

Compendium of Plant Genomes
Series Editor: Chittaranjan Kole

Takeshi Nishio
Hiroyasu Kitashiba *Editors*

The Radish Genome

Compendium of Plant Genomes

Series Editor

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The Radish Genome

 Springer

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ISSN 2199-4781

ISSN 2199-479X (electronic)

Compendium of Plant Genomes

ISBN 978-3-319-59252-7

ISBN 978-3-319-59253-4 (eBook)

DOI 10.1007/978-3-319-59253-4

Library of Congress Control Number: 2017948205

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Printed on acid-free paper

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The registered company is Springer International Publishing AG

The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

*This book series is dedicated to
my wife Phullara, and our children Sourav,
and Devleena*

Chittaranjan Kole

Preface to the Series

Genome sequencing has emerged as the leading discipline in the plant sciences coinciding with the start of the new century. For much of the twentieth century, plant geneticists were only successful in delineating putative chromosomal location, function, and changes in genes indirectly through the use of a number of ‘markers’ physically linked to them. These included visible or morphological, cytological, protein, and molecular or DNA markers. Among them, the first DNA marker, the RFLPs, introduced a revolutionary change in plant genetics and breeding in the mid-1980s, mainly because of their infinite number and thus potential to cover maximum chromosomal regions, phenotypic neutrality, absence of epistasis, and codominant nature. An array of other hybridization-based markers PCR-based markers, and markers based on both facilitated construction of genetic linkage maps, mapping of genes controlling simply inherited traits and even gene clusters (QTLs) controlling polygenic traits in a large number of model and crop plants. During this period a number of new mapping populations beyond F₂ were utilized and a number of computer programs were developed for map construction, mapping of genes, and for mapping of polygenic clusters or QTLs. Molecular markers were also used in studies of evolution and phylogenetic relationship, genetic diversity, DNA-fingerprinting and map-based cloning. Markers tightly linked to the genes were used in crop improvement employing the so-called marker-assisted selection. These strategies of molecular genetic mapping and molecular breeding made a spectacular impact during the last one and a half decades of the twentieth century. But still they remained ‘indirect’ approaches for elucidation and utilization of plant genomes since much of the chromosomes remained unknown and the complete chemical depiction of them was yet to be unraveled.

Physical mapping of genomes was the obvious consequence that facilitated development of the ‘genomic resources’ including BAC and YAC libraries to develop physical maps in some plant genomes. Subsequently, integrated genetic-physical maps were also developed in many plants. This led to the concept of structural genomics. Later on, emphasis was laid on EST and transcriptome analysis to decipher the function of the active gene sequences leading to another concept defined as functional genomics. The advent of techniques of bacteriophage gene and DNA sequencing in the 1970s was extended to facilitate sequencing of these genomic resources in the last decade of the twentieth century.

As expected, sequencing of chromosomal regions would have led to too much data to store, characterize, and utilize with the-then available computer software could handle. But development of information technology made the life of biologists easier by leading to a swift and sweet marriage of biology and informatics and a new subject was born—bioinformatics.

Thus, evolution of the concepts, strategies and tools of sequencing and bioinformatics reinforced the subject of genomics—structural and functional. Today, genome sequencing has traveled much beyond biology and involves biophysics, biochemistry and bioinformatics!

Thanks to the efforts of both public and private agencies, genome sequencing strategies are evolving very fast, leading to cheaper, quicker and automated techniques right from clone-by-clone and whole-genome shotgun approaches to a succession of second generation sequencing methods. Development of software of different generations facilitated this genome sequencing. At the same time, newer concepts and strategies were emerging to handle sequencing of the complex genomes, particularly the polyploids.

It became a reality to chemically—and so directly—define plant genomes, popularly called whole-genome sequencing or simply genome sequencing.

The history of plant genome sequencing will always cite the sequencing of the genome of the model plant *Arabidopsis thaliana* in 2000 that was followed by sequencing the genome of the crop and model plant rice in 2002. Since then, the number of sequenced genomes of higher plants has been increasing exponentially, mainly due to the development of cheaper and quicker genomic techniques and, most importantly, development of collaborative platforms such as national and international consortia involving partners from public and/or private agencies.

As I write this preface for the first volume of the new series “Compendium of Plant Genomes”, a net search tells me that complete or nearly-complete whole-genome sequencing of 45 crop plants, eight crop and model plants, eight model plants, 15 crop progenitors and relatives, and three basal plants are accomplished, the majority of which are in the public domain. This means that we nowadays know many of our model and crop plants chemically, i.e., directly, and we may depict them and utilize them precisely better than ever. Genome sequencing has covered all groups of crop plants. Hence, information on the precise depiction of plant genomes and the scope of their utilization is growing rapidly every day. However, the information is scattered in research articles and review papers in journals and dedicated web pages of the consortia and databases. There is no compilation of plant genomes and the opportunity of using the information in sequence-assisted breeding or further genomic studies. This is the underlying rationale for starting this book series, with each volume dedicated to a particular plant.

