in the prior one (Caco-2 cells). *Brassica* silver nanoparticles (*Brassica* Ag-NPs)-induced NF-B mediated autophagy in Caco-2 cells was accelerated by the Bj leaf aqueous extract. According to results the Ag-NPs reduced Caco-2 cell viability by producing oxidative stress and DNA damage [34]. High-quality gold nanoparticles (AuNPs) were produced using aqueous Chinese lettuce (CL) leaf extract as a reducing agent in this study. According to FTIR studies, the excellent stability of AuNPs can be linked to the presence of CL leaf extract on particle surfaces. AuNPs concentrations ranging from 0 to 2 g/ml, but no significant variations in cell viability were observed; however, A549 cell proliferation was severely reduced in a dose-dependent manner at doses more than 4 g/ml. The viability of cells was 90% after 48 hours of treatment with AuNPs, according to cytotoxicity data. After 48 hours of therapy, a healthy nucleus was found with decreased chromatin condensation. These studies indicate that AuNPs had no negative effects on A549 cells' chromatin or DNA. AuNPs eventually caused necrosis and apoptosis in A549 cells, according to the results of the apoptosis experiment [35]. The green approach of nanoparticle synthesis, which is both environmentally and biologically friendly, is a new topic that has emerged as a viable alternative to traditional methods such as physical and chemical synthesis. The green synthesis of magnetic iron oxide nanoparticles (IONPs) from iron (III) chloride was described in this study employing *B. oleracea* aueous peel extract. The MTT assay was used to investigate the cytotoxic effects of IONPs on the MCF-7 breast cancer cell line. The polydispersity index of IONPs was found to be 0.265, and the mean particle size was 675 25 nm. The effective

synthesis of nanoparticles is supported by characterization results, and nanoparticles' dose-dependent lethal effects on MCF-7 cells make them a promising chemotherapeutic agent for breast cancer treatment [36].

6. Future perspectives

According to previous research *Brassica* species have potential biomedical properties because different species of *Brassica* genus were found to have several significant phytochemicals like phenols, alkaloids, carbohydrates, tannins, saponin, flavonoids, proteins etc. Owing to these biologically important phytochemicals the extract of *Brassica* species exhibits strong biomedical application. Several potential biological activities of different *Brassica* species have been discovered such as antioxidant activity, antibacterial activity, antifungal activity, antidiabetic activity, anticancer activity, anti-inflammatory activity, antinoceptive activity, antiviral activity, antihyperlipedemic activity, antiobesity, antihyperglycemic activity and antidepressant activity [37].

On the other hand *Brassica* species mediated nanoparticles have been explored less as compared to the pure extract of different *Brassica* species. These kind of nanoparticles are used against few activities such as antibacterial, antifungal, anticancer, and antioxidant activities with enhanced performance as discussed earlier but still there is a plenty of room in exploration of *brassica* mediated nanoparticles against the remaining biological activities. In future the *Brassica* mediated nanoparticles can used for these activities with enhanced performance and which can act as potential materials in the treatment of such critical diseases.

7. Conclusions

Brassica species have some potential phytochemicals which not only act as reducing agents but also act as capping agents owing to the stability of prepared nanoparticles. Several species of *Brassica* genus and different parts of each plant has been used for the synthesis of nanoparticles of different metals such as gold, silver, copper, zinc, iron and selenium. The particles prepared using extract of various species of nanoparticles were found to be safer as compared to prepared by conventional chemical methods with enhanced biological properties. Recent studies showed that the *Brassica* species mediated nanoparticles have strong antifungal, antibacterial, anticancer and antioxidant activities. However, more study is required for the extension of biomedical applications of these nanoparticles because the pure extract of several species of *Brassica* genus has been explored for having metabolites with profound biological properties. Further, due to absence of toxic chemicals in this method synthesis these kind of nanoparticles can be used for other biomedical, environmental and electrochemical purposes.

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Conflict of interest

The authors have no conflicts of interest to declare. All co-authors have seen and approved the contents of the manuscript and have no financial interest to report. We certify that the submission is original work and not under review in another publication.

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Chapter 4

Secondary Metabolites of *Brassica juncea* (L.) Czern and Coss: Occurence, Variations and Importance

Aditya Pratap Singh, Ponaganti Shiva Kishore, Santanu Kar and Sujaya Dewanjee

Abstract

There are numerous secondary plant metabolites found in the crop *B. juncea*, especially glucosinolates. Isothiocyanates, the by-products of glycosinolate breakdown, are beneficial to human health. A number of studies have also called attention to phenolic compounds and carotenoids, both well known for their anti-oxidant properties. A notable feature is that the profiles and concentrations of secondary plant metabolites vary greatly between varieties and that genetic factors are thought to be the most significant factors. In addition, environmental and agronomic factors have also been noted to change the concentrations of secondary plant metabolites. Secondary plant metabolites are primarily produced for defense purposes. Consequently, the intrinsic quality of Indian mustard, including color, aroma, taste, and medicinal properties, is profoundly influenced by its secondary metabolite profile. The health benefits of glycosinolates and the cancer prevention properties of their breakdown products make them of specific interest. Plant cells that have been injured undergo enzymatic decomposition of glucosinolate by endogenous enzymes such as myrosinase, which releases degradation products such as nitriles, epithionitriles, or isothiocyanates. The main phenolic compounds found in *B. juncea* are flavonoids and hydroxycinnamic acid derivatives. A diverse secondary metabolite pool is also essential for plant-environment interactions.

Keywords: *brassica*, glucosinolate, myrosinase, metabolites, phenolics

1. Introduction

Among the largest groups of autotrophs on this planet are plants. There are many organisms that feed on them, including bacteria, fungi, invertebrates, and vertebrates. It is remarkable that plants are able to support such a large group of organisms. In spite of this, some plants still manage to survive on this earth, even in very hostile environments. In order to defend themselves against herbivores and attackers, they possess a variety of mechanisms [1]. Indian mustard (*Brassica juncea*), an annual herb

that belongs to the brassicaceae family, is one such plant. Affordable, healthy foods like mustard contain bioactive ingredients like glucosinolates, their breakdown products, and polyphenols. It is also high in ascorbic acid, fibre, chlorophyll, minerals, and volatile organic compounds. Mustard is utilised as a spice because of its strong, fiery, pungent flavour. The leaves of the mustard plant are used as stimulants, expectorants, and diuretics in folk medicine [2].

2. Chemical compounds in mustard

There are several important molecules present in mustard leaves, including chlorophyll, beta-carotene, ascorbic acid and potassium [3]. Mustard seeds include a lot of dietary fibre and lipids in addition to carbohydrates and proteins. Furthermore, they contain vitamin K and C, electrolytes such as sodium and potassium and trace minerals such as Mg, Ca, Mn, Fe, Zn, Cu, and Se [4, 5]. Varieties, locations, growing areas, and methods of processing influence mustard's specific nutrients and content. The lower and upper leaves of mustard have similar nutrient contents [6]. Compared to the rest of the plant, seeds have higher protein, carbohydrate, and fat content, while dietary fibre content is lower [5]. Indian mustard, or *B. juncea* L., is an oilseed as well as leafy vegetable crop bearing seeds that are high in both protein and oil (37–49%) with promising possibilities [7, 8].

2.1 Polyphenol types and contents in mustard

An important group of secondary metabolites in plants, polyphenols are found in cortex, skin, roots, fruits, and leaves of plants. They are phenolic compounds with multiple hydroxyl groups. A polyphenolic compound is a hydrophilic compound in the cell, and when combined with carbohydrates, it is predominantly a glycosidic compound. Polyphenols, which comprise flavonoids and tannins, have been proven to have anticancer and antioxidant properties [9, 10]. It is evident that mustard variety, plant part, preparation techniques, and detection technique all had a significant impact on the types and contents of polyphenols. The Mustards' total phenolic content range between 404.33 and 3.26 milligrams of gallic acid equivalent/g [9]. Various mustards contain polypheonols, including epigallocatechin gallat, proanthocyanidins, epicatechin gallat, rutin, naringin, protocatechuic acid, p-hydroxybenzoic acid, catechin, chlorogenic acid, vanillic acid, gallic acid, sinapic acid, caffeic acid, p-Coumaric acid, ferulic acid, vanillin, and p-hydroxybenzaldehyde. There is substantial variation in the polyphenol content of mustard greens. Mustard greens were found to have the greatest content of sinapic acid and then chlorogenic acid. In general, lateral buds were found to have more polyphenols than other parts of the plant [11]. Various plant sections have different polyphenol contents, which are arranged in the following order: seeds, leaves, roots, and stems [12, 13]. Furthermore, mustard contains a high level of flavonoids. Indian mustard (*B. juncea*) contained flavonoids in amounts ranging from 56 to 2893 μg kaempferol-3-O-hydroxyferuloyldiglucoside-7-O-glucoside equivalents/g [14]. There was a large variation in flavonoids content between mustard varieties and detection methods.

Polyphenols can be detected in mustard by several methods, including: 1. high-performance liquid chromatography (HPLC) [15], 2. Reversed-phase HPLC (reverse-phase high-performance liquid chromatography) [16], 3. Qualitative HPLC-ESI-MSn analysis [13], 4. UHPLC-DAD-ESI-MSn analysis and quantification [17],

5. Folin-Ciocalteu reagent [18], 6. Ultra-sonication [19], 7. spectrophotometry methods, 8. Others- paper chromatography, Column chromatography and thin-layer chromatography [16]. Microwave extraction, Soxhlet water bath extraction, and ultrasonic extraction are the methods used to extract mustard's total polyphenols [17, 20, 21]. Using three solvents, Huang et al. [22] extracted mustard polyphenols. Overall, ethanol extracted polyphenols were higher than methanol extracted polyphenols followed by water extracted polyphenols. About 8000 different polyphenolic compounds have been identified in Indian mustard [23]. As new technology and further research are developed, it will be possible to find more polyphenols in mustard.

2.2 Types and contents of glucosinolates and their degradation products in mustard

Glycosinolates are mostly made up of three components (sulfonium sulfonate, D-glucose and an amino acid side chain R) in plants. The glucosinolates are categorised as aliphatic, aromatic, and Indole glucosinolates based on the difference in R, i.e., functional group [24, 25]. Three components are involved in glucosinolate biosynthesis: lengthening of the side chains of amino acids, the development of core structures, and alteration of secondary side chains [26]. In addition to various biological functions, glucosinolates play a vital role in determining how cruciferous vegetables smell and taste. The non-volatile flavour precursors nitriles, thiocyanates, isothiocyanates, and glucosinolates are what gives mustard its spicy flavor [27, 28]. Glucoseglucoside can result in isothiocyanate breakdown products with fresh, aromatic, or bitter and spicy flavours through the three degradation processes of enzymatic, chemical, and thermal degradation [29].

Kim et al. [21] detected 13.0 mg of glucosinolates per gram of mustard. It has been found that sinigrin is present in all mustards reported to date [12]. According to Sun et al. [11], Sinigrin made up 41.7% of the total glucosinolates in Korean leaf mustard (*B. juncea* var. Integrifolia). The study of Nugrahedi et al. [30] concluded that over 90% of the glucosinolate content in fresh mustard was sinigrin, which was reduced by 95% after 3 days of fermentation, while the levels of neo glucobrassicin and 4-methoxy glucobrassicin dropped to 80–90%.

It was found that Potherb Mustard (*B. juncea*) contained progoitrin and gluconapin as the main glucosinolates [31]. Nutrient composition and content differed significantly between plant organs/tissues. Compared to other baby mustard edible portions, the skin of *B. juncea* var. gemmifera contains more aromatic glucosinolates. Korean Dolsan Leaf mustard (*B. juncea*) seeds were more likely to contain sinigrin than stems, roots, and leaves, according to Tsukamoto et al. [12]. Mustard has also been qualitatively found to comprise other glucosinolates and their breakdown products [26], including progoitrin, glucoerucin, and glucoraphanin. More mustard types need to be explored, along with the glucosinolate alterations and processes in mustard processing.

In cruciferous vegetables, there are over 200 known glucosinolates [29], but no systematic study has been conducted on mustard glucosinolates.

3. Developments in the research of mustard's medicinal properties

Consumption of mustard leaves has been associated with several possible health advantages in Asia and Africa [32]. Literature reports that mustard extracts have

antiinflammatory, antioxidant, antidepressant, antimutagenic, and antibacterial activities. The extract from mustard also inhibits angiotensin-converting enzymes, lowers blood cholesterol levels, increases HDL cholesterol levels, and protects against renal ischemia. There is also evidence that the risk of developing numerous malignancies, including breast, colon, lung, and gastric cancers, is decreased by the mustard extract.

