Chapter 11

Raphanus

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11.1 Introduction

Members of *Raphanus* are annual and outcrossing plant species of the Brassicaceae family. They are believed to have originated in the regions from Mediterranean to Black Sea. The genome was described as RR and RrRr for *Raphanus sativus* and *R. raphanistrum*, respectively (Baranger et al. 1995; Chevre et al. 1996; Darmency et al. 1998). The somatic chromosome number of *Raphanus* species is $2n = 18$ (Banga 1976; Harberd 1976; Prakash et al. 1999). Genome size of *R. sativus* was reported to be ca. 526 Mbp (Arumuganathan and Earle 1991) and 573 Mbp of 0.583 pg of genome content (Johnston et al. 2005). These values are intermediate between 529 Mbp (0.539 pg) for *Brassica rapa* and 696 Mbp (0.710 pg) for *B. oleracea* (Johnston et al. 2005), although genome size of *R. raphanistrum* is not yet clear.

Seven species compose the genus *Raphanus* classified under two sections of *Raphanis* DC. and *Hesperidopsis* Boiss. Section *Raphanis* comprises six species including *R. sativus*, *R. raphanistrum*, *R. microcarpus*, *R. rostras*, *R. maritimus,* and *R. landra*, and section *Hesperidopsis* includes only one species, *R. aucheri* (Kitamura 1958). On the other hand, Fujii (1977) and Hida (1990) supposed six species except for *R. aucheri*, and even eight species were proposed by Cheam and Code (1995). Banga (1976), George (1985), and Hida (1990) independently presumed that four wild species, *R. raphanistrum*, *R. maritimus*, *R. landra,* and *R. rostras,* might be involved in the development of cultivated radish. These wild radishes are growing as the dominant weedy plants around the coastal areas of the Sea of Marmara and the Bosphorus Straits of Mediterranean (Dixon 2007). Harberd (1976) proposed that the genus *Raphanus* could be included into a cytodeme based on the somatic chromosome number, chromosome configuration at MI in pollen mother cells (PMCs) and fertility supported by Prakash et al. (1999) and Dixon (2007). Tsunoda (1979) and Hinata (1995) proposed that these wild species were included in *R. raphanistrum* and the genus *Raphanus* was classified into two species, *R. sativus* and *R. raphanistrum.*

11.2 Application of Wild Species for Radish Breeding

Potential of wild radishes and their hybrid lines was evaluated by Matsuzawa et al. (1996), but the genetic resources of wild species have not been exploited to achieve divergent taste, tolerances to new disease and insect damage, effective seed production system, and so on. The agricultural traits of the genus *Raphanus* were summarized by Warwick (1993). These include cytoplasmic male-sterility (CMS) (Ogura 1968; Yamagishi and Terachi 1996), resistance to pod shattering (Agnihotri et al. 1991), tolerance to saline soils (Warwick 1993), and resistance to blackleg (Salisbury 1987).

11.3 Domestic and Wild Radish

Brassica juncea and *B. rapa* grow in a natural state at the banks and roadsides. *B. napus* was also growing in the semi-wild at the Meiji Period. A wild radish

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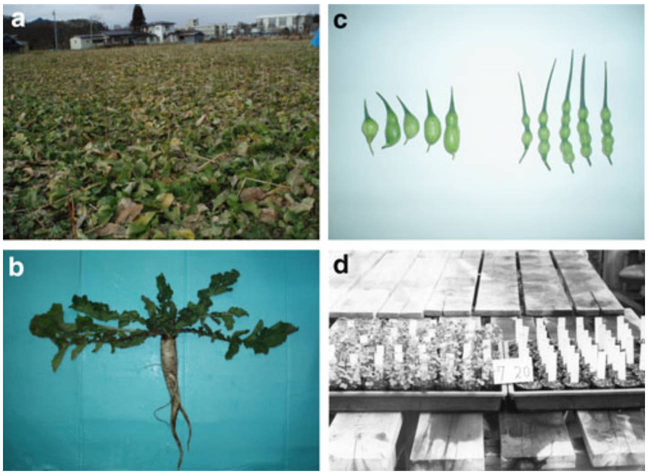