Plant genome science has emerged as an important subject in academia, and the present compendium of plant genomes will be highly useful both to students and teaching faculties. Most importantly, research scientists involved in genomics research will have access to systematic deliberations on the plant genomes of their interest. Elucidation of plant genomes is not only of interest for the geneticists and breeders, but also for practitioners of an array of plant science disciplines, such as taxonomy, evolution, cytology,

physiology, pathology, entomology, nematology, crop production, bio-chemistry, and obviously bioinformatics. It must be mentioned that information regarding each plant genome is ever-growing. The contents of the volumes of this compendium are therefore focusing on the basic aspects of the genomes and their utility. They include information on the academic and/ or economic importance of the plants, description of their genomes from a molecular genetic and cytogenetic point of view, and the genomic resources developed. Detailed deliberations focus on the background history of the national and international genome initiatives, public and private partners involved, strategies and genomic resources and tools utilized, enumeration on the sequences and their assembly, repetitive sequences, gene annotation, and genome duplication. In addition, synteny with other sequences, comparison of gene families, and, most importantly, potential of the genome sequence information for gene pool characterization through genotyping by sequencing (GBS) and genetic improvement of crop plants have been described. As expected, there is a lot of variation of these topics in the volumes based on the information available on the crop, model, or reference plants.

I must confess that as the series editor it has been a daunting task for me to work on such a huge and broad knowledge base that spans so many diverse plant species. However, pioneering scientists with life-time experience and expertise on the particular crops did excellent jobs editing the respective volumes. I myself have been a small science worker on plant genomes since the mid-1980s and that provided me the opportunity to personally know several stalwarts of plant genomics from all over the globe. Most, if not all, of the volume editors are my longtime friends and colleagues. It has been highly comfortable and enriching for me to work with them on this book series. To be honest, while working on this series I have been and will remain a student first, a science worker second, and a series editor last. And I must express my gratitude to the volume editors and the chapter authors for providing me the opportunity to work with them on this compendium.

I also wish to mention here my thanks and gratitude to the Springer staff, Dr. Christina Eckey and Dr. Jutta Lindenborn in particular, for all their constant and cordial support right from the inception of the idea.

I always had to set aside additional hours to edit books besides my professional and personal commitments—hours I could and should have given to my wife, Phullara, and our kids, Sourav, and Devleena. I must mention that they not only allowed me the freedom to take away those hours from them but also offered their support in the editing job itself. I am really not sure whether my dedication of this compendium to them will suffice to do justice to their sacrifices for the interest of science and the science community.

Kalyani, India

Chittaranjan Kole

Preface

Radish (*Raphanus sativus* L.) is an important vegetable in East Asia, e.g., Japan, China, and Korea, but not so much in Europe, America, Africa, or Oceania. Therefore, studies on radish are predominantly performed by Asian researchers, and not by Europeans or Americans. In the East Asia, radish roots are thick, long, and mostly white, whereas those are tiny and mostly red in Europe. The East Asian type is called East Asian big long radish, white radish, or daikon (in Japanese), and the European radish type is called European small radish or European garden radish. In this e-book, we use terms “Asian big radish” and “European small radish” for them. In Asian big radish, there is a great variation in root size and shape, especially in Japanese radish. In Southeast Asia, pods are consumed as a vegetable, and seeds are used as oil seeds. On the other hand, *Raphanus raphanistrum*, a species in the same genus as radish, is a serious weed in America and Australia, but not so much in Asia. The recent globalization will change the food culture, and Asian big radish may become an important vegetable in the western countries.

Radish is closely related to the genus *Brassica*, which contains many important crops such as rapeseed, cabbage, broccoli, Chinese cabbage, turnip, and mustard. Genetic and genomic studies of *Brassica* have been intensively carried out, but these studies of radish have been left behind those of *Brassica*. Phylogenetic relationship of radish with *Brassica* species is not established. Although radish is crossable with *R. raphanistrum* and hybrids between radish and *R. raphanistrum* are fully fertile, they have different species names. Asian wild radish growing at seashores is *R. sativus* var. *raphanistroides*. Relationships between the wild radish and cultivated radish are controversial. Besides the importance of radish as a crop itself, radish and wild radish are important genetic resources for breeding of *Brassica* crops.

Development of the next-generation sequencers accelerated whole-genome sequencing of many crop species. Two groups in Japan and one Korean group carried out the whole-genome sequencing of radish independently. Although the group of Tohoku University and Kazusa DNA Research Institute, where high-density linkage maps of DNA markers were constructed to form a standard linkage map, published a draft genome sequence of radish first, the other Japanese group and the Korean group published radish genome sequences with more data soon after that. These genome sequence data will contribute to identification of genes important for breeding of radish and

Brassica crops. Since these three groups joined in publishing this e-book, we hope this cooperative book publication accelerates collaboration of them for integration of three radish genome sequences to make a standard reference genome sequence of radish.