3.1 Anticancer activity

Several bioactive components of mustard, including polyphenols, flavonoids, glucosinolates, and their degradation products, are believed to play a role in its antiproliferative and preventative effects on tumor cells. This is especially true for glucosinolates such as sulforaphane, indole-3-methanol, sinigrin, isothiocyanate allyl acid, and their degradation products. ACI/N rats were found to inhibit tongue cancer when administered sinigrin and inhibit liver cancer when administered sinigrin [33]. Further, Kim et al. [34] found that, especially for SNU-C4 and SNU-251 cells, red mustard had greater anticancer properties than green mustard. In this study, the glucosinolates of both Korean red and green mustard were tested against the cancer cells, SNU-251, SNU-C4, SNU-354, and MCF-7. In the red and green mustard extract, sinigrin was determined to be the primary active ingredient. As a result of glucosinolate degradation products [35, 36], studies have demonstrated that considerable inhibition of cancer cells of lung by phenethyl isothiocyanate, benzyl isothiocyanate, allyl isothiocyanate, and sulforaphane. Sulforaphane effectively suppressed the growth of esophageal adenocarcinoma cells [37], cancer cells of colon [38], and lung cancer cells [35]. As reported by Tanaka et al. [33], indole-3-methanol inhibits cancer cell of colon, breast, and tongue s in male ACI/N rats [38]. A study found that the ethyl acetate extract of *B. juncea* var. Raya is anti-cancerous and can prevent the spread of colon cancer cells, (HCT116), breast cancer cells (MCF-7, MDA-MB-231), lung cancer cells (A-549), cervical cancer cells (HeLa), and prostate cancer cells (PC-3). As a result, the extract has potential to supress the growth of cancer cells was dose-dependent, affecting the breast carcinoma cell line the most. Studies have shown that mustard extract has therapeutic potential in addition to its cacinopreventative properties. The extract causes cancer cell lines to undergo apoptosis, which kills them, as a result of reactive oxygen species being generated by the mitochondrial pathway. Several compounds were identified in *B. juncea* var. Raya. Among the isothiocyanates present in *B. juncea* var. Raya include allyl isothiocyanates (23%, degraded from sinigrin), 2-phethyl isothiocyanates (20%, generated from gluconasturiin) and 3-butyl isothiocyanates (18%, degraded from gluconapin). Additionally, it has been claimed that mustard extracts in methanol and water can suppress the growth of cancer cells [3, 39, 40], however, their main active components are unknown. It will be necessary to continue identifying the components of mustard extract, examine dose-resistance relationships, and examine how structure affects anticancer activity in order to advance in this field.

3.2 Antioxidant activity

A number of antioxidant compounds have been found in mustard [18], including phenolic compounds, vitamin A, glucosinolates, vitamin C, and other compounds. It was found that the 50% acetonitrile extract of Korean Dolsan Leaf mustard was shown to have somewhat better antioxidant activity than that of other sites in the

study. In addition to ABTS, EDA, and FRAP (Ferric ion-reducing antioxidant power) were shown to have antioxidant activity. As shown in Oh, Kim et al. [21], it was linearly correlated with flavonoid concentration, indicating that flavonoids and polyphenols may act as mediators for their antioxidant activity. However, aerobic environment, temperature, fermentation time, solvent, and pH may affect the antioxidant activity of fermented mustard [15, 18, 41]. Several mustards have different antioxidant activities, and different mustards have various antioxidant capacities. Tests conducted *in vivo* and *in vitro* can identify antioxidant activity. A primary method for evaluating antioxidant activity *in vitro* is DPPH (2,2-Diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl; 1,1-Diphenyl-2-picrylhydrazyl radical). For the determination of antioxidant activity *in vivo*, LPO is the most common method. Although Free radicals are scavenged by both methods, DPPH [42] is faster, simpler and more economical than other test methods. The antioxidant activity of mustard extracts may also be determined using the ABTS-free radical scavenging and iron reduction antioxidant capacity (FRAP) tests [13, 22]. A number of malignancies have been closely associated with excessive nitrate intake [19, 43].

The methanol-based mustard extract shows nitrite scavenging activity at a higher level than water extract and ethanol extract [18, 44]. *In vitro* pH value studies of microbiological thiobarbital acid-free fatty acids were also carried out to determine the antioxidant potential of mustard leaf ethanol extracts on raw meat lipid oxidation protection. The findings showed that while the samples' pH declined after storage, their contents of free fatty acids and thiobarbital considerably rose significantly ($P < .05$).

In samples treated with ethanol extracts of mustard leaf pickles of 0.1% or 0.2%, bacteria were significantly less prevalent than in samples treated with control ascorbic acid (0.02% ascorbic acid), demonstrating mustard's antioxidant properties [22, 32, 45]. Animals are often shielded from oxidative stress throughout testing in order to evaluate *in vivo* detection. Live mice were treated to oxidative stress brought on by urethane, cyclophosphamide, and mustard leaf extract, as well as new radiation-induced chromosomal damage. When given at 50–250 mg/kg body weight over the course of 7 days, mustard leaf extract decreases the micronuclei brought on by radiation and genotoxic substances. Furthermore, glutathione levels and glutathione S-transferase levels rose, shielding the mice against genotoxicity and chromosomal damage. In streptozotocin-induced diabetic rats, mustard BuOH extract fraction was also evaluated for its effect on oxidative stress. As a result of thiobarbituric acid fraction administration (100 or 200 mg/kg of body weight every day for 10 days), superoxide levels, glycosylated protein, serum glucose, thiobarbituric acid levels, and nitrite/nitrate both the amount of reactive substances and the amount of thiobarbituric acid-reactive compounds were dramatically decreased as well. As a result of reduced lipid peroxidation and oxygen-free radical levels in mustard leaf BuOH fractions, oxidative stress associated with diabetes is improved [46]. Several studies have examined the antioxidant potential of mustard extracts using various methodologies; however, further research is needed to determine the antioxidant components of mustard, their assimilation, metabolism, and antioxidant processes in the human body.

3.3 Anti-obesity

A limited amount of research has been conducted in this field, but some studies have shown, that *B. juncea* L. leaf extracts with 80% (v/v) ethanol had positive effects on obese Sprague-Dawley rats on a high-cholesterol diet. A number of lipid

parameters were improved in rats, including serum and organ lipid levels. Gene/protein expression related to fat metabolism and cholesterol production was also regulated. In rats given the extract, the weight of the organs significantly decreased, and the bulk of the mesentery, epididymis, and total adipose tissue all decreased ($p < 0.05$). Those enzymes produced a significant amount of mRNA expression being given a dose of leaf extract from *B. juncea*.

Based on the findings, 80% (v/v) ethanol extract of *B. juncea* L. leaf has the potential to alleviate obesity, likely by inhibiting the expression of G6pdh, Acc, and Fas genes [47].

3.4 Anti-inflammatory, antiviral, and antibacterial properties

An antiviral effect is obtained from brassinosteroids, which are polyhydroxy steroids found in *B. juncea* extract [48]. In comparison to ethanol, n-hexane, and hot water extracts (80°C), mustard subcritical water extract showed higher antiviral activity. Mustard subcritical water extracts were reported to exhibit 50.35 percent antiviral activity in influenza A/H1N1 influenza virus-infected cells, whereas a milk extract containing 0.28 mg/mL subcritical water extract showed 39.62% antiviral activity [48]. *B. juncea* extract, when diluted to 1.25 mg/mL, showed strong antiviral activity against influenza virus A/H1N1, according to Lee et al. [45]. At a dosage of 1.25 mg/mL, mustard extract significantly reduced the spread of virus particles.

There was a selective antibacterial effect of crude Oriental mustard seed meal extracts and purified polyphenols on both Gram-negative and Gram-positive bacteria (*Listeria monocytogenes* and *Staphylococcus aureus*). The hydrolyzed extract was found to be effective against *Bacillus subtilis*, *S. aureus*, *Escherichia coli*, *L. monocytogenes*, and *Pseudomonas fluorescens* when the minimum inhibitory level was 0.1 g/L.

The mustard extract has also been shown to be a successful anti-inflammatory agent. *B. juncea* 50% ethanol extract has been demonstrated to lessen both acute inflammations (12-o-tetradecanoylphorbol-acetate (TPA) generated mouse ear edoema and arachidonic acid (AA) produced mouse ear edoema) and chronic inflammation (many applications of croton oil (CO) induced) in mice [49]. TPA-treated mice's ears were found to be significantly thinner and MPO activity significantly decreased by extracts, as was mRNA and protein levels of IL-6. Researchers discovered that *B. juncea* has anti-inflammatory properties. Mustard ethyl acetate and n-butanol fractions have also been examined for their effects on peritoneal macrophages stimulated with lipopolysaccharide. Neither fraction produced nitric oxide (NO) or nitrite, and both inhibited nitric oxide (NO) generation. Compared with mustard leaf n-butanol, mustard leaf ethyl acetate appears to perform better as a protective agent against lipopolysaccharides and inhibits nitrite synthesis more strongly. A study demonstrated by Kim et al. [50] showed that mustard leaf inhibits the production of nitrites and nitric oxide, possibly contributing to its anti-inflammatory properties.

3.5 Therapeutic effect on diabetic cataract

Studies have been conducted on mustard extract and dietary cataract Albino Wistar rats administered streptozotocin. Scientists found that administering extracts to subjects for 8 weeks at doses of 250 and 500 mg/kg body weight prevented cataract development, as well as improving protection against diabetic cataracts at high concentrations [51]. In a study by Yokozawa et al. [52], The effectiveness of a mustard ethyl acetate (EtOAc) extract in preventing diabetes and its consequences was examined. Research was conducted on diabetic rats induced by streptozotocin. After

oral treatment of EtOAc fractions (200 mg/kg body weight/d and 50 mg/kg body weight/d) for 10 days, a dose-dependent reduction in blood glucose glycosylated protein levels and thiobarbital acid reactive substance levels was seen. Additionally, serum, liver, and kidney mitochondrial levels of superoxide and nitrite/nitrate reduced. Based on these results [52], mustard leaf extract may be beneficial in reducing diabetic complications.

3.6 Anti-hyperglycemia effect

A study of extracts from green and red mustard leaves (*B. juncea* var. Integrifolia) examined their phenolic and glucosinolate concentrations as well as their blood sugar reducing abilities. According to the findings, green and red mustard leaves had total phenolic contents of 1228.48 36.81 and 850.75 28.88 mg/100 g, respectively, while green mustard leaves had sinigrin contents of 953.19 41.11 and 1319.62127.95 mg/100 g, respectively. Sinigrin is more abundant in red mustard leaves than green mustard leaves, which have a greater amount of total phenolic content. Red mustard leaves were shown to suppress the activity of -glucosidase but to have no influence on that of alpha-amylase. Accordingly, red mustard leaves reduce blood sugar levels more effectively than green mustard leaves [53].

3.7 Antidepressant effect

As a result of diabetes, rodents demonstrate changes in their behavior, brain structure, and biochemical characteristics [54]. *B. juncea* leaf methanolic extract has been studied because of its antidepressant properties. Tests for tail-hanging, behavioural despondency, learned helplessness, and motor activity were used to measure the therapeutic efficacy. Furthermore, serum levels of serotonin, norepinephrine and dopamine were determined following extract treatment. *B. juncea* was discovered to have anti-depressive properties in behavioural experiments using diabetic rats and mice as well as biochemical examinations., dopamine, norepinephrine, and Serotonin levels in the brain were elevated by mustard extract in a dose dependent manner in comparison to diabetic-depressed baseline values. In order to fight diabetes-related depression, *B. juncea* might prove to be a valuable nutritional alternative [54].

4. Genetic engineering methods to augment Glucosinolate content

Brassical plants contain glycosinolates, which contain nitrogen and sulfur. Myrosinases hydrolyze these glucosinolates into various compounds when plant tissue is damaged, such as by mechanical injury or by pathogens or insect pests attacking the plant. *B. juncea* is able to defend itself against pathogenic insects and pathogens using aromatic and indole glucosinolates [55]. Aside from its pungency, mustard oil also features distinctive flavors due to glucosinolates. It is this pungency that has made mustard oil so popular. Chemoprotective and anticancer properties have been observed for many glucosinolates and their degradation products. *B. juncea* seedmeal contains high levels of glucosinolates (80–120 mol/g) which are nutritional antagonists and reduce the palatability. As a result, seedmeal is not palatable to poultry. Augustine et al. [56] found that the degradation products of these compounds are also goitrogenic in nature.

Since Indian mustard seedmeal contains such high levels of glucosinolates, it is less expensive in the international market. Indian mustard breeding programs target

glucosinolate content reductions to 30 mol/g of dry seed weight (DSW). As a result of negative linkage drag between seed glucosinolates and seed yield, whenever quality lines are developed through conventional breeding methods, yield penalty occurs. A genetic engineering approach was required to improve this trait. Using RNAi-based targeted suppression of the BjMYB28 transcription factor gene involved in aliphatic glucosinolate biosynthesis, A high-yielding Indian mustard cultivar Varuna was reduced in its glucosinolate content by Augustine et al. [57]. As low as 11.26 moles/g of DSW of glucosinolate-containing transgenic Indian mustard lines can be developed. However, the desirable non-aliphatic glucosinolate content and composition did not change following targeted silencing of BjMYB28 transcription factor. There are many anti-cancer properties found in the glucosinolates in Indian mustard that offer great health benefits.

Sulphoraphane, produced by glucosinolate glucoraphanin, has anticancer and healing properties. Glucosinolate glucoraphanin is converted in this process by the enzyme AOP as well as the enzyme GSL-ALK, which leads to certain undesirable degradation products like gluconapin and progoitrin, which are present in greater amounts in *B. juncea*. The GSL-ALK gene family has four functional homologs and Augustine and Bisht [56] used constitutive gene silencing to silence all four homologs. As a result, the transgenic *B. juncea* plants contained more glucoraphanin, a desirable glucosinolate, and fewer antinutritional glucosinolates. Transgenic mustard lines were also more resistant to *Sclerotinia sclerotiorum*, the fungus that causes stem rot. Indian mustard seeds contain various types of aliphatic glucosinolates, including sinigrin, which can be used medically and therapeutically in mammals. A Bju.CYP79F1 gene overexpression study by Sharma et al. [58] demonstrated that Bju.CYP79F1 gene is functionally important for sinigrin biosynthesis in a *B. juncea* line having high glucosinolate content in the seeds, but no sinigrin in them. Through overexpression of the Bju.CYP79F1 gene, transgenic mustard lines were able to synthesize sinigrin in their seeds. An antinutritional compound found in Indian mustard seedmeal is sinapine, a type of glucosinolate.