Fig. 11.1 Characteristics of Japanese wild radish. (**a**) Koubou-daikon population at Yonezawa, Yamagata Pref., (**b**) Morphology of Koubou-daikon, (**c**) Pods of Koubou-daikon (*right*) and cultivated radish (*left*), (**d**) Dormancy of Hama-daikon in about 1 month after sown

(*R. sativus* L. var. *hortensis* f. *raphanistroides* Makino) growing in the coastal area of Japan, Korea, and South China is known as Hama-daikon (Furusato and Miyazawa 1958; Kitamura 1958; Ohnishi 1999). This species have been considered to be involved in the development of domestic varieties such as Moriguchi, Hatano, and Hosone (Fujii 1977). The other wild radish, known as Nora-daikon or No-daikon, was grown at the restricted inland areas in Tohoku district (Aoba 1967, 1988), and was suggested to be the island type of Hama-daikon by Ohnishi (1999). No-daikon has been grown around the areas 70 km away from the coast being isolated by the mountainous region. This radish was called Koubou-daikon, since Saint Koubou had introduced it to this area about 1,200 years ago. In the 1950s, large colonies were identified in Yamagata and Fukushima Prefectures (Aoba 1981). The plants of this wild radish grow naturally in the field where other crops such as buckwheat was harvested and plowed over (Fig. 11.1a). Their leafy rosettes vigorously grow covering the field and cannot be harvested by hand for development of lateral roots (Fig. 11.1b).

No-daikon was assumed to be relative to Kozena that was locally cultivated at the Kozena Area in the Onoda Village, Miyagi Pref. Kozena is one of the important resources for CMS (Namai 1991; Sakai and Imamura 1994, 2003), although people do not take the plumped root but harvest the fresh leaf of this radish (Furusato and Miyazawa 1958; Sasaki 1994).

Both Hama-daikon and No-daikon are sharply pungent and develop solid roots, and constricted pods yielding a seed (Fig. 11.1c). The 100 seeds weights are ca. 1.306 g, 2.463 g and 2.823 g for Hama-daikon, Minowase, and Horyo, respectively. Wild radishes showed seed dormancy as shown in Tables 11.1–11.3. Most of the seeds did not germinate immediately after sowing (Table 11.1; Fig. 11.1d). Within a month after sowing, 13% of seeds germinated and about 50% of them germinated within 45 days after sowing (Table 11.2). Seed dormancy was broken by chilling treatment of more than 12 h (Table 11.3). The seed dormancy was also observed for wild radish, Koubou-daikon (Aoba 1967) and *R. raphanistrum* examined at Victoria in Australia (Cheam and Code 1995).

Recent studies on wild radishes are focused on analysis of origin of cultivated radishes and evolution of wild radish, model system of competition and suppression between wild radish and their hybrid groups in California, and gene flow from transgenic rapeseed (*B. napus*) to wild radishes or other relatives. For the progress of works on genetics, breeding, and ecology of radishes, it might be important to analyze the genomic feature of wild radishes including Japanese wild ones.

11.4 Researches in Origin of Radish Using Gene Markers

The origin of cultivated radish is not so clear even though the molecular biology studies have been performed.

Table 11.1 Dormancy of Japanese wild radish (Hama-daikon)

No. of strain observed	Date sown	
	1-Jul	31-Jul
3[a]	0	13.3
Cultivated radishes		
Minowase	46.7	93.3
Horyo	63.3	100

[a] Seeds were harvested from Chiba Pref. on 25 June 1993

Table 11.2 Seed germination rate in Japanese wild radish

No. of strain observed	Date of observation[b]				Total germination rates (%)
	27 Jul	11 Aug	22 Aug	02 Sept	
3[a]	10	11.7	20	6.7	48.4
Cultivated radishes					
Minowase	100				
Horyo	100				

[a] Seeds were harvested from Chiba Pref. on 25 June 1993
[b] Seeds were sown on 18 July 1993

Table 11.3 Effects of chilling treatment on dormancy in Japanese wild radish

No. of strain observed[a]	Hours of chilling	Date of observation[b]		Total germination rates (%)
		10 Aug	25 Aug	
3	12	47	29.1	76.1
3	24	51.7	20.8	72.5
3	72	38.1	15.4	53.5