Dr. Chittaranjan Kole recommended us to edit a book of “The Radish Genome”. For publishing this book, we have been supported by Dr. Christina Eckey of Springer. We are much grateful to them for their helpful suggestions and encouragements.

Miyagi, Japan

Takeshi Nishio
Hiroyasu Kitashiba

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Takeshi Nishio

Abstract

Radish is important as a root vegetable, a leafy vegetable, a fruit vegetable, an oil crop, and also as a cover plant. The economic importance and characteristics of radish differ between the East and the West of the world. In the East, there are radish cultivars having large roots with various shapes called “Asian big radish” and those grown for production of immature pods or oil seeds, whereas radish is a small vegetable grown within one month in the West. Asian big radish is expected to eventually become popular in the West. Radish belongs to the genus *Raphanus*, but is similar to the *Brassica* species except for the shape of pods and seeds. Despite their similarities, the order of genes in chromosomes is quite different between *Raphanus* and *Brassica*. Radish genome sequences have been published from three groups using similar cultivars, and therefore, collaboration for combining sequence data is considered to be effective for determination of more reliable genome sequences. Some radish lines have high salt tolerance and disease resistance different from *Brassica* crops. Radish also has a characteristic glucosinolate composition. Since radish can be crossed with *Brassica* species, it is also important as a genetic resource for *Brassica* crop breeding.

1.1 Introduction

Radish is a Brassicaceae crop, mainly used as a root vegetable. Roots of radish have large variations in size and shape. European small radish has a small round root ca. 2–3 cm in diameter, whereas “Sakurajima-daikon” in Japan has a large round root of more than 30 cm. Most widely grown radish cultivars in Asia have roots of cylindrical shape ca. 10 cm in diameter and ca.

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40 cm in length, whereas “Moriguchi-daikon” in Japan has a long cylindrical root of more than 2 m with a diameter of ca. 3 cm. The color of radish roots is also various. The major Asian radish has a white thick root and is called “East Asian big long radish,” “white radish,” or “daikon” in Japanese (“Asian big radish” hereafter), whereas European small radish, which is commonly called “radish,” and the root surface of some landraces in Asia are red, although the red part develops from a hypocotyl. There are also purple, green, and black colors on the root surface in other landraces. Some cultivars in China are red or green inside the roots.

Young plants removed by thinning are used as a leafy vegetable. “Kosena-daikon,” a Japanese radish landrace, which has a small root, is a cultivar specialized as a leafy vegetable. Immature pods of rat’s tail radish, which is cultivated in Southeast Asia, e.g., Thailand and India, are used as a vegetable. The root of rat’s tail radish is not thick. A radish sprout called “Kaiware-daikon” is popular as a vegetable in Japan, and the recent vogue of Japanese cuisine has also made it popular in Western countries. Since radish seeds contain a high amount of oil, ca. 40%, radish is also produced as an oil crop (Ahuja et al. 1987). Furthermore, cultivation of radish as a cover crop to avoid soil erosion and to suppress weeds has become popular in the USA and Canada (Weil and Kremen 2007).

The species name of radish is *Raphanus sativus*, its genus being different from that of the major Brassicaceae oil crops and vegetables, i.e., *Brassica*. European small radish is similar to red turnip landraces in *Brassica rapa*. “Shogoin-daikon,” a Japanese radish landrace having a large round root, is very similar to “Shogoin-kabu,” a Japanese turnip landrace. It is not easy to distinguish radish from turnip at a vegetable market. The greatest morphological difference between radish and turnip is the shape of siliques (Fig. 1.1). Siliques of Brassicaceae plants have two parts, i.e., a beak formed from the sub-stylar region and a part having valves, called the valvar portion (Gomez-Campo 1980). Although *Brassica* and most Brassicaceae species have seeds in the valvar portion without

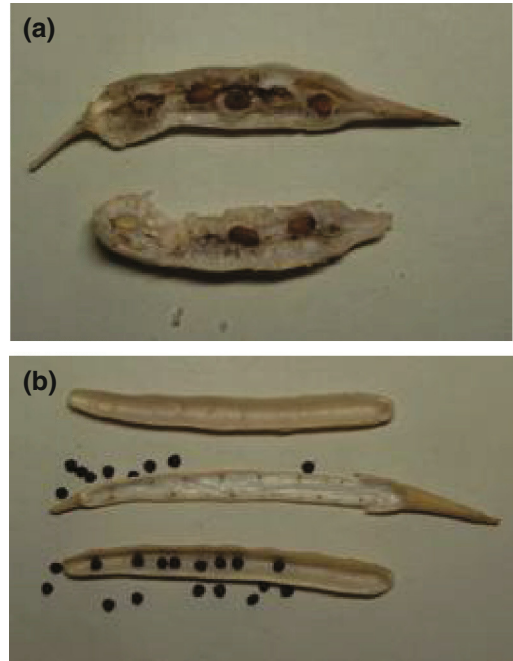


Fig. 1.1 Seed pods (siliques) of radish (a) and turnip (b)