As a result, it gives chicken eggs a fishy flavour and contributes a gritty quality to meat, decreasing customer interest in both products. Sinapine levels in the germplasm of *Brassica* lines range from 6.7 to 15.1 mg/g of DSW. Kajla et al. [59] tried to silence two genes to reduce the amount of sinapine in Indian mustard. The three SGT-encoding enzymes that catalyse the crucial stages in the sinapine production pathway are sinapate glucosyltransferase, sinapoylglucose, and choline sinapoyltransferase. To stop the production of the target genes, they employed three distinct methods of gene silencing, including RNA interference (RNAi), antisense gene, and synthetic micro RNA. The RNAi gene silencing method was used to assess a decrease in seed sinapine content in these transgenic lines that ranged from 15.8% to 67.2%. A transgenic mustard line only contained 3.79 mg/g of DSW sinapine.

5. Conclusion

As a cruciferous plant and a primary raw material for kimchi, mustard (*B. juncea*) is widely used as a food and spice. The dietary fiber, minerals, chlorophyll, vitamins, glucosinolates and their degradation products, volatile components, polyphenols, and phytochemicals found in mustard are just some of the phytochemicals it contains. Varieties, growth environments, extraction technologies, and food processing affect the content and type of food. As well as anti-cancer properties, mustard also has antioxidative properties, anti-inflammatory properties, antibacterial properties, antiviral properties, anti-obesity properties, antidepressant properties, diabetes treatment,

and cataract prevention and treatment. Currently, mustard seeds are fermented, fried, steamed, microwaved, and extracted from their oils. As mustard is primarily processed by fermentation, it does not have intensive processing or utilization, while the functional components of mustard do not get the best use. Most products do not have a high level of value-addition. The bioactive components of mustard include glucosinolates and polyphenols. The exact amount and structure of glucosinolates and polyphenols found in mustards are still unknown, despite extensive research on their qualitative determination. Based on the data, glucosinolates and polyphenols in mustards are not reported to change and degrade during processing. There have been many studies examining the anticancer and antioxidant effects of mustard extracts, but their exact mechanism of action and structure–activity relationship have not yet been determined. In addition to mastering the metabolic pathways of bioactive components, improving research into the degradation mechanism will ensure that bioactive components are retained during processing. Utilizing modern metabolomics to study and adjust specific components of plants to produce mustard plants that have stable genetic properties, are high in glucosinolates, polyphenols, and other beneficial chemicals. These products can be improved with novel technologies, and their applications can be expanded to include functional foods for health.

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Conflict of interest

The authors declare no conflict of interest.

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Chapter 5

Host Plant Resistance in Brassicaceae against Aphids

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Abstract

This chapter deals with *brassica* plants and their resistance to sucking pests—aphids. *Brassica* plants are known to synthesize a number of plant secondary metabolites which impart resistance to insect-pests and diseases. Aphids are known to feed primarily on sieve elements. The sieve elements in vascular bundles of angiosperms are important channels for nutrition. They are the channels of transport of photoassimilates from source to the sink. Because of the high nutrition content of the sap inside sieve elements, they are the target for many insect-pests and bacterial and fungal pathogens. Aphids are one such group of insects which target SE elements of phloem for nutrition. They are among the most important insect pests in agriculture particularly serious in temperate and sub-tropical climates. In addition to direct damage by feeding as well as toxic effects of saliva, the withdrawal of nutrients is detrimental to plant growth and development. In addition to this, aphids also cause indirect damage to plants by acting as vectors of plant pathogenic viruses. Furthermore, honeydew excreted by aphids provides suitable substrate for sooty molds that interfere with normal plant photosynthesis. In this chapter work on host plant resistance in *Brassica* plants against aphids has been reviewed.

Keywords: *Brassica*, host plant resistance, oilseed, phloem feeder, aphids

1. Introduction

Brassicaceae family is one of the earliest group of cultivated plants [1] which are a source of vegetables, oilseeds and condiments. Various biotic and abiotic stresses limit the production and productivity of these crops. Out of various insect-pests, aphids are important pests. Turnip aphid alone is known to cause 35.4 to 91.3% reduction in yield with the average yield losses of around 56.2% [2]. At present, systemic insecticides are used to manage aphid pests. Although these insecticides are very effective, but they have the associated problems like residue problem in oil and cake, environmental pollution and development of insecticide resistance. Past two decades have witnessed an increased interest in finding alternate solutions for aphid management. One such strategy is host plant resistance. It is an effective, economical and environment friendly option for pest management. The first step in development of insect resistant cultivar is the identification of source of resistance. In this chapter we have

attempted to review literature on screening of plants to find source of resistance and latest developments in host plant resistance in Brassicaceae against aphid pests.

2. Species complex of aphids on Brassicaceae plants

Members of the family Brassicaceae serve as suitable hosts to a number of aphid species. The main aphid species reported to infest *Brassica* plants are mealy cabbage aphid, *Brevicoryne brassicae* (L.); turnip/mustard aphid, *Lipaphis erysimi* (Kaltenbach)/*Lipaphis pseudobrassicae* (Davis); green peach aphid, *Myzus persicae* (Sulzer); shallot aphid, *Myzus ascalonicus* Doncaster; potato aphid, *Macrosiphum euphorbiae* (Thomas); corn root aphid, *Aphis maidiradicis* Forbes; and root feeding aphids, namely, bean root aphid, *Smynthurodes betae* Westwood and cabbage root aphid/poplar petiole gall aphid, *Pemphigus populitransversus* Riley [3]. Among these, three species viz. *B. brassicae*, *L. erysimi* and *M. persicae* cause serious damage to *Brassica* crops in one or other part of the world. *B. brassicae* is native to Europe with worldwide distribution. It is a serious pest of *Brassica* vegetables in most European countries and results in significant yield losses. It is a specialist pest of Brassicaceae that feeds on phloem sap of its host plants [4]. Although it is a primary pest of *Brassica* vegetables, it also feeds on other species in genus *Brassica* [4–7]. *L. erysimi*, the most important pest of oilseed *Brassica* in Indian subcontinent, is native to eastern Asia [3].

Unlike *B. brassicae* and *L. erysimi*, peach-potato aphid, *M. persicae* is a generalist pest and feeds on more than 400 plant species [8] including broccoli, cabbage, carrot, cauliflower, egg plant, lettuce, papaya, peach, peppers, sweet potato, tomato, etc. There are two views about its origin. Many workers believe it to be native of China-the native place of its host plant *Prunus persica*, while others believe it to have originated from Europe [9]. In addition to direct feeding damage, it is an efficient vector of 100 plant pathogenic viruses including potato virus Y, potato leaf roll virus and various mosaic viruses including western yellows [10, 11]. The pest is polyphagous and cosmopolitan in distribution. It possesses very high genotypic plasticity for color, life cycle, host plant relationships and mechanisms for insecticide resistance.

Initially there were doubts about the origin and identity of *Lipaphis pseudobrassicae*. Till 1914, it was confused with *B. brassicae* in North America. Davis [12] recognized it as a distinct species and named it *Aphis pseudobrassicae*. Later, it was transferred to the genus *Rhopalosiphum* [13] because of weakly clavate siphunculi and was referred to as *Rhopalosiphum pseudobrassicae* (Davis) till 1964. In 1932, Börner and Schilder [14] found that species *pseudobrassicae* should be placed in *Lipaphis*—a genus erected by Mordvilko [15] for a *Brassica* feeding aphid, *erysimi*. While attempting to discriminate *pseduobrassicae* from *erysimi*, Hille Ris Lambers [16] could not find any characters that can differentiate the two and stopped short of making it a synonym. However, other workers considered *pseudobrassicae* as a subspecies of *erysimi* [17, 18]. Despite this, *erysimi* continued to be used from 1975 onwards.

3. Aphid-plant interactions

Aphids are specialized phloem sap feeders which insert their needle like stylets in the plant tissue avoiding/counteracting the different plant defenses and withdraw large quantities of phloem sap while keeping the phloem cells alive. In contrast to

the insects with biting and chewing mouthparts which tear the host tissues, aphids penetrate their stylets between epidermal and parenchymal cells to finally reach sieve tubes with slight physical damage to the plants, which is hardly perceived by the host plant [19]. The aphid stylets play major role in host plant selection [20]. The long and flexible stylets mainly move intercellular in the cell wall apoplasm [21], although stylets also make intracellular punctures to probe the internal chemistry of a cell. The high pressure within sieve tubes helps in passive feeding [19]. During the stylet penetration and feeding, aphids produce two types of saliva. The first type is dense and proteinaceous (including phenoloxidases, peroxidases, pectinases, β-glucosidases) that forms an intercellular tunneled path around the stylet in the form of sheath [22]. In addition to proteins this gelling saliva also contains phospholipids, and conjugated carbohydrates [23–25]. This stylet sheath forms a physical barrier and protects the feeding site from plant's immune response. When the stylet come in contact with active flow of phloem sap, the feeding aphid releases digestive enzymes in the vascular tissue in the form of second type of 'watery' saliva. The injection of watery saliva (E1) prevents the coagulation of proteins in plant sieve tubes and during feeding the watery (E2) saliva gets mixed with the ingested sap which prevents clogging of proteins inside the capillary food canal in the insect stylets [19]. Though, the actual biochemical mode of action of inhibition of protein coagulation is unknown, the calcium binding proteins of aphid saliva are reported to interact with the calcium of plant tissues resulting in suppression of calcium-dependent occlusion of sieve tubes and subsequent delayed plant response [26, 27]. This mechanism of feeding is more specialized and precise which avoids different allelochemicals and indigestible compounds abundant in other plant tissues [28]. In addition to this, aphid saliva also contains non-enzymatic reducing compounds which in the presence of oxidizing enzymes inactivate different defense related compounds produced by plants after insect attack [24].

The early response of plants to feeding by insects or infection by pathogens share some common events such as protein phosphorylation, membrane depolarization, calcium influx and release of reactive oxygen species (ROS, such as hydrogen peroxide) [29], which leads to activation of phytohormone dependent pathways. In response to infestation/infection different phytohormone-dependent pathways are activated. The ethylene (ET) and jasmonate (JA) pathways are activated by different necrotrophic pathogens [30] and grazing insects [31], while salicylate (SA) dependent responses are activated by biotrophic pathogens [30]. These responses lead to production of various defense related proteins and secondary metabolites with antixenotic or antibiotic properties. In the case of infestation by aphids, a SA-dependent response appears to be activated, while the expression of JA-dependent genes is repressed [32–35]. All these responses lead to the manipulation of the plant metabolism to ensure compatible aphid-plant interactions.

4. Stages and extent of damage

Damage is caused by both nymphs and adults. Wing dimorphism leads to two different morphs—*alatae* (winged) and *apterae* (wingless). Apterae are small to medium sized pale to yellowish, gray or olive green with body covered with small waxy coating (not as waxy as *B. brassicae*). However, under cold and humid conditions this waxy covering becomes dense coat of white wax. Small to large colonies of *L. erysimi* suck plant sap from flower buds, flowers, siliquae, pods and underside of

leaves which leads to their curling, crinkling and yellowing. Continuous feeding and consequent resource restriction leads to drying of the plant part being fed upon.

Parthenogenetic viviparity limits the need for males to fertilize females and eliminates the egg stage from life cycle. Further, the development of an aphid begins even before its mother's birth—a phenomenon known as telescoping of generations. Thus, the generation time is considerably reduced to as low as 5–7 days under favorable conditions [36] leading to rapid increase in population growth. Under varying population levels, prevailing agro-climatic conditions and phenological stage of the crop damage by *L. erysimi* has been reported to vary from as low as 10 to as high as 90% [37–46]. In addition to direct feeding damage, *L. erysimi* is also vector of 10 non-persistent viruses including turnip mosaic virus, cauliflower mosaic virus and cabbage black ring spot virus [43, 47]. Like *B. brassicae*, it is also a *Brassica* specialist. *Brassica rapa* and *B. juncea* are generally better hosts compared to other *Brassica* species [43]. It is cosmopolitan in distribution and is found wherever *Brassica* plants are grown. Host range may include many species and genera of Brassicaceae, including *Brassica*, *Barbarea*, *Capsella*, *Erysimum*, *Iberis*, *Lepidium*, *Matthiola*, *Nasturtium*, *Raphanus*, *Rorippa*, *Sinapis*, *Sisymbrium* and *Thiaspi* [48, 49].

5. Bioecology and different control interventions

Many workers have attempted to study the bioecology of the pest in an effort to find weak links in pest's life cycle so that this information can be used in devising an effective pest management strategy. Though good information has been generated, but keeping a view the changed spectrum of mustard varieties over the time, cultural practices and the global environmental change, there are still many gaps in our knowledge. There is no egg or other resting stage in its life cycle and the mustard aphid is reported to survive on some wild crucifers and some vegetables during summer months [50, 51] particularly in submountaineous regions. On the other hand, in plain region of Rajasthan, Sachan and Srivastava [52] could not locate the pest from July to October on cabbage. Similarly, Lal [53] also stated that this pest is not traceable in plains of India during summer months. Thus, it was hypothesized that aphids migrate from hilly areas to plains of India to avoid extremely low temperatures in winter season. However, this 'Hills to Plain Hypothesis' failed to highlight the exact route of aphid migration and the exact source of aphid population. Recently, Ghosh et al. [54] have studied the migration behavior of *L. erysimi* over Indo-Gangatic plains through 24 h backward air-mass trajectory and found that mountainous regions of Kashmir are the source of aphid migration in North-Indian plains in winter season. Studies on aphid migration and their development on host plants help in developing effective forewarning and forecasting models which have implications in precise timing of control interventions. Generally, the *alatae* of *L. erysimi* start appearing on the crop in October when the crop is still young. They generally remain at low levels in winter season and start increasing from December till mid March in different regions of the country (**Table 1**) after which a decline in population is observed due to maturity of the crop and rise in temperature [52, 65, 66]. Though a number of natural enemies are reported to be associated with *L. erysimi* (*Diaeretiella rapae*, coccinellids, chrysopids and syrphids) which increase in abundance with the warming of temperature after winter season, but they are generally ineffective in suppressing the aphid population. There is a lack of phenological synchrony between their peak populations since natural enemies are active late in the season when most of the damage by aphids has already occurred [67, 68].