[a] Seeds were harvested from Chiba Pref. on 25 June 1993
[b] Seeds were sown on 01 Aug 1993

For this purpose, Yamagishi and Terachi (1994, 1996) studied on the *orf138* gene of mitochondrial DNA (mtDNA) in cytoplasm (Ogura type; Ogura 1968) that induced CMS. Distribution of *orf138* gene was surveyed for radish cultivars and wild radishes (Hama-daikon) were collected in Japan. However, *orf138* gene could not be detected in the cultivars except for a few ones (MS Gensuke, Kozena, Nou-No.1 bred in Okinawa Pref., Sabaga). On the other hand, this gene was widely distributed in more than 40% of wild radish populations growing in East Asia including Japan and could be identified in both *R. raphanistrum* and *R. maritimus* (Yamagishi and Terachi 1997, 2003). It was assumed that the Ogura cytoplasm carrying the *orf138* gene might be originated in the course of cytoplasmic differentiation to wild radish after the genus *Raphanus* had evolved (Yamagishi 2006).

The nucleotide sequence of an entire coding region of *orf138* was examined for 107 plants of Japanese wild radish, 29 cultivars, and seven strains of *R. raphanistrum* (Yamagishi and Terachi 2001). Six sites of nucleotide change, and one single insertion/deletion (39 bp) were observed in the *orf138* region from wild and cultivated radishes. By the analysis of these seven mutations, the *orf138* sequences of 143 plants were classified into nine types (Type A to Type I) and two types (B and C), eight types except for H type and five types (A, B, E, F and H) were identified in *R. raphanistrum*, Japanese wild radishes, and cultivars, respectively. The type B was detected in 41% of Japanese wild radishes, and found in 13 out of 19 populations growing in Japan. In addition, this type was more frequent (86%) in *R. raphanistrum* suggesting that Type B is one of the ancestral types of *orf138*. Giancola et al. (2007) recently found the mutated *orf138* gene in three populations from France and one from South England, although it was of low frequency. The Ogura-type cytoplasm carrying Type B and Type C sequences might have evolved in *R. raphanistrum*. Furthermore, these cytoplasms might introgress into Japanese wild radish along with the nuclear *Rf* gene(s). By the aid of the *Rf* gene, Ogura-type cytoplasm was transmitted into wide range of radishes in Japan. Several independent mutations occurred in *orf138* of Japanese wild radish, and the variants including Type A have been maintained in populations. Ogura-type cytoplasms with specific types of *orf138* have also been introgressed into some native varieties (Yamagishi and Terachi 2001; Yamagishi 2006). Iwabuchi et al. (1999) suggested *orf125* to be CMS-inducing gene for domestic variety Kozena grown in Miyagi Pref. of Tohoku district. On the other hand, Yamagishi (2006) described that *orf125* might be equivalent to Type F of Japanese wild radish growing in Yamagata and Aomori prefectures of Tohoku district.

Out of nine types of *orf138,* five types – A, B, D, F and H – were ascertained to induce male-sterility. On the other hand, it is important to survey the nuclear restorer gene (*Rf* gene; Brown et al. 2003) inhibiting the expression of *orf138* for pollen fertility. *R. raphanistrum* and most of the Japanese wild radishes had the restorer gene, although a half of the cultivated radishes were carrying it (Yamagishi 1998). Twenty-three out of 28 Chinese and European varieties possessed the restorer gene, although 20 out of 28 Japanese varieties did not carry it. Koizuka et al. (2003) isolated the *Rf* gene, *orf687*, from the Chinese radish var. Yuan hong, and Giancola et al. (2007) also found *Rf* gene in *R. raphanistrum* from France. In addition, Yasumoto et al. (2008) investigated the distribution of the *Rf* gene in Japanese wild radish including 226 individuals collected from 15 regions in Japan and two regions of Korea. The examined 95 plants (42%) of the Japanese wild radish possessed *orf138*, 207 (91.6%) had the *Rf* gene in both Japanese wild radish and F_1 hybrids bred by CMS variety with *orf138*. By polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the nucleotide sequence of between the *Rf* gene and *orf687*, it was clarified that plants with an identical RFLP pattern of *orf687* were restrictively found in three populations collected from southern region of Japan including Iriomote, Zanpazaki, and Imajuku.