seeds in the beak, radish has seeds in the beak and the valvar portion of radish is absent. Seeds of radish are arranged in a line in a silique and are much larger than those of *Brassica* and most other Brassicaceae species, which are arranged in two lines in a silique. Radish siliques do not dehisce and can float on water.

Raphanus sativus is closely related to *Raphanus raphanistrum*, which is a wild radish distributed as a weed in farmlands, roadsides, and coastal dunes in Europe, North America, and Australia. *R. sativus* has white or pale purple flowers, whereas *R. raphanistrum* has yellow flowers, although some lines of *R. raphanistrum* have white flowers. Siliques of *R. raphanistrum* are longer than those of *R. sativus* and can be fragmented into pieces containing a single seed. *R. raphanistrum* can be crossed with *R. sativus*, and hybrid progeny have normal seed fertility, indicating that these two species have the same genome. In Asian countries, *R. raphanistrum* is not common as a farmland weed, whereas *R. sativus* var. *raphanistroides* is widely distributed in coastal dunes. *R. sativus* var. *raphanistroides* can grow near the seaside but is rarely found in

farmlands or riversides, suggesting that it has high salt tolerance with low competitive ability as a weed in farmlands or riversides.

Although radish and *Brassica* species are distantly related, intergeneric hybrids between them can be obtained and radish is a useful genetic resource for breeding of *Brassica* crops especially in breeding for disease resistance and stress tolerance. In this chapter, biological and economical characteristics of radish are outlined.

1.2 Classification of Radish

Raphanus sativus and *R. raphanistrum* belong to the subtribe *Raphaninae*, which is different from the subtribe containing *Brassica*, *Sinapis eruca*, and *Diploaxis*, i.e., *Brassicinae*. The morphology of silique is an important taxonomic criterion to separate *Raphanus* from *Brassica*. However, recent molecular taxonomic studies using DNA polymorphism data of chloroplast DNA (cpDNA) and nuclear DNA do not support the grouping of *Brassica*, *Sinapis eruca*, and *Diploaxis* excluding *Raphanus*, but suggest that *Raphanus* is closely related to *Brassica rapa* and *Brassica oleracea* (see Chap. 2).

The tribe *Brassicaceae* contains seven subtribes: *Brassicinae*, *Cakilinae*, *Moricandiinae*, *Raphaninae*, *Savignyinae*, *Vellinae*, and *Zillinae*. Molecular phylogeny based on cpDNA restriction site polymorphism has indicated that the tribe *Brassicaceae* is separated into seven lineages, i.e., Rapa/Oleracea lineage, Nigra lineage, Crambe lineage, Cakile lineage, Vella lineage, Savingya lineage, and Zilla lineage. Cakile lineage, Vella lineage, Savingya lineage, and Zilla lineage correspond to *Cakilinae*, *Vellinae*, *Savignyinae*, and *Zillinae*, respectively, but species in *Brassicinae*, *Raphaninae*, and *Moricandiinae* are intermingled and reclassified into Rapa/Oleracea lineage containing *B. rapa*, *B. oleracea*, *Eruca vesicaria*, *Erucastrum abyssinicum*, *Diploaxis erucoides*, *Moricandia arvensis*, etc., Nigra lineage containing *Brassica nigra*, *Brassica fruticulosa*, *Sinapis arvensis*, *Sinapis alba*, *Erucastrum varium*, etc., and Crambe lineage (Warwick and Black 1997; Warwick and Hall

2009). *R. sativus* and *R. raphanistrum* belong to the Rapa/Oleracea lineage. Phylogenetic studies using nucleotide sequence data of the nuclear *S*-locus related 1 (*SLRI*) gene (Inaba and Nishio 2002) and the nuclear ribosomal internal transcribed spacer (ITS) and chloroplast *trnL* intron sequences (Warwick and Sauder 2005) have shown a slightly different classification of species, but *R. sativus* has always been found to belong to a group of *B. rapa* and *B. oleracea*. These findings suggest that morphological change of the silique from the *Brassica* type to the *Raphanus* type in the Rapa/Oleracea lineage occurred independently from that in the Crambe lineage, the Cakile lineage, and the Zilla lineage, which also have siliques of the *Raphanus* type.