State	Period of peak activity	Crop	Reference(s)
Rajasthan	End January	*B. juncea*	[55]
Punjab	Mid February	*B. juncea*	[56]
	Jan–Feb	*B. campestris*	
	Jan–Mar	*B. juncea*	
	Mar	*Brassica napus*	
		B. carinata	[57]
Haryana	Jan–Feb	*Brassicas*	[58, 59]
Delhi	Feb	*Brassica rapa*	[60]
Bihar	Jan–Feb	Rape/mustard	[61]
Orissa	January	Rape/mustard	[62]
Uttar Pradesh	January	*B. juncea*	[63]

Source: Bakhetia et al. [64].

Table 1.
Period of peak activity of Lipaphis erysimi *as influenced by different types of cruciferous plants.*

Cold and cloudy conditions are generally favorable for the development of mustard aphid [69], while extreme weather events like sub-zero temperature, fog, frost, rains and thunderstorms and very high temperature are the leading abiotic mortality factors. Mean maximum temperature of 17–18°C favors rapid population multiplication [58] while very low temperature during December and high temperature after March have detrimental effect on its multiplication. Hsiao [70] reported that *L. erysimi* manifests maximum intrinsic rate of increase, higher net reproductive rate and longer mean generation time at 25°C compared to other range of temperatures tested. In Nagpur, India, Kulat et al. [71] reported that a combination of maximum and minimum temperature in the range of 26.4–29.0°C and 8.4–12.6°C along with relative humidity of 75–85% in January resulted in conditions favorable for *L. erysimi* population development. On the other hand, a declining trend in population was observed at relative humidity ≤65%.

5.1 Cultural management

5.1.1 Sowing time

Time of sowing has a significant influence on the damage caused by aphids on oilseed *Brassica*. In India, *L. erysimi* generally causes maximum damage at flowering stage of the crop [72] which spans from end December/first fortnight of January to mid-February in different parts of the country. Thus, alteration in sowing date can help crop escape from the damage caused by *L. erysimi* as it leads to phenological asynchrony between the most susceptible crop stage and peak period of aphid activity. Phenological asynchrony can be achieved either through breeding by incorporating genes for earliness or alterations in sowing time. It has been observed that crop sown early (before October 20) escapes damage by *L. erysimi* in India [73–85]. However, care should be taken to avoid sowing too early especially in dry regions such as Rajasthan as it can result in more damage by painted bug [86]. On the other hand, crop sown late suffers heavy damage by *L. erysimi* [41, 42, 79, 87–90].

5.1.2 Fertilizer application

Optimum nutrient application is an essential and often ignored component in both integrated pest as well as disease management. Excessive use of nitrogenous fertilizers can make plants more succulent [91] and susceptible to insect attack [92]. *L. erysimi* population was 4–8 times more in mustard crop that received 40 and 60 kg ha^{-1} N as compared to that in the crop which received no fertilizer application [93, 94]. On the other hand, Bakhetia and Sharma [95] reported that increase in nitrogen application upto 80 kg ha^{-1} had no effect on *L. erysimi* population development, however a negative correlation was observed between sulfur application and *L. erysimi* population development [96]. Similarly, increase in K application adversely affected reproduction and honey dew excretion of *L. erysimi* [97]. Increased application of P and K reduced aphid incidence on mustard plants [98].

5.1.3 Irrigation

In an agroecosystem, plants encounter multiple stresses that can influence their physiology and chemical composition including plant secondary metabolites. Drought/water stress not only influences plant physiology leading to decreased growth, but also leads to changes in profile of secondary metabolites and allocation of resources [99–102]. Water stressed mustard plants were reported to support lower population of *Brassica* specialist *L. erysimi* [103, 104], while opposite trend was observed for generalist aphid *M. persicae* [100]. Similarly, Mewis et al. [102] reported rapid growth of *M. persicae* on water stressed *Arabidopsis thaliana* plants while *B. brassicae* performed equally well both on water stressed and well watered plants. However, heavy infestation of *B. brassicae* on water stressed plants of *Brassica napus* compared to unstressed plants was reported by some workers [105, 106]. This may partly be due to increase in concentration of amino acids in phloem sap [107] which makes it more nutritious. Miles et al. [108] reported increase in concentration of amino acids in water stressed rape plants leading to enhanced development of *B. brassicae*.

Besides changes in primary metabolites, water stress also leads to changes in plant secondary metabolites. Variations in glucosinolates may be in part responsible for observed variation in insect performance. Previous studies have reported decrease in glucosinolate levels in water stressed plants [100, 101]. Unlike generalist aphids, specialist aphids may tolerate glucosinolates in their host plants. However, there is general lack of complete understanding w.r.t. to effect of drought stress on secondary metabolite accumulation in relation to impact on plant resistance against aphids with different feeding specializations. Mewis et al. [102] reported a general trend of increase in levels of sucrose, several amino acids such as glutamic acid, proline, isoleucine and lysine while decrease in the levels of 4-methoxyindol-3-yl methyl glucosinolate was observed in water stressed plants. On the other hand, Chadda and Arora [107] observed a reduction in amino acids concentration in water stressed plants which in turn resulted in amino acid imbalance in aphid excretion resulting in reduced fecundity.

Bakhetia and Brar [109] reported a heavy aphid infestation on mustard grown under rainfed conditions with very high damage while irrigated crop maintained a good crop stand despite high aphid pressure partly due to differences in plant vigor.

5.2 Biological control

The term biological control covers a broad range of macro and microorganisms (e.g. parastitoids, predators, bacteria, virus, fungi, etc.), botanical extracts, semiochemicals and secondary metabolites from living organisms. The entomopathogenic fungus *Verticillium lecanii* has been found promising against *L. erysimi* [110, 111]. In a 2 years field study, spray of *V. lecanii* @ 10^8 cs/ml followed by spray of neem seed kernel extract (5%) resulted in 60% reduction in *L. erysimi* population on Indian mustard as against 49% increase in aphid population in untreated control [110]. Field efficacy of neem seed kernel extract (5%) and neem leaf extract (5%) against *L. erysimi* has also been reported by other workers [112]. Many plant based materials have been evaluated against *L. erysimi* including neem/azadirachtin, nicotine sulphate, rotenone and pyrethrins. Extracts from common plants such as *Azadirachta indica*, *Lantana camara*, *Melia azedarach*, *Solanum xanthocarpum* exhibited variable toxicity against *L. erysimi* [113]. Tetrahydroazadirachtin-A, a thermo and photostable derivative of azadirachtin provided superior control of mustard aphid on *B. juncea* compared to azadirachtin [114]. Despite variable efficacy of botanicals, there is much needed to be done for their commercial exploitation. For example, the application rates of neem seed formulation (0.5–2.0 kg/ha) or fresh leaves (10–20 kg/ha) are too high to be acceptable by growers [115]. In developing countries, the use of biopesticides in pest management is low due to a number of factors such as low efficacy, speed of action, limited spectrum of activity, availability and affordability [116] and there is a need to create awareness among farmers about the ill effects associated with the use of chemical insecticides.

Aphid natural enemies can also be used for its management under field conditions. Like other agricultural systems, *Brassica* agroecosystem is also prone to pest outbreaks compared to natural ecosystems primarily due to loss of biodiversity [117]. However, Hawkins et al. [118] stated that one or two particularly effective natural enemies are all that are needed for effective pest control. Bakhetia and Sekhon [38] reported six Coccinellid species, 16 Syrphids, one chamaemyiid, hemerobiid (predators), four species each of parasitoids and entomopathogenic fungi and one predatory bird to be associated with *L. erysimi* as its natural enemies in India. Coccinellids are the predominant predators of this aphid species, among which *Coccinella septempunctata*, *C. repanda*, *transversalis*, *Brumoides suturalis*, *Menochilus sexmaculatus* and *Hippodamia variegata* are abundant in *Brassica* agroecosystem. Kumar [67, 119] observed that these natural enemies generally become active very late in the season when most of the damage by aphids has already occurred. Thus, despite their abundance these natural enemies fail to provide satisfactory control of mustard aphid due to phenological asynchrony between the peak activity period of *L. erysimi* and its natural enemies [67, 68]. Limited efficacy of *C. septempunctata* @ 5000 beetles ha^{-1} and *V. lecanii* @ 10^8 conidial spores ml^{-1} against *L. erysimi* was reported on Indian mustard upto 10 days after release [111]. Although a number of syrphids are known to predate on mustard aphid, but they are generally very low in number to provide effective population suppression. The common associated syrphids are *Episyrphus balteatus*, *Sophaerophoria scutellaris*, *Metasyrphus adligatus*, *M. corollae*, *Eristalis obscuritarsis*, *E. tenax*, *Xanthogramma scutellaris*, *Syrphus serarius* and *S. issaci*. Similarly, the green lacewings (*Chrysoperla carnea* and *Chrysopa scaslastes*) have limited scope for use in population suppression of insect-pests.

In addition to predators, small aphid parasitoids, *Diaeretiella rapae* and *Encyrtus* sp. are also associated with mustard aphid. Just like predators, the parasitoids also

appear late in the season (around mid February). Atwal et al. [120] reported that *D. rapae* causes more than 70% aphid parasitization in Punjab, India. However, recently Kumar [119] reported only 15.6% aphid parasitization on *B. rapa*. Biological control has the potential to offer sustainable solution for pest problems in agriculture [121] but its success rate is very low compared to chemical control [122]. With globalization and intensification of agriculture, the pest problems will increase resulting in increased use of pesticides [123]. For sustainable pest management, the expectations from biological control agents will increase.

5.3 Chemical control

At present, there is no stable resistant cultivar available against aphid pests in rapeseed-mustard. Thus, in their absence, insecticides are and will continue to be the major component of any pest management programme. In a developing country like India, farmers use them as the primary method of pest management as they find it easily available, economical and effective method of pest management. However, in an ideal pest management programme, insecticides should be used as the last option when all other alternative methods fail to provide satisfactory control since there are many problems associated with their use including environmental pollution, insecticide resistance and resurgence and pesticide residues in oil and cake. In India, pesticides are extensively used in rapeseed-mustard, but their application is mostly erratic. The fields requiring pesticide application are left unsprayed while other fields are sprayed indiscriminately and unnecessarily [124]. Even in a developed country like UK, the indiscriminate use of insecticides of vegetable *brassicas* was common due to lack of economic thresholds for many pests in the 90s [125]. The introduction of neonicotenoids played a significant role in pest management, but these were not introduced on a large scale in *brassica* crops unlike other crops [126]. In European Union, the ban on use of neonicotenoid insecticides as seed treatment on crops that attract pollinators, implemented in December 2013, adversely affected the pest control in oilseed rape [127, 128]. There were serious crop losses in 2014, 2015 and 2016 due to flea beetle, *Psylliodes chrysocephala* and peach-potato aphid, *M. persicae* which were already resistant to the alternative pyrethroid insecticides [129]. However, Blacquière and van der Steen [127] argue that decline of honey bees and wild pollinators is not likely caused by use of neonicotenoids and suggested for comprehensive studies on interactions with non-pesticide stressors. In India, neonicotinods are still used for pest management. Single application of imidacloprid provided 99% *L. pseudobrassicae* control [130]. Although, very high level of aphid control is obtained by use of synthetic insecticides, but high fecundity and short generation time of aphids leads to rapid population growth to levels similar to those in untreated fields within just 2–3 weekds [131]. To avoid indiscriminate use and prophylactic application of insecticides, the pesticide application decisions should be based on action (economic) threshold. But economic threshold levels are available for only a few major insects and there is need to calculate the same for other pests as well.

5.4 Integrated pest management

The sustainable solution to pest problems revolves around amalgamation of all the available and viable pest management strategies. However, in developing

countries including India farmers are largely dependent on the use of synthetic chemicals due to their easily availability and quick results. The well known example of control failures of diamondback moth, *Plutella xylostella* was attributed to widespread and indiscriminate use of insecticides. This not only disturbed the natural control by parasitoids and predators in *Brassica* ecosystem, but also exerted high selection pressure on insect population leading to development of insecticide resistance to almost all groups of insecticides [132, 133]. In northern India, early sowing of rapeseed-mustard in October is advocated to create phenological asynchrony between the peak activity period of *L. erysimi* and flowering stage of the crop. However, growers in most parts of the North-Western India particularly Punjab and Haryana are unable to sow their crop in October due to late harvesting of rice crop in the preceding season. Action thresholds for control decisions are available, but control interventions are rarely made based on these thresholds. In the developing countries including India, there is a functional extension system to educate growers about importance of IPM and ill effects of insecticides, but farmers do not follow the advice of extension personnel. They follow the recommendations only if they are made into law [49].

Even in the developed country like UK, guidelines to manage aphids and insecticide resistance management have been published [134, 135], but insecticides are not selected on the basis of being less harmful to aphid natural enemies [136, 137]. A well established pest monitoring and forecasting system also exists in UK. In contrary to developing countries, it is supposed that growers in UK will follow the advice of extension functionaries—but this is not true. Most of the *Brassica* growers do not follow the recommendations of extension functionaries and go for prophylactic application of insecticides [136].