1.3 Genome of Radish

Radish is a monogenomic species having a chromosome number of gametes (n) of nine. Although it is monogenomic, most genes have three similar copies in a genome as in the *Brassica* monogenomic species, suggesting that genome triplication has occurred after divergence from an ancestor of *Arabidopsis thaliana*. The chromosome number is the same as that of *B. oleracea*, but the genome structure of radish is quite different from those of *B. oleracea* and closely related *B. rapa*, as has been suggested by a classical genome study analyzing meiotic chromosome pairing (Harberd and McArthur 1980; Mizushima 1980). Genome synteny of radish with *B. rapa* or *B. oleracea* can be observed in short regions, but there are few overall synteny (Li et al. 2011; Shirasawa et al. 2011), suggesting that genome rearrangements have occurred many times in the speciation processes of these species (Mun et al. 2015; see Chap. 5). This is in contrast to the relationships between rice and other Poaceae species (Benetzen and Freeling 1997) and between tomato and other *Solanum* species (Peters et al. 2012).

Genome size of radish has been estimated to be from 526 (Arumuganathan and Earle 1991) to 573 Mb (Johnston et al. 2005) by flow cytometry. Unigene sequences of radish have been

published in the RadishBase (<http://bioinfo.bti.cornell.edu/cgi-bin/radish/index.cgi>) (Shen et al. 2013). The sequences of the whole radish genome were first reported by Kitashiba et al. (2014) and are available in “*Raphanus sativus* Genome Data Base” (<http://radish.kazusa.or.jp>). Using short-read genomic sequences of 191.1 Gb (246.5 times the radish genome size estimated by them) obtained by next-generation sequencers and both end sequences of 20,736 BAC clones, 76,592 scaffolds of 402 Mb spanning 75.9% of estimated genomic size containing 61,572 predicted genes were obtained. To a high-density linkage map of 2553 DNA markers, 1345 scaffolds were assigned. Using next-generation sequencing data 121.8 times the radish genome size, 40,123 scaffolds spanning 393.3 Mb have been constructed (Mitsui et al. 2015). Jeong et al. (2016) have also read the radish genome sequences and assembled them into 10,674 scaffolds spanning 426 Mb. In these studies, similar cultivars of Asian big radish were used for determination of the genome sequence. Reassembling of sequences or overlapping of scaffolds obtained in these studies may enable construction of much longer scaffolds covering large parts of the radish genome. Although these studies have so far been performed with the support of different organizations, collaboration of these teams is expected (see Chaps. 3 and 4).

1.4 Characteristic Traits of Radish

Raphanus sativus is an allogamous species having self-incompatibility. Therefore, populations of radish landraces and wild radish have high genetic polymorphism, which is reflected by DNA polymorphism in a cultivar (see Chap. 6). Self-incompatibility can be overcome by bud pollination or high-concentration CO₂ treatment (Niikura and Matsuura 2000), and selfed progeny can be obtained. However, they show inbreeding depression, making it difficult to obtain inbred lines.

Like self-incompatibility of *Brassica* species, S-receptor kinase (SRK) is the recognition molecule of the stigma, and SP11, also called

SCR, is the recognition molecule of pollen (Okamoto et al. 2004). The genes of these recognition molecules, i.e., *SRK* and *SP11/SCR*, have multiple alleles and are inherited as one set, which is called *S* haplotype, by progeny. There are many *S* haplotypes in *R. sativus* (Sakamoto et al. 1998; see Chap. 13), and nucleotide sequences of *SRK* and *SP11/SCR* of some *S* haplotypes in *R. sativus* are similar to those of *B. rapa*, suggesting that *S* haplotypes possessed by an ancient species were inherited by species in both *Raphanus* and *Brassica* without great alteration of nucleotide sequences (Okamoto et al. 2004).

Most radish cultivars are vegetables having thick roots. Size, shape, and color of roots are important traits of radish. Quantitative trait loci (QTLs) controlling root shape and color have been reported (Tsuru et al. 2008; Hashida et al. 2013). However, root thickening is influenced by other traits, such as flowering. In our QTL analysis of root thickness using a progeny obtained by crossing between “Aokubi” having a white thick root with green color on the top and a cultivar of rat’s tail radish, a QTL having the highest LOD score corresponded to a QTL for bolting time (unpublished). This may be natural because early flowering is considered to have an adverse effect on root thickening. Transcriptome analysis of growing roots has been performed, and genes related to root thickening were identified (see Chaps. 8 and 9).

The color of root surface in radish is derived from anthocyanins. Pigments of red color and purple color of radish cultivars are pelargonidin and cyanidin, respectively. A hybrid between a red root line and a white root line was found to have purple roots, suggesting that the red and the white have knockout mutations in different genes responsible for cyanidin synthesis and the functional alleles in the red and the white acted as complementary genes. Alleles of the gene for flavonoid 3'-hydroxylase (F3'H) in red root cultivars have been revealed to have insertions of a Ty3/gypsy transposon or a helitron (Ozeki 2010). A gene for dihydroflavonol reductase (*RsDFR*) and a gene for anthocyanidin synthase (*RsANS*) are expressed in the epidermal tissues of roots of

a red-skinned cultivar, whereas these genes are not expressed in those of a white-skinned cultivar (Park et al. 2011).