Though, aphid natural enemies are active in *Brassica* ecosystem but their peak activity lags behind the peak aphid activity [67] and they fail to provide effective aphid control. Rapeseed-mustard cultivars with less susceptibility/tolerance to aphids are available, but the resistance levels are still not high enough to induce growers to use them as a sole control measure. At present, semiochemicals are not applied to disrupt aphid pests or to attract their natural enemies in the agroecosystem. Thus, there are very limited control options available that can be made component of integrated pest management module. A resistant cultivar can serve as the core component of IPM module which will not only reduce reliance on insecticides but will also reduce pest management cost in addition to reduction in environment pollution and pesticide residues in oil and cake.

6. Traditional approaches in breeding for aphid resistance

Brassica plants are among the oldest cultivated plants with documented records dating back to ca. 1500 BC [138]. Their domestication over the years has lead to narrowing of genetic base. The breeding efforts were focussed on high yield and quality traits (low glucosinolates and erucic acid). Thus, little/no attention was paid to maintain insect and/or disease resistance. Thus, over the course, the defense related genes in ancestral *Brassica* plants were lost. However, in the recent times, there is renewed interest in remobilizing these defense related genes. All this requires, rigorous screening of large *Brassica* germplasm for insect resistance and an efficient screening technique is the very prerequisite for this.

6.1 Screening methodology

Screening for source of resistance is the first step in development of an insect resistant cultivar. A very large number of attempts have been made in the past to identify sources of resistance in primary gene pool of crop *brassica* species [139–143]. The literature on the screening techniques for aphid resistance has been reviewed extensively by Bakhetia and Sekhon [38]. The different techniques used for screening are discussed in the following text.

6.1.1 Seedling stage screening

Screening at seedling stage is more desirable than field screening at adult plant stage because of the cost and efforts involved. However, resistance at seedling stage may not express at adult plant stage and no serious effort has been made to correlate seedling stage resistance with the adult plant resistance. Earlier, Bakhetia and Bindra [144] had attempted to develop seedling stage screening method against *L. erysimi* which is compatible with adult plant evaluation. It is based on seedling mortality at a defined aphid population level. For efficient resistance screening, population levels of 11, 20, 20, 30 *apterae* and, 1 and 3 ml aphids (1 ml = ~600 numphs + *apterae*) per plant are optimum for resistance screening at cotyledonary, 2-leaf, 4-leaf, flower bud initiation and flowering stages, respectively [143]. Despite the advantages of seedling stage screening, this method is not widely used for screening of *Brassica* germplasm against aphids.

6.1.2 Adult plant stage screening

Contrary to the seedling stage screening, this is the most widely used method in screening for aphid resistance in *Brassica* germplasm, since it reflects the actual resistance exhibited by plants under field conditions. Though, it is laborious and time consuming method, but it does not undermine its usefulness. It is based on the injury symptoms exhibited by aphid feeding which range from yellowing, curling and crinkling of leaves to drying of floral buds, flowers and shriveling of pods. Different grading systems have been adopted by different workers, but the one suggested by Bakhetia and Sandhu [145] is the most practical and widely accepted for mustard aphid screening.

Based on the aphid injury level, different injury grades for field screening are given to the plants as follows:

Aphid infestation index (AII)	**Description**
0	Free from aphid infestation. Even if a single wingless aphid is present, the plant is considered infested. Plants showing excellent growth.
1	Normal growth, no curling or yellowing of the leaves, except only a few aphids along with little or no symptoms of injury. Good flowering or pod setting on almost all the branches.
2	Average growth, curling and yellowing of a few leaves. Average flowering and pod setting on all the branches.
3	Growth below average, curling and yellowing of the leaves on some branches. Plants showing some stunting, poor flowering and little pod setting.
4	Very poor growth, heavy curling and the yellowing of leaves, stunting of plants, little or no flowering and only a few pods forming. Heavy aphid colonies on plants.
5	Heavy stunting of plants; curling, crinkling and yellowing of almost all the leaves. No flowering and pod formation. Plants full of aphids.

Bakhetia and Sandhu [145].

Based on the degree of damage, an injury grade is given to every observed plant. The Aphid Infestation Index (AII) is calculated by multiplying the number of plants falling under each injury grade with their respective grade number. AII is calculated at pre-flowering, flowering and pod formation stages as:

$$\text{Aphid Infestation Index} = \frac{(0\times a)+(1\times b)+(2\times c)+(3\times d)+(4\times e)+(5\times f)}{a+b+c+d+e+f}$$

where a, b, c, d, e, and f are the number of plants falling under each injury grade.

The different test entries are classified into different resistance categories based on the AII as:

Aphid infestation index (AII)	**Reaction**
0.00–1.50	Resistant
1.51–2.50	Moderately resistant
2.51–3.50	Susceptible
>3.50	Highly susceptible

Higher the AII, lower is the level of resistance in an entry.

6.1.3 Other screening methods

Recently, Dhillon et al. [146] evaluated twig cage, whole plant cage, plot cage and uncaged plants methods to look for efficient screening method against *L. erysimi*. They concluded that no-choice twig cage method is the most appropriate of all for field screening of *Brassica* genotypes. However, there were many flaws in their methodology followed. The authors infested the test plants artificially with pieces of infested *Brassica* twigs pinned to the plant. Pinning of host plants inflicts mechanical injury which activates the myrosinase-glucosinolate defense system in *Brassica* plants which in turn interferes with expression of natural resistance. Further, twig cage alters the microclimate leading to physiological and nutritional deviation from naturally grown plants. Generally, caged twigs/plants exhibit abnormal growth which may interfere with their natural expression of resistance. Furthermore, authors have worked out both Aphid Population Index (API) and Aphid Damage Index (ADI) each on 0–5 scale. Aphid Resistance Index (ARI) is worked out after taking mean of the two. While, ADI is based on the degree of damage done to host plants, API is based on the aphid population—higher the pest numbers, more the API will be. Some plants may harbor high aphid population without exhibiting significant damage and vice versa. Thus, inclusion of API in calculations of Aphid Resistance Index does not represent the true resistance exhibited by host plant. While, Bakhetia and Sandhu [145] recorded both aphid population and injury grade at same point of time (though they have not used population data for calculation of Aphid Infestation Index), Dhillon et al. [146] recorded API after 21 days of artificial infestation and ADI at completion of pod formation, which puts another question mark on the methodology followed.

In addition to this, aphid population at a particular stage and an increase in population during a given time interval can also be used in germplasm screening [38]. Kumar et al. [147] attempted to screen a diverse array of wild crucifers based both on the adult plant resistance and effect on aphid demographic parameters (survival, development and fecundity) and reported one wild *Brassica fruticulosa* to be resistant to *L. erysimi*. Only limited attempts have been made to develop screening technique based on aphid biology, despite its significance in identifying sources of resistance. It is possible to develop such a criterion for aphid screening since nymphal survival, fecundity, longevity and reproduction are similar at all the plant growth stages [144]. Singh et al. [148] and Malik [149] have also reported fecundity to be inversely related to resistance.

6.2 Conventional breeding

The three modalities of resistance include antixenosis, antibiosis and tolerance. Although, antixenosis does not exert any selection pressure on insect population and there is no risk of biotype development, it is rarely effective under no choice conditions as insects can learn to feed on less preferred host plant. In contrast, antibiosis exerts high selection pressure on the insect population leading to high risk of biotype development, a danger not applicable to tolerance. Insect population can be allowed to feed on the crop and growers would not need to control them, but they would breed population to infest their neighbors' crops. Thus, an ideal resistance is a combination of all the three mechanisms [150].

Earlier workers have attempted to develop resistant cultivars using different breeding methods viz. intervarietal hybridization, induced mutagenesis or autotetraploidy. *B. napus* strains and colchicine induced tetraploid toria (*B. rapa*) were found to be resistant to mustard aphid as compared to diploids with antibiosis mechanism of resistance [148, 151–154]. However, these were cytogenetically unstable. Many workers have attempted to artificially synthesize *B. napus* and *B. rapa* x *Eruca sativa* alloploids [155] but these were not resistant.

In an attempt to develop aphid resistant cabbage variety, Lammerink [156] made selections from F_3 generation of the cross (Broad Leaf Essex rape x Colder Swede) x giant rape. In addition to this, he also made recurrent selection in the crosses involving purple top white globe and Sjodin turnip. Kumar et al. [147] screened a diverse array of wild crucifers and found one wild *B. fruticulosa* to be resistant to *L. erysimi*. They further attempted to introgress the resistance gene to *B. juncea* background. In addition to *L. erysimi*, *B. fruticulosa* has been earlier reported to be resistant to mealy cabbage aphid, *B. brassicae* [5, 6, 157, 158]. It was reported to possess antixenosis and antibiosis mechanisms of resistance against *L. erysimi* along with the *B. juncea-fruticulosa* introgression lines [159]. Further, monitoring of feeding behavior of *B. brassicae* by electrical penetration graph (EPG) revealed large reduction in duration of passive phloem uptake in *B. fruticulosa* compared to *Brassica oleracea* var. *capitata* cv. 'Offenham Compacta'. Aphids either showed quick withdrawl of stylets from sieve tubes or there was disrupted phloem uptake [5]. The mechanism of resistance was a combination of both antixenosis and antibiosis [157]. In addition to resistance against aphid pests, *B. fruticulosa* has also been reported to possess resistance (antibiosis) against *Delia radicum* [160].

In addition, efforts have also been made to induce mutation in *B. juncea* for resistance against aphid pests both by chemical [161] and physical mutagens [162, 163] but

no significant results were obtained. Recently, Agrawal et al. [164] have attempted to use γ-irradiation on a set of introgression lines to optimize the introgressed segment.

6.3 Use of transgenic technology

Transgenic technology has emerged as an alternative breeding strategy to conventional breeding. Different strategies such as expression of protease inhibitors, RNA interference (RNAi), antimicrobial peptides and repellents can be employed for sap sucking insects such as aphids. Since aphids are phloem feeders, thus, phloem specific promoters can be used for expression of defense related compounds against them. This would lead to target specific expression of defense compounds with little/no effect on non-target insects. Further, it will also limit the GM-associated resource investment by plants to the plant tissues that are not attacked by the insect. The *SUC2* promoter that regulates the *AtSUC2* sucrose-H^+ symporter gene is restricted to plant phloem which produces aphid toxic proteins. This protein is transferred through sieve tubes to actual aphid feeding site [165].

Likewise Protease Inhibitors (PIs) can also be targeted to confer resistance in transgenic plants to insects, which inhibit/reduce the activity of enzymes involved in protein digestion (proteases). Toxic effects of PIs on insect-pests have been well demonstrated particularly those from order Coleoptera, Lepidoptera and Orthoptera [166]. In aphids, PIs ingested along with plant sap inhibit protein digestion in insect gut leading to disruption in amino acid assimilation subsequently leading to adverse effect on insect growth and its ability to cause plant damage. Successful attempts have been made to express PIs such as trypsin inhibitors and chymotrypsin inhibitors in phloem of transgenic plant [167, 168]. Barley cystein proteinase inhibitor, HvCPI-6 is reported to inhibit performance of *M. persicae* and *Acyrthosiphon pisum* in artificial diet [169] while, cysteine protease inhibitor, oryzacystatin I (OC I) inhibited growth of *M. persicae*, *A. gossypii* and *A. pisum* [170]. *M. persicae* fed on transgenic *B. napus* plants expressing (OC I) suffered reduction in adult weight, biomass and fecundity in comparison to those fed on control plants. Thus, protease inhibitors have a good potential to be used as an effective strategy to confer aphid resistance in plants.

In addition to PIs, lectins also exhibit high toxicity against sap sucking insects including aphids. Lectins are proteins that selectively bind to carbohydrates and carbohydrate moieties of glycoproteins leading to poisonous effect on the insect. The poisonous effects of lectins have been demonstrated on a number of insects, especially the sap sucking insects [171, 172]. A number of genes coding for different lectins have been introduced in *B. juncea* that confer resistance against *L. erysimi* such as wheat germ agglutinin from *Triticum* spp. [173], agglutinin ACA from *Allium cepa* [174], fusion lectin ASAL from *Allium sativum* and ACA from *A. cepa* [174]. Laboratory bioassays have confirmed significant toxic effect of these transgenic plants on *L. erysimi*.

RNAi is gaining increased attention as another potential strategy to confer resistance against insects. It involves suppression of genes at the level of RNA (posttranslational RNA-mediated gene silencing). Transgenic plants that delivered dsRNA to *M. persicae* resulted in inhibition of Rack1 protein located in gut and C002 protein located in the salivary glands of the aphid [175]. The transformed tobacco and *A. thaliana* plants resulted in adverse effect on aphid fecundity with upto 60% silencing in aphids that fed on these plants. Although salivary and gut proteins are

the promising targets for sucking insects including aphids, the other targets can be transporters in the bacteriocyte plasma membrane required for transport of nutrients between aphids and symbionts, *Buchnera aphidicola*.

7. Induced resistance

Plants are known to increase the level of many defense related compounds post insect infestation. This induction of resistance after insect feeding has also been reported in *Brassica* plants. During infestation of plants by insects, major defense related plant hormones are salicylic acid (SA), jasmonic acid (JA), ethylene (ET) and abscisic acid (ABA) which are involved in induced resistance of many plants against insects [176]. Aphid feeding is known to activate SA signaling pathway in a number of host plant species [35, 177, 178]. However, no involvement of SA signaling against aphids was reported in Arabiopsis [179]. Kettles et al. [180] reported an increase in *M. persicae* population in ET-insensitive *Arabidopsis* mutant *ein2*, indicating the role of ET in conferring resistance to aphids. Kerchev et al. [181] reported that resistance of *Arabidopsis* to aphids also depends upon ABA biosynthesis and signaling. Recently, Palial et al. [182] have reported in induction in glucosinolates content in *B. fruticulosa* and total phenols content in *B. juncea-fruticulosa* introgression lines after *L. erysimi* feeding.