The large seed size is a remarkable trait of radish among Brassicaceae species. Seed size of radish is about five times in weight that of *B. rapa*. Due to the large seed size, cotyledons and hypocotyls of seedlings are larger than those of *Brassica*. The larger size of seedlings facilitates cultivation by direct sowing of seeds in the field and produces bigger sprouts than *Brassica*. The large seed trait is related to the shape of siliques. Amphidiploid plants of intergeneric hybrids between *R. sativus* and *B. rapa* have siliques of an intermediate type, which has both the beak and the valvar portion with a few seeds (Fig. 1.2). The amphidiploid plants are partially sterile, but a small number of seeds can be

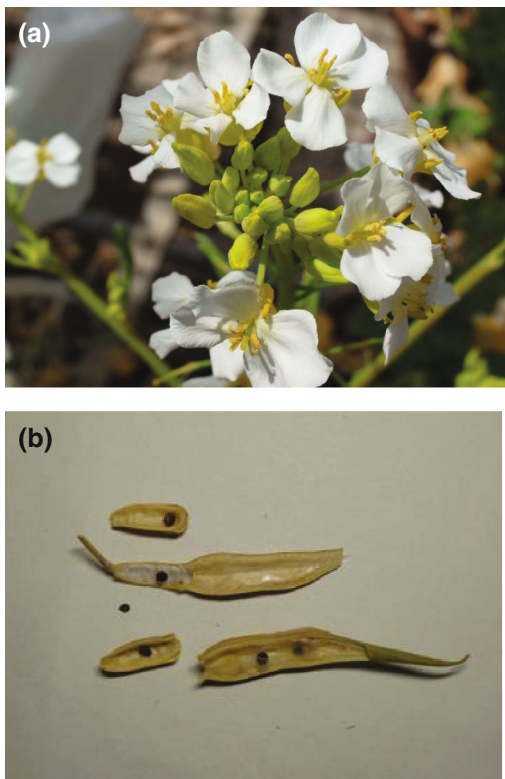


Fig. 1.2 Flowers (a) and a silique (b) of an amphidiploid intergeneric hybrid between turnip and radish. Diploid hybrid plants between them are sterile, whereas amphidiploid (tetraploid) hybrid plants are fertile and set seeds. (Provided by Dr. K. Tonosaki)

obtained. The size of the seeds is also intermediate between *Raphanus* and *Brassica*.

The pungency of radish is due to isothiocyanates, which are hydrolyzed products of glucosinolates. The content of glucosinolates has a great influence on the taste of grated fresh radish and radish salad. The major glucosinolate in radish roots is glucoraphasatin (also called 4-methylthio-3-butenyl glucosinolate, dehydroerucin), and variation of the glucosinolate composition is limited. However, there is a great variation in the contents of glucoraphasatin in Japanese radish cultivars (Ishida et al. 2015). QTLs controlling glucosinolate content in the root have been reported (Zou et al. 2013), and candidate genes responsible for the content have been inferred. Recently, a mutant having a high amount of glucoerucin without glucoraphasatin has been selected (Ishida et al. 2015), and the gene responsible for this mutation has been identified (Kakizaki et al. 2017).

Although radish generally has high salt tolerance, such tolerance of *R. sativus* var. *raphanistroides* is especially high (Nasu et al. 2012). Although genetic analysis of salt tolerance of *R. sativus* var. *raphanistroides* has not yet advanced, the genes for salt tolerance of radish will be useful for breeding of *Brassica* crops. In the production of radish, high-temperature stress has become a serious problem. The core of a radish root turns reddish brown by the high-temperature stress, resulting in unmarketable products. However, since there is a variation in sensitivity to the high-temperature stress, it should be possible to develop a tolerant cultivar.

After bolting, radish roots become fibrous and unsuitable for markets. Therefore, a late bolting trait is preferred. One the other hand, rat's tail radish or cultivars for oil production are requested to flower even in tropical regions. For floral induction, vernalization is required, but rat's tail radish can flower without vernalization. There is a large variation in vernalization requirement in radish as in many other winter crops. In a QTL analysis of bolting time using a progeny obtained by a cross between "Aokubi" and rat's tail radish, a QTL having a significant LOD score was

detected in a region containing an *FLC* gene (see Chap. 11).