8. Conclusion

The continuous coevolutionary history of aphids and members of family Brassicaceae have enabled these plants to evolve an array of defense related genes. However, plant breeding efforts have largely focused on selection for yield related and quality traits such as low glucosinolates and erucic acid traits with little attention to retain the adequate levels of insect and disease resistance. This lead to the loss of defense related genes in these crops over the time. Further, availability of chemical control measures at that time downgraded the importance of host plant resistance since chemical control was thought to be satisfactory and invulnerable. However, later it was realized that though insecticides can provide a short term pest control and host plant resistance can provide effective, economical and environment friendly pest management option. Thus, early plant breeders focused on host plant resistance as a single component of pest management and laid more emphasis on screening for virtual immunity to aphids. Immunity/high level of resistance can result from very high level of toxic substance (toxic to aphids) in host plant which can exert high selection pressure on aphid population leading to the development new biotypes, possible side effects on non-target organisms including honeybees and yield drag. Thus, partial resistance has potential role in sustainable pest management as varieties with partial resistance can be integrated with other pest management methods. At present, there is no effective IPM strategy against aphids due to lack of aphid resistant variety. Although, various workers have developed *Brassica* transgenics that offer some degree of resistance against aphids, but they have primarily evaluated under laboratory settings and field testing of such transgenics is still awaited.

To maintain sustainability of pest control and production systems, IPM should be seen as the best approach and host plant resistance can serve as core component of any IPM module. Rather than complete resistance, it is partial resistance that has greater potential to maintain such sustainability.

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Chapter 6

Perspective Chapter: Capitalizing on the Host Suitability of *Brassica* Biofumigant Crops to Root-Knot Nematodes (*Meloidogyne* spp.) in Agroecosystems – A Review on the Factors Affecting Biofumigation

Philip Waisen and Koon-Hui Wang

Abstract

The use of *brassica* biofumigant crops for the management of plant-parasitic nematodes in agroecosystems has been extensively studied. However, the effects of biofumigation against root-knot nematodes (*Meloidogyne* spp.) remain inconsistent, owing to the factors including but not limited to biofumigant crops, edaphic factors, termination methods, cultural practices, and sensitivity of *Meloidogyne* life stages to biofumigation. This review chapter argues that 'host suitability' or the susceptibility of biofumigant *brassica* crops, which is often considered an important management challenge, could in actuality maximize the performance of biofumigation against *Meloidogyne*. Each of these factors has been reviewed with an emphasis on the host's suitability as an opportunity to capitalize on to maximize the biofumigation effect. This can be achieved by synchronizing the termination time in relation to the nematode development and *Meloidogyne* degree-days. The logic is that the cultivation of susceptible biofumigant crops would stimulate *Meloidogyne* egg hatch and the resulting infective juveniles would be at the most vulnerable stage to biofumigation kill. From a plethora of published research and a myriad of information available on biofumigation, and integration with host suitability, it trickled down to six steps as necessary to maximize biofumigation effects to successfully manage *Meloidogyne* spp. in agroecosystems.

Keywords: cover crops, glucosinolates, isothiocyanates, management, susceptibility

1. Introduction

1.1 Root-knot nematode

More than 4100 species of plant-parasitic nematodes are known worldwide, collectively posing an important threat to global food security [1]. Globally, crop losses

inflicted by plant-parasitic nematodes are estimated at $125 billion annually, with at least $10 billion in the United States [1, 2]. Those nematodes in the genus *Meloidogyne*, the root-knot nematodes, are ranked among the most serious plant-parasitic nematodes estimated based on their economic and scientific significance [3]. To date, 98 species of *Meloidogyne* have been described including the major species—*M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* [4]. Root-knot nematodes are sedentary endoparasites and obligatory biotrophs, infecting a wide range of crops [5]. Second-stage juveniles (J2s) are infective, thus mobile and actively seek hosts (**Figure 1**). In doing so, the J2s are attracted to growing root tips by exudates, enter roots intercellularly behind the root cap, and migrate to the cell elongation region, where they initiate feeding sites by secreting effector proteins synthesized in esophageal glands [6, 7]. The effector proteins hijack routine cellular functions and expedite nuclear division but without cell division (cytokinesis). These events lead to the formation of feeding sites, the multinucleated and hypertrophied giant cells, which are active metabolic sinks diverting photosynthates away from storage organs [8]. The infection of the root system by root-knot nematodes results in characteristic gall formation. Root galling interferes with water and nutrient uptake, resulting in water stress, nutritional deficiency, and stunting of infected plants. The infected plants are predisposed to opportunistic soil-borne pathogens that can exacerbate the severity of the disease.

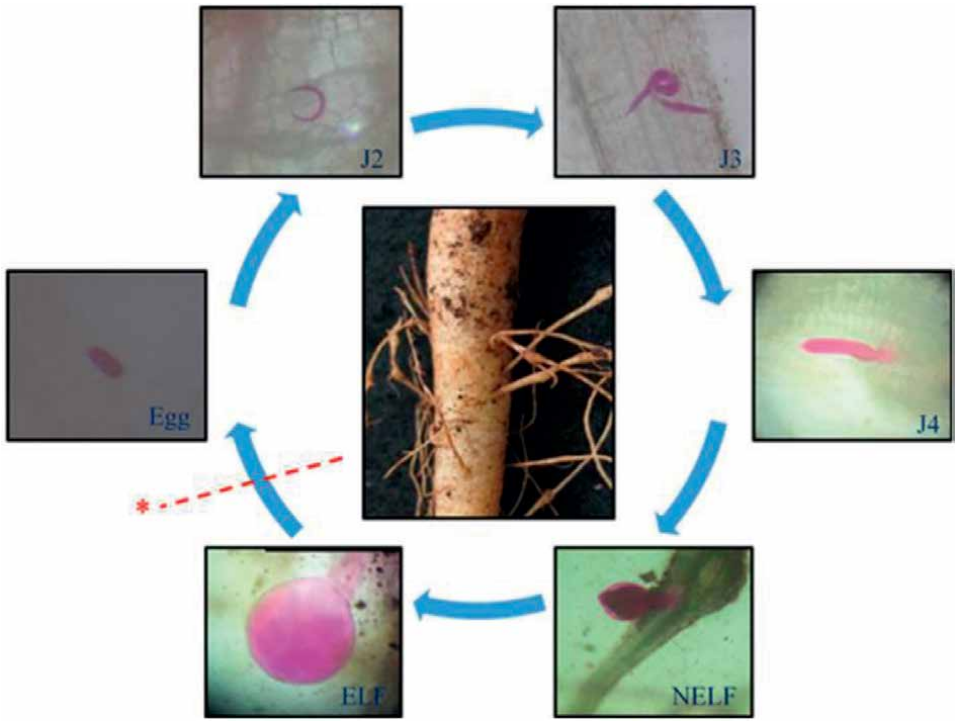

Figure 1.
The life cycle of a root-knot nematode. J2 = second-stage infective juvenile; J3 = third stage juvenile; J4 = fourth stage juvenile; NELF = non-egg laying female or mature female; ELF = egg-laying female. Red perforated line and asterisks indicate when biofumigant crops can be terminated to stop egg production.

1.2 Management

Management of root-knot nematodes relies primarily on the use of synthetic nematicides. Since the onset of the Green Revolution, soil fumigation has been an effective and non-discriminant approach to combat soil-borne pests and pathogens, including plant-parasitic nematodes, in agroecosystems. However, fumigants such as methyl bromide have been banned and the use of other effective nematicides is being restricted as with restricted-use pesticides such as Vapam (metam sodium) and Telone (1,3-dichloropropene) [9]. The banning and restricted use of effective nematicides have led to a worldwide search for nematicide alternatives.

Cover crops with allelopathic compounds offer an alternative to managing plant-parasitic nematodes in a user-friendly and environmentally sound manner. Some examples of allelopathic compounds being investigated include monocrotaline in sunn hemp, *Crotalaria juncea* [10], α-tertienyl in French marigold, *Tagetes* spp. [11], dhurrin in sorghum-sudangrass, *Sorghum* × *drummondii* [12], L-dopa in velvet bean, *Mucuna pruriens* [13], and glucosinolates in members of Brassicaceae [14–18].

This review focuses on the factors affecting the effectiveness of biofumigation against root-knot nematodes, highlighting host suitability as an opportunity to maximize biofumigation effect in agroecosystems.

2. Biofumigation

Biofumigation is a collective term used for all plant-derived volatiles utilized in pest and disease management. The term biofumigation was originally coined by Kirkegaard et al. [19] to refer to the use of plant-derived volatiles exclusively by the members of Brassicaceae for pest and disease management in agroecosystems. In particular, glucosinolates (GLs), β-d-thioglucose thioglycosides, are the naturally occurring secondary metabolites synthesized by members of Brassicaceae, and are stored in vacuole of sulfur-rich S-cells (**Figure 2**). The GLs are spatially separated from myrosinase (Myr) enzymes, β-thioglucosidases, which are stored as myrosin grains in the vacuole of a particular idioblast known as myrosin cell (**Figure 2**) [20–22]. To date, at least 200 GLs have been identified from plants, of which more than 80% occur in members of Brassicaceae [22–25]. Each GL constitutes a β-thioglucose moiety ($C_6H_{12}O_6S$), a sulfonated oxime moiety, and a thiohydroximate-O-sulfonate moiety (**Figure 3**) [26]. Glucosinolates are categorized as aliphatic, aromatic or indole, if the amino acid side chain denoted as R, is methionine, phenylalanine, or tryptophan, respectively (**Figure 3**) [27]. Upon tissue damage during termination or by herbivory, Myr comes in contact with GL and hydrolyzes the thioglucoside linkage (carbon-sulfur bond), yielding *D*-glucose and an aglycone, thiohydroxymate-O-sulfonate, an unstable intermediate (**Figure 3**). Being unstable, the aglycone spontaneously undergoes a non-enzymatic rearrangement to form volatile products including isothiocyanates (ITCs), nitriles, and thiocyanates as well as non-volatile products including sulfate and sulfur [26, 28]. Isothiocyanates have biocidal properties [29] like the synthetic counterpart, methyl ITC from metam sodium in Vapam and Dazomet [30]. Thus, a successful biofumigation partly depends on cultural and termination practices that favor more ITC production.

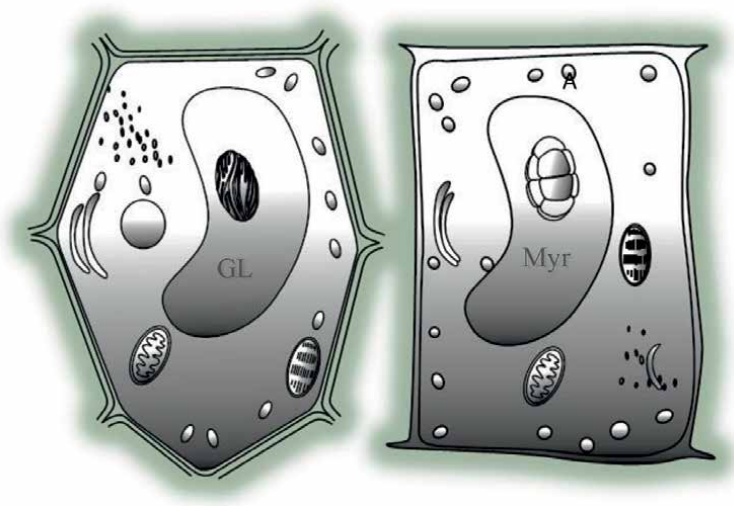

Figure 2.
Sulfur-rich S-cell contains glucosinolate (GL), and B) myrosin cell contains myrosinase (Myr) [20].

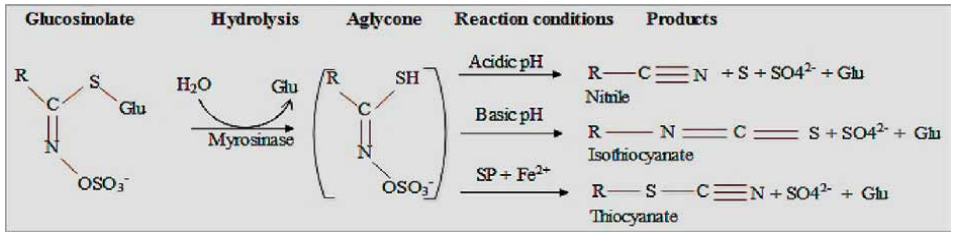

Figure 3.
Glucosinolate hydrolysis pathway modified from Kirkegaard [19]. Glu = glucose; R−N═C═S is isothiocyanate; R-C≡N is nitrile; SP = specifier proteins; R−S−C≡N is an ionic thiocyanate.

The effectiveness of biofumigation broadly depends on (1) *brassica* cover crops, (2) edaphic factors, (3) termination methods, (4) cultural practices, and (5) nematode species and life stages. In addition, this chapter argues that 'host suitability' to root-knot nematodes is another critical factor that has become evident in recent years, which is associated with stimulating egg hatch and open-end trap cropping.