Although clubroot caused by *Plasmodiophora brassicae* is a serious problem in the production of *Brassica* vegetables, Japanese and Korean cultivars of radish are generally tolerant to clubroot. Radish can be used as a source of resistance genes for the breeding of *Brassica* vegetables (Akaba et al. 2009). A QTL controlling clubroot resistance has been mapped on LG1 (Kamei et al. 2010), corresponding to Rs5 (Kitashiba et al. 2014, Chap. 3). In radish cultivation, *Fusarium* yellow caused by *Fusarium oxysporum* is one of the most serious diseases. Leaves wilt and the vascular tissue in a root is browned by this disease. Some cultivars and landraces have relatively high resistance to *Fusarium* yellow (see Chap. 12).

Radish is recalcitrant in tissue and cell cultures because of its low plant regeneration ability. There have been few reports on successful protoplast culture, anther or isolated microspore culture (Takahata et al. 1996), and plant transformation (Park et al. 2005; Cho et al. 2008). The difficulty of plant transformation makes it impossible to demonstrate the function of isolated genes in radish. Development of an efficient plant transformation technique is indispensable for basic and applied studies in radish. Since there must be genetic variation in plant regeneration ability, identification of cultivars or lines having high regeneration ability would be the first step for the development of in vitro culture techniques.

1.5 Use of Radish as a Vegetable, a Feed, and Materials

Although radish was once the most cultivated vegetable in Japan, its production has been decreasing because consumption of radish pickles has recently decreased. Exploring novel ways of utilization is required for increasing the economic value of radish. European small radish is used for salad after slicing or rough cutting. Asian big radish has not been used for salad, but shredded white radish, which is a garnish of

sliced raw fish, is increasingly used in a salad. Since crispiness is important for the shredded radish, white radish cannot be replaced by turnip roots. The white radish root can be stored and is inexpensive, and therefore, it is a good material for use in salad.

Tempura and shabu-shabu, popular Japanese dishes, are garnished with grated radish, which contains amylase and other enzymes to help digestion. Grated radish is also served with grilled fish or dried young sardines. Grated radish is pungent because of its high content of isothiocyanates. By disruption of cells by grating, glucosinolates are hydrolyzed by the enzyme myrosinase, which is stored in the vacuoles of myrosin cells. Isothiocyanates have anti-bacterial and anti-fungal activity, and some isothiocyanates have been revealed to have anti-carcinogenic activity (Ishida et al. 2015). The isothiocyanate produced from the major glucosinolate in roots, i.e., 4MTB-GSL, has also been reported to be able to reduce cell proliferation and induce apoptosis in cancer cell lines with very limited toxicity toward normal human T-lymphocytes (Papi et al. 2008). Due to the increasing popularity of Japanese cuisine, consumption of grated radish will most probably increase.

A popular boiled dish containing white radish is “oden,” which is served even in convenience stores in Japan. In Chinese cuisine, Asian big radish is mainly served in boiled dishes. Although digestive enzymes and isothiocyanates in radish roots are lost, pieces of radish roots absorb soup, resulting in good taste. There are various types of radish pickles in Japan, e.g., takuanzuke, bettarazuke, asazuke, and fuku-jinzuke, but consumption of radish pickles has decreased. In Korea, Asian big radish is used as a material of kimuchi.

Radish sprouts, called “kaiware-daikon,” are consumed with sushi. Consumption of kaiware-daikon has decreased in Japan after the incident of bacteria O157 tainting of kaiware-daikon in 1996. As fresh green vegetables, only kaiware-daikon, cucumber, and labiate leaves are served with sushi. The international popularity of sushi may result in increased

consumption of kaiware-daikon. As in radish roots, kaiware-daikon contains 4MTB-GSL, a major glucosinolate (Barillari et al. 2005; Papi et al. 2008).

Like *Brassica* seed oil, radish seed oil contains a high amount of erucic acid, about 30–40% (Ahuja et al. 1987), which is considered to be harmful as a food. Erucic acid is known to be suitable for industrial use, such as for use as a lubricant. It is promoted to make hair velvety, and radish seed oil is marketed as cosmetics. Residue of oil extraction can be used for extraction of glucosinolates. The major glucosinolate in radish seeds has been reported to be glucoraphenin, which is hydrolyzed into sulforaphene (Barillari et al. 2005). Sulforaphene has been indicated to have 1.3–1.5 times higher activity to inhibit mutagenesis induced by cooked food mutagens than sulforaphane (Shishu 2009).

Availability of radish as a winter cover crop to avoid soil erosion and loss of soil nitrogen has been reported (Weil and Kremen 2007). Radish can alleviate soil compaction by drilling with thick roots, which are killed by cold temperature, resulting in holes. Radish can suppress weeds more effectively than other cover crops, e.g., rye and rape. Furthermore, cover crops of *Brassica* can reduce soil nematodes (Weil and Kremen 2007), and radish is also considered to be effective in control of nematodes.