3. *Brassica* cover crops

Members of Brassicaceae constitute some 350 genera and 3500 species [31]. *Brassica* biofumigant crops that are commonly utilized for biofumigation purposes include brown mustard (*Brassica juncea*), yellow or white mustard (*Sinapis alba;* Syn. *Brassica hirta*), rapeseed (*Brassica napus*), field mustard (*Brassica rapa* var. *rapa*), and oil radish (*Raphanus sativus*) [29]. The selection of biofumigant crop species and cultivars or accessions is crucial because the types and concentrations of GLs vary among species, cultivars, and even tissues within a cultivar [15, 32]. Sinigrin (allyl GL) is a dominant GL in *B. juncea* and *B. nigra*, and varies by cultivar and tissues [29]. For example, total GL and allyl GL levels of *B. juncea* 'Terrafit', 'Terratop', 'Terraplus', and 'ISCI99'

Biofumigant crop				Total (ITC-generating) GL		Nematode		
Species	**Cultivar/accession**	**Form[a]**	**Amendment rate[b]**	**μmol g^{-1} dw[c]**	**nmol g^{-1} soil[d]**	**Species**	**Suppression[e]**	**References**
B. carinata	Acc. 94044	GM	2.0%	21.7 (21.5)	86.8 (85.3)	*Pratylenchus neglectus*	32.6%	[37]
	BRK-147A	GM	na	30.6	135.4	na	na	[32]
	BRK-147A	S	na	116.0	na	na	na	[34]
	ISCI7	SM	2.5 t/ha	163.4 (160.1)	na	*Meloidogyne chitwoodi*	>80.0%	[38]
	ISCI7	SM	3.0 t/ha	150.7 (147.7)	na	*M. incognita*	<RGI	[39]
	na	LF	6.0% (v/v)	90.0	na	*M. incognita*	81.0%	[40]
B. hirta	Martegena	GM	na	73.1	na	*M. javanica, T. semipenetrans*	na	[41]
B. juncea	Acc. 99Y11	GM	2.0%	20.4	81.6	*P. neglectus*	40.9%	[37]
	Caliente 99	GM	230.0*	62.5 (49.2)	na	*Globodera pallida*	Effective	[35]
	Caliente 61	GM	0.1 t/ha	49.1 (36.3)		*M. incognita*	No effect	[42]
	Cutlass	GM	na	11.7	135.4	na	na	[32]
	ISCI99	GM	9.9 t/ha	29.0 (25.0)	100.5 (91.4)	*Trichodorus, Tylenchorynchus*	No effect	[33]
		GM	1.1 t/ha	72.1 (58.4)	na	*M. incognita*	No effect	[42]
	JR049	GM	5.6 t/ha	6.7 (4.9)	44.6 (40.4)	na	na	[15]
	Nemfix	GM	10.3 t/ha	22.5 (20.2)	169.9 (161.6)	*M. javanica*	9.0 fold	[15, 43]
	Nemfix	SM	2.0 t/ha	na	na	*M. javanica*	9.0 fold	[43]
	Pacific Gold	SM	1.2 t/ha	153.2 (152.0)	na	*M. incognita, P. penetrans*	>90.0%	[17]
		GM	1.2 t/ha	57.7 (45.9)	na	*M. incognita*	No effect	[42]
	Pacific Gold	SM	>2.2 t/ha		na	*G. pallida*	100.0%	[17, 18]
	Pacific Gold	SM	>4.5 t/ha			*G. ellingtonae*	>92.1%	[18]

Biofumigant crop				Total (ITC-generating) GL		Nematode		
Species	**Cultivar/accession**	**Form[a]**	**Amendment rate[b]**	**μmol g^{-1} dw[c]**	**nmol g^{-1} soil[d]**	**Species**	**Suppression[e]**	**References**
	Pacific Gold	S	na	61.0	na	na	na	[34]
	Pacific Gold	SME	1.1 t/ha	278.0 (278.0)		*G. ellingtonae*	100.0%	[18]
	Terrafit	GM	6.9 t/ha	22.2 (19.3)	61.1 (55.8)	*Trichodorus, Tylenchorynchus*	No effect	[33]
	Terraplus	GM	7.5 t/ha	20.1 (15.4)	63.4 (54.5)	*Trichodorus, Tylenchorynchus*	No effect	[33]
	Terratop	GM	8.4 t/ha	16.7 (13.1)	61.8 (52.5)	*Trichodorus, Tylenchorynchus*	No effect	[33]
B. napus	BQ Mulch	GM	7.0 t/ha	25.7	164.5 (91.9)	na	na	[15]
	Dunkeld Acc. 94713	GM	2.0%	7.5 (6.8)	28.8 (24.0)	*Pratylenchus neglectus*	44.5%	[37]
	Dwarf Essex	SM	5.0 t/ha	41.9 (35.6)	na	*M. incognita*	90.0%	[17]
	Dwarf Essex	SM	50.0 t/ha	41.9 (35.6)	na	*P. penetrans*	90.0%	[17]
	MaximaPlus	GM	7.7 t/ha	16.6 (9.0)	78.1 (21.3)	na	na	[15]
	Sunrise	SM	15.0 t/ha	14.8 (3.0)	na	*M. incognita, P. penetrans*	No effect	[17]
B. nigra	Acc. 95067	GM	2.0%	16.4 (16.4)	65.4 (65.4)	*P. neglectus*	28.1%	[37]
	Giebra	GM	na	22.5	647.6	na	na	[32]
	Giebra	S	na	193.0	na	na	na	[34]
B. oxyrrhina	Acc. 95060	GM	2.0%	34.0 (33.4)	136.1 (133.8)	*P. neglectus*	71.8%	[37]
B. rapa	Harmoni	GM	na	3.6	15.7	na	na	[32]
	Harmoni	S		<30.0	na			[34]
	na	GM	2.0%	3.2 (2.9)	12.9 (11.4)	*P. neglectus*	33.1%	[37]
E. sativa	Nemat	GM	77.7 t/ha*	61 (36)	na	*G. pallida*	No effect	[35]

Biofumigant crop				Total (ITC-generating) GL		Nematode		
Species	**Cultivar/accession**	**Form[a]**	**Amendment rate[b]**	**μmol g^{-1} dw[c]**	**nmol g^{-1} soil[d]**	**Species**	**Suppression[e]**	**References**
R. sativus	Bento	GM	124.7 t/ha*	31.7 (27.8)	na	*G. pallida*	No effect	[35]
S. alba	IdaGold	SM	20.0 t/ha	163.9 (156.8)	na	*P. penetrans*	65.0%	[17]
	IdaGold	SM	20.0 t/ha	163.9 (156.8)	na	*M. incognita*	90.0%	[17]
	IdaGold	SM	100.0 t/ha	163.9 (156.8)	na	*P. penetrans*	90.0%	[17]
	Zlata	GM	30.7 t/ha	na	na	*G. rostochiensis*	na	[44]

[a]*GM = green manure; S = intact seed; SM = defatted seed meal; SME = defatted seed meal extract in powder; LF = liquid formulation (prepared from defatted seed meal and liquid phase) mixed in water; na = data not available.*
[b]*Tissue amendment is based on dry weight unless indicated with * which is identified as fresh weight.*
[c]*Values outside of parentheses are the average of total GL in dry shoot and root tissues of biofumigant crops, and values inside of parentheses are GL that only generate ITC.*
[d]*Values outside the parentheses are determined based on total GL in root and shoot per dry weight of soil (based on 10-cm soil depth, and 1.08 g cm^{-3} soil bulk densities). Values inside the parentheses are GL that only generate ITC.*
[e]*<RGI = reduced root gall index; effective = nematode suppression was statistically significant; na = data not available; No effect = nematode suppression was not significant.*

Table 1.
Nematode suppressive effects of different biofumigant crop species affected by their cultivars/accessions, a form of application, amendment rates, glucosinolate concentration, and target nematodes.

were different among cultivars and tissues [33]. The cultivar 'ISCI99' generated more biomass and accumulated higher concentrations of both total and allyl GLs in roots than in foliage [33]. The concentration of GL in roots and stems decreased gradually as the plant develops; it increased in the leaves and reproductive organs of *B. juncea* [34]. The growing season also affected the concentration of GL in *brassica* crops [35]. The highest GL production was achieved in summer followed by spring growing seasons, indicative of higher growing degree-days and corresponding biomass production [35, 36]. Hence, the selection of *brassica* crops is important for successful biofumigation. **Table 1** shows *brassica* biofumigant crop species and cultivars or accessions, forms of application, amendment rates, GL concentration, and target plant-parasitic nematodes.

4. Edaphic factors

Edaphic factors play an important role in the performance of biofumigation against plant-parasitic nematodes in agroecosystems. The edaphic factors include soil's physical, chemical, and biological properties. The impact of each soil property has on the effectiveness of biofumigation are discussed.

4.1 Soil physical properties

Soil moisture, texture, and temperature are recognized as the main players affecting biofumigation processes in the soil. Soil moisture mediates GL hydrolysis, impacts ITC half-life, and renders GL prone to leaching. The half-life of benzyl GL, for example, increased from 6.8–15.5 hours at a 1:1 soil to water ratio to 17.5–19.5 hours at 8–11.6% soil moisture levels [45]. Excessive soil moisture can cause GL to leach from the biologically active rhizosphere because GL adsorbs weakly to soil particles [46, 47]. Soil moisture is recommended to be maintained at optimum levels to achieve desired outcome [48]. When it comes to soil texture, GL degrades more rapidly in clay topsoil than in sandy topsoil. However, in the clay subsoil, GL degradation reduced due to the lack of biological activities to an extent of no degradation in sandy subsoil [15]. In terms of soil temperature, volatility of ITC increases with temperature, especially short-chained aliphatic GLs are more prone to volatilization loss if proper measures are not taken to contain them in the soil [49–51].

4.2 Soil chemical properties

Soil pH, the redox states of iron, and soil organic matter (SOM) are regarded as important soil chemical properties known to influence ITC production in the soil [52]. Aglycone, an unstable intermediate of GL hydrolysis, undergoes a non-enzymatic rearrangement and depending on the occurrence of these chemical properties, either ITCs, nitriles or thiocyanates are produced. The rearrangement is regulated by these soil chemical properties (**Figure 3**). Low pH favors nitrile production whereas high pH favors ITC production [53, 54]. At soil <pH 6, the aglycone undergoes proton (H^+) dependent desulfuration to yield nitrile and elemental sulfur [52, 55]. In contrast, aglycone experiences a concerted loss of sulfate ($SO4^{2-}$) at soil ≥pH 6, which is independent of H^+ in Lossen rearrangement and produces ITC [52]. Thus, maintaining soil ≥pH 6 is desirable for the purposes of biofumigation. With regards to redox states of iron, ferrous (Fe^{2+}) and ferric (Fe^{3+}) irons promote nitrile production [56, 57], thus reduces ITC production. Hanschen et al. [57] autoclaved soil to increase Fe^{2+} content,

and they observed an antagonistic effect on the performance of biofumigation. The presence of Fe^{3+} can nearly terminate both allyl nitrile and allyl ITC production [52, 54, 58]. In terms of SOM, hydrophobic ITCs are adsorbed to SOM, thus reducing their biofumigation activities [46, 59]. Sorption of ITC to SOM increases with their non-polar nature [45]. Price et al. [49] incorporated *B. juncea* tissue in sandy soil with less SOM and found less ITC in the headspace than in clay soil with high SOM. Matthiessen and Shackleton [59] also noted that higher SOM at a low temperature significantly reduced ITC volatility, resulting in a low biofumigation effect.

4.3 Soil microbiota

Some soil microorganisms produce Myr, the enzyme that catalyzes GL hydrolysis. For example, *Aspergillus niger,* a ubiquitous soil-borne facultative pathogen [60, 61], and *Enterobacter cloacae*, a bacterial antagonist of *Fusarium oxysporum* and *Pythium* spp. [62], produce Myr when GL was added to the soil. One reason these microbes produce Myr could be to break down GL to obtain glucose, as glucose is one of the hydrolysates of GL hydrolysis (**Figure 3**). Albaser et al. [63] found a strain of soil-borne bacterium, *Citrobacter* WYE1, to possess an inducible β-glucosidase capable of transforming GL into ITC. Soils treated with γ-irradiation, that did not inactivate Myr enzyme, degraded benzyl GL whereas autoclaved soils, where Myr enzymes were denatured, arrested the GL degradation [15]. This suggests that biofumigation in soils treated by solarization, fumigation, or sterilization could compromise its effectiveness.

5. Termination methods

Method of termination is how the *brassica* crop residues are terminated for biofumigation purposes. Different termination methods generate different regimes of biofumigation efficacy. At least nine biofumigation methods are described in the literature. These methods are presented from low to high efficacy levels in **Figure 4**. These include (1) intercropping, (2) crop rotation, (3) soil incorporation, (4) soil incorporation + solarization, (5) tissue maceration + soil incorporation, (6) tissue maceration + soil incorporation + watering, (7) tissue maceration + soil incorporation + plastic mulch, (8) tissue maceration + soil incorporation + rolling soil surface or compaction, (9) open-end trap crop + tissue maceration + soil incorporation + plastic much [64]. Waisen et al. [65] alluded to the fact that using biofumigant crops which are susceptible to root-knot nematodes can in fact stimulate egg hatch, thus making biofumigation of these crops more effective. This concept is also known as the "open-end trap crop" approach where targeted nematodes are allowed to infect the biofumigant crops prior to being trapped and fumigated.

The incorporation of *brassica* biofumigant crop tissues in the soil by tillage is a popular method of biofumigation, where ITCs are generated from the mechanical damage of tissues during soil incorporation [35, 66, 67]. During the growth of *brassica* crops either in rotation or intercropping scenarios, negligible quantities of ITCs are generated through leaf washings, root exudates, or through physical damage by herbivorous pests, which have shown promise to suppress soil-borne pathogens [22, 35]. Oil radish roots released ITC in the rhizosphere following feeding damage by cabbage root fly larvae (*Delia radicum*), which was claimed to be toxic to encysted eggs of potato cyst nematode (*Globodera pallida*) [35]. As the research on biofumigation expands, knowledge of the mechanism of ITC production becomes apparent,

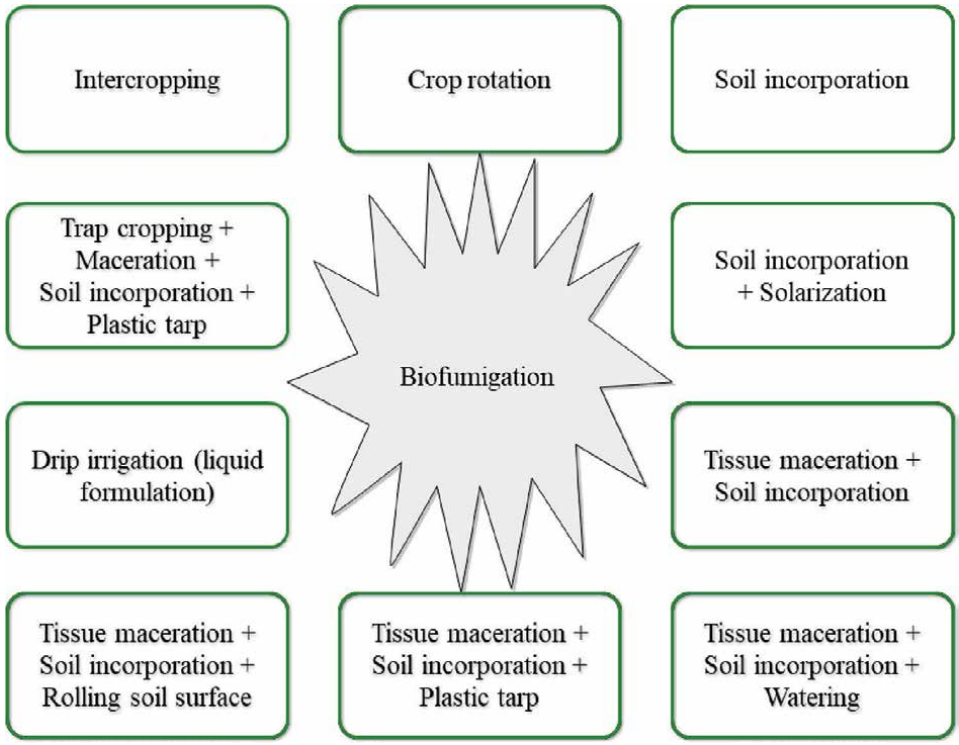

Figure 4.
Biofumigation methods using brassica *biofumigant crops.*

and the conventional method of biofumigation has shifted to include tissue maceration, irrigation, and mulching with an impermeable plastic film. The fact that GL and Myr are spatially separated in intact plant cells (**Figure 2**), tissue maceration would enhance GL hydrolysis, thus maximizing ITC production and biofumigation effect [48, 68]. Effective biofumigation occurs when hydrolysis of GL generates more than 100 nmol of ITC/g soil [46]. In addition, with the knowledge that water mediates GL hydrolysis, it is beneficial to add water after tissue maceration and soil incorporation to maximize hydrolysis while washing the ITC into the rhizosphere to be in contact with target nematodes. It has been reported that irrigation with 34 mm in a field after pulverizing *B. juncea* tissues produced 100 nmol/g soil of propenyl ITC [48], with a biofumigation effect equivalent to the 200 nmol methyl ITC/g soil from metam sodium [30]. Furthermore, with the understanding that aliphatic ITCs are volatile [4], maximum biofumigation effectiveness requires sealing the soil with impermeable plastic film immediately after tissue maceration and soil incorporation [69]. Mulching with black plastic was shown to be more advantageous than clear solarization mulch because of its low solar radiation transmittance, which would be less destructive to Myr and beneficial to soil microorganisms [60]. Stapleton and Duncan [70] also recommended to tarp the soil for no more than 7 days to avoid anaerobic soil disinfestation [71, 72]. This is because under anaerobic soil conditions, redox potential decline and generate Fe^{2+} as well as organic acids that would interfere with ITC production [73].

6. Cultural practices

The application of sulfur (S) and nitrogen (N) fertilizers to *brassica* biofumigant crops is important because N and S are integral elemental constituents of GL (**Figure 3**) [74, 75]. Low N and high S fertilizer application enhanced aliphatic GL in *Brassica rapa* [76, 77]. Li et al. [78] noted that while total GL concentration was not affected by fertilizer inputs, individual GL concentration was affected by S or N supply. Nitrogen-containing tryptophan-derived indole GL was directly proportional to N supply whereas S-containing methionine-derived aromatic GL was inversely proportional to N supply [78]. Application of N-containing fungicide, metconazole increased total GL concentration in *B. juncea* and *R. sativus* [35]. In addition, cultivation of biofumigant *brassica* crops can recruit microorganisms that produce Myr enzymes to break down GL. It is important to be mindful of when to cultivate *brassica* biofumigant crops as they are photosensitive and flower when the day length is long. This means brassicas intended for biofumigation will quickly flower before sufficient biomass production necessary for biofumigation. In temperate climates such as in California, grow *brassica* biofumigant crops during winter months and not during spring or summer months. For example, 'Caliente 199' brown mustard planted for biofumigation in Coachella Valley in Southern California prematurely flowered at 5-6 weeks barely producing any biomass (**Figure 5**).

Figure 5.
*Showing brown mustard (*Brassica juncea*) 'Caliente 199' field planted in Spring of 2022 bolting prematurely in Coachella Valley (Southern California, USA).*

7. Nematode life stages

Sensitivity to ITC varies by species and developmental stages of nematodes [41, 50]. Mojtahedi et al. [50] observed J2s of *M. chitwoodi* were more vulnerable to biofumigation than their egg counterparts. In another study, J2s of *M. incognita* were more sensitive to defatted seed meals of brassicas compared to a mixed stage of root lesion nematode, *Pratylenchus penetrans* [17]. The J2s of root-knot nematodes are more mobile, and their metabolic or respiration rates are elevated and the likelihood of ITC intake is higher than any later other developmental life stages. Fumigation with metam sodium (methyl ITC) is more effective against the target pest when it is actively respiring [79], suggesting that biofumigation would be most effective when the nematodes are active. Thus, J2s are more prone to biofumigation than egg stage or when the nematodes are in survival stage. Thus, the cultural practices aimed at triggering nematode egg hatch are crucial.

8. Host suitability

Most *brassica* crops that are utilized for biofumigation purposes are good hosts for root-knot nematodes. Thus, the use of susceptible biofumigant crops as a pre-plant cultural nematode management tactic has been cautioned or their use has been considered an important management challenge because of the high likelihood of increasing the target nematode population before planting cash crop [80–82]. The cultivars of *B. juncea* and *B. rapa* were reported to be good hosts of root-knot nematodes while that of *Eruca sativa* 'Nemat' and *R. sativus* 'Boss' including 'TerraNova' were ranked among the poorest hosts [80, 81, 83]. Host suitability of a list of *Brassica* species and cultivars to root-knot nematodes is presented in **Table 2**. The use of biofumigant crops that are good hosts of root-knot nematodes has been advised against in attempts to address undesired nematode reproduction. Instead, the use of *brassica* cultivars that are poor or non-hosts to root-knot nematodes has been recommended [4, 16, 81]. Alternatively, cultivating nematode-susceptible *brassica* crops during winter to limit nematode development and delay egg production was recommended [83]. However, this approach would be impractical in tropical climatic regions where temperatures remain above the nematode development thresholds all year round.

In the past, the host suitability has perceived negative implications for biofumigation associated with increasing the target nematode population and compromising the performance of biofumigation. This review argues that host suitability could, in fact, be beneficial, especially when it comes to stimulating egg hatch and trapping J2s as an open-end trap crop [65]. The hatchlings or J2s are now at the most sensitive or vulnerable stage to be killed by ITC through biofumigation. Melakeberhan et al. [87] found that *M. hapla* accumulated 450–500 degree-days (with a base temperature of 10°C) to develop from undifferentiated eggs to egg-laying females on oil radish and proposed that terminating the crop before completion of the nematode's life cycle might be best used as a trap crop. Waisen et al. [65] found *M. incognita* J2s accumulated 283 degree-days to reach egg-laying females on *B. juncea* 'Caliente 199' in greenhouse conditions, and reduced soil population of *Meloidogyne* spp. in two field trials.

The key to maximizing biofumigation kill is to grow root-knot nematode susceptible biofumigant crop with an aim to activate or stimulate egg hatch and subsequently terminate the crop right before the nematode completes its life cycle. The termination time is critical and it must be done based on nematode degree-days

Biofumigant crop		***Meloidogyne* species**			**References**
Species	**Cultivar**	***M. hapla***	***M. incognita***	***M. javanica***	
Brassica carinata	Bc007	Poor	Moderate	Poor	[81]
Brassica juncea	ISCI99	Good	Good	Good	[81]
	Nemfix	Good	Good	Good	[81, 83]
	Pacific Gold	Moderate/good	Good	Moderate	[80, 81]
Brassica napus	Humus	Poor/moderate	Poor/ moderate	Poor/ moderate	[81]
	Winfred	Poor	Moderate/ good	Good	[81]
Brassica rapa	Rondo	Good	Good	Good	[81]
	Samson	Good	Good	Good	[81]
Eruca sativa	Nemat	Poor	Poor	Poor	[81, 84, 85]
Raphanus sativus	Adagio	Poor	Poor	Poor	[81, 86]
	Adios	Poor/moderate	Moderate/ good	Poor/ moderate	[81]
	Boss	Poor	Poor	Poor	[81, 84]
	Colonel	Good	Poor	Poor	[81]
	Comet	Poor	Good	Poor	[81]
	Defender	Poor	Poor	Poor	[81]
	TerraNova	Good	Poor	Poor	[81]
Sinapis alba	Abraham	Poor/moderate	Poor	Poor/ moderate	[81]
	Absolut	Poor	Moderate	Moderate	[81]
	Accent	Poor	Poor	Poor	[81]
	Achilles	Poor/moderate	Moderate/ good	Moderate/ good	[81]
	Condor	Poor	Moderate/ good	Poor	[81]
	IdaGold	Good	Moderate/ good	Moderate	[81]
	Maxi	Moderate	Poor/ moderate	Poor	[81]
	Santa Fe	Poor/moderate	Moderate	Poor/ moderate	[81]

Table 2.
Host suitability of common biofumigant crops to major Meloidogyne species.

or heat units as demonstrated by Waisen et al. [65] and Melakeberhan et al. [87]. At the average soil temperatures of 22°C in winter or 29°C in summer in Hawaii, USA, *brassica* crops were recommended to grow for 5–6 weeks to achieve the dead-end trap cropping effect [64, 65]. At this time, root-knot nematodes will reach mature females but before laying eggs.

9. Conclusions

An important question to address is what is the best possible combination with respect to the abovementioned factors affecting biofumigation to maximize the biofumigation performance against root-knot nematodes in agroecosystems? This chapter highlights that the exploitation of *brassica* host suitability to root-knot nematodes can enhance the biofumigation effect with a time-sensitive process of stimulating egg hatching and trapping J2s with a subsequent targeted release of ITC at the most vulnerable life stage. Ideally, coupling open end trap crop tactic with an effective termination method that releases maximum ITC would enhance biofumigation effect on target nematodes [88]. Based on the abovementioned factors and the contemporary knowledge on the mechanism of biofumigation, it trickles down to six steps as necessary to maximize biofumigation effects. These include (1) Select a potent and susceptible biofumigant crop - Selecting a cultivar or an accession of *Brassica* species that produce high ITC-generating GL (e.g., *B. juncea* 'Caliente 199'). Most importantly, the selected *brassica* biofumigant crop must be susceptible to your target plant-parasitic nematode, the root-knot nematode; (2) Field preparation—Till the field, direct seed the selected biofumigant crop (e.g., *B. juncea* 'Caliente 199' at 10–12 kg/ha). In temperate climates such as in California, Grow *brassica* biofumigant crops in winter but not in spring or summer as they are photosensitive and flower before biomass production necessary for biofumigation. Adjust the soil pH to near neutral (pH 6–7) because lower pH favors nitrile production, instead of ITC. Irrigate and fertilize the biofumigant crops as needed. (3) Termination—Terminate the biofumigant crop 5–6 weeks after planting. Based on field research conducted in tropical climates in Hawaii, termination can be done at 5-week old in summer or 6-week old in winter. Termination involves comprehensive maceration of aerial tissues using a flail mower; (4) Tissue incorporation - Immediately incorporate the macerated tissues to 10–15 cm deep to minimize volatilization losses of ITC and maximize ITC contact with nematodes. Soil incorporation must target rhizosphere where the nematodes are; (5) Sealing the soil—covering the soil with impermeable plastic mulch (opaque or black plastic is recommended as soil is cooler under black plastic mulch and chances of Myr denaturation is minimized) immediately after the tissue incorporation to retain ITC from volatilization loss; and (6) uncover and transplant cash crop—uncover the plastic mulch after 7 days and transplant the cash crop. The one-week time window is recommended to avoid phytotoxicity.

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Brassica crops, including broccoli, cauliflower, cabbage, and mustard, are among the most important crops worldwide with oilseed *Brassica* among the largest traded agricultural commodities. They play a vital role in agriculture, horticulture, and human nutrition. The health-promoting and disease-preventing properties of *Brassica* vegetables are well documented, and their nutritional value makes them an essential part of a balanced diet. *Brassica - Recent Advances* provides a comprehensive overview of the recent research on *Brassica* crops. The book covers such topics as the breeding of *Brassica* crops, agronomic practices, pest management, and plant secondary metabolites. It is an essential resource for researchers, students, and professionals interested in *Brassica* crops and their applications.

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