1.6 Breeding of Radish and Use of Radish as a Breeding Material

Most modern cultivars of radish are hybrid cultivars. Old cultivars and landraces are mass selected cultivars having some genetic variations within a cultivar. Since inbreeding depression is remarkable, efforts to enhance uniformity of a cultivar result in decrease of plant vigor. As a root vegetable cultivar, uniformity of root shape and color is necessary, but some variations in leaf shape and other traits can be allowed. To develop a cultivar having high uniformity, hybrid breeding has been used (see Chap. 15). The parents of a hybrid cultivar are inbred lines. Although

repeated selfings to develop inbred lines make plants less vigorous, some lines showing less inbreeding depression can be selected from a large number of inbred lines. These inbred lines are maintained and propagated by bud pollination by hand. Since the number of seeds obtained by one time of the hand pollination is small, double cross or three-way cross is preferred to single cross, which can provide more uniform progeny than double cross.

In the production of hybrid seeds, self-incompatibility or male sterility is used for avoiding self-fertilization. Self-incompatibility is sometimes unstable under some stress conditions, e.g., high temperature, and contamination of hybrid seeds by selfed seeds may increase. Since such contamination spoils the quality of hybrid seeds, examination of contaminated seeds using isozyme or DNA markers is indispensable. To reduce the percentage of selfed seeds, selection of parental lines having strong and stable self-incompatibility is required. The strength of self-incompatibility depends on *S* haplotypes. Therefore, a simple technique for *S* haplotype identification is required. The methods developed for identification of *S* haplotypes in *B. rapa* and *B. oleracea* can also be used in radish (Nishio et al. 1996; Sakamoto et al. 1998; Niikura and Matsuura 1998; Lim et al. 2002).

Although it is difficult to use male sterility in hybrid seed production by double cross, risk of contamination of selfed seeds in single cross or three-way cross is lower in the use of male sterility than that in the use of self-incompatibility. Furthermore, restoration of pollen fertility in male-sterile lines under some growing conditions, e.g., high temperature, can be easily noticed by visual inspection of plants. The source of the well-known cytoplasmic male sterility used in rapeseed breeding is radish, i.e., Ogura cytoplasm (see Chap. 7). The same cytoplasm has been found in landraces in radish, *R. sativus* var. *raphanistroides*, and *R. raphanistrum* (Yamagishi and Terachi 1996). Although a restorer gene is not necessary in hybrid breeding of root vegetables, a gene useful in the breeding of rapeseed, possibly also of rat's tail radish, has been identified (Koizuka et al. 2003).

Mutation breeding of radish has not been successfully performed because it is difficult to distinguish induced mutations and variations present in radish cultivars. Furthermore, most mutated traits are controlled by recessive alleles and are not suitable for hybrid breeding, which combines dominant alleles of parents. However, all the landraces having characteristic traits have been developed by spontaneous mutations. Recently, a cultivar having a new composition of glucosinolate has been developed by selecting a spontaneous mutation in a landrace (Ishida et al. 2015). Techniques for reverse-genetic selection of mutants have been developed. Although TILLING (McCallum et al. 2000), which has been successfully used for reverse-genetic selection of mutants in many plant species, is not suitable for use in radish cultivars, which has many gene variations in a cultivar, mutant selection using a next-generation sequencer, e.g., KeyPoint® of Keygene, may be available for use in mutation breeding of radish. Another cost-effective technique for reverse-genetic selection is required.

Because of the difficulty of plant regeneration, genetic engineering has not been used in radish breeding. However, many GMO cultivars have been developed in soybean, which is also recalcitrant in tissue and cell culture. The reason why such efforts have not been made in radish may be lower economic value of radish than that of soybean. Another problem of using genetic engineering in radish breeding may be wide distribution of wild radish in the world. Stress tolerance genes and insect resistance genes can be transmitted to wild plants, providing higher competitive ability. Since flowering is not necessary in root vegetable production, the use of a sterility gene, e.g., pollen sterility gene used in the seed production technology (DuPont-Pioneer), may be useful.

Intergeneric hybrids between *R. sativus* (as a female parent) and *B. oleracea*, named *Raphanobrassica* (Karpechenko 1924), and between *B. rapa* (as a female parent) and *R. sativus*, named *Brassicoraphanus* (Terasawa 1932), have long been studied (see Chap. 14). Efficiency of hybrid production without embryo

culture between *B. rapa* and *R. sativus* depends on cultivars of *B. rapa*, and three QTLs controlling hybrid formation ability have been identified (Tonosaki et al. 2013). Crossability of *R. sativus* with *Brassica* suggests that *Brassica* genetic resources may be available for use in radish breeding. Since *R. sativus* has valuable traits for *Brassica* breeding, e.g., disease resistance (Akaba et al. 2009), nematode resistance (Lelivelt et al. 1993), stress tolerance, and a different composition of glucosinolates, the economic value of radish as a breeding material of several *Brassica* crop species can be emphasized.

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