From these studies, it was confirmed that the *orf138* gene (Ogura-CMS gene; Ogura 1968) identified in Japanese native variety is now widely distributed among *R. raphanistrum* in Europe and Japanese wild radish. Moreover, the origin of cultivated radish could be demonstrated by the molecular analysis of the wild radish species.

11.5 Origin of Japanese Wild Radish

Huh and Ohnishi (2001, 2006) investigated the genetic diversity of Japanese wild radish growing around East Asia using allozyme and amplified fragment length polymorphism (AFLP) marker analysis. The Korean populations were of smaller size being isolated like dots, but they maintained a high level of genetic diversity. The average percentage of polymorphic loci, the mean number of alleles per locus, and the average heterozygosity for Korean populations were 63.1%, 2.27, and 0.281, respectively, whereas these parameters for Japanese populations were 53.3%, 2.26, and 0.278, respectively. These results might show the higher genetic diversity in the Japanese wild radishes. These parameters for *R. raphanistrum* in Europe were 94%, 3.25, and 0.45, respectively. In AFLP analysis, both Japanese and Korean populations of Japanese wild radishes showed a wide range of variation where the average percentage of polymorphic loci and the average heterozygosity were 66.3% and 0.128, respectively. *R. raphanistrum* and two lines of *R. sativus* from Kazakhstan were confirmed to be closely related. Therefore, *R. raphanistrum* might have an involvement in the origin of *R. sativus*.

As has been mentioned, plants with cytoplasm carrying *orf138* and nuclear *Rf* gene are widely distributed in Japanese wild radishes, but most of the Japanese cultivars have not incorporated these genes. The European and Chinese varieties had the *Rf* gene, but many varieties except for those in Taiwan have not yet carried the *orf138* gene. Therefore, Yamagishi (2006) suggested that Japanese wild radish might be the descendants from *R. raphanistrum*. Terachi et al. (2001) investigated the nucleotide sequence of the entire coding and flanking regions of *orfB* using *R. raphanistrum*, Japanese wild radishes and cultivated ones. The plants with Ogura male-sterile (MS) cytoplasm carried only the type 1 sequence, whereas the plants with normal cytoplasm had either type 2 or type 3. As regards to the distribution of these three types among varieties and populations, 78% of cultivated radishes exclusively possessed type 2 and 92% of Japanese wild radishes had two or three types (Yamagishi 2004). These facts might suggest that the Japanese wild radishes were not the escape from cultivated radish but the descendants from *R. raphanistrum* (Yamagishi 2006).

11.6 Roots of the Cultivated Radish

Yamagishi and Terachi (2003) analyzed the structural variations in the mitochondrial *cox*I and *orf*B regions by PCR using three wild species (*R. raphanistrum*, *R. landra*, *R. maritimus*) and 43 cultivated radishes. Five (I–V) and four (I–IV) types of mtDNA were identified for *R. raphanistrum* and *R. maritimus*, respectively. They considered that the cytoplasmic differentiation of these two species might be too small to discriminate them as independent species. All of the European cultivars were type II and 60% of Chinese ones were type III. Japanese cultivars classified into type I (42%) and type III (42%) were common to the northern Chinese cultivars. Yamagishi et al. (2005) also analyzed the sequences of *trn*L to *psb*G region in the chloroplast genome by PCR-RFLPs using 118 plants of 22 strains in three wild species (*R. landra*, *R. maritimus*, and *R. raphanistrum*), 219 plants of 13 populations in Japanese wild radishes and 80 plants of 48 varieties in the cultivated radishes. They identified eight haplotypes, although type 1 and type 2 could not be classified. All the seven types were identified in *R. raphanistrum* and five types in *R. maritimus* showed the highest rate (40.6%) of type 3 in the two species. Japanese wild radish contained 39.3% of type 8, 33.6% of type 7 and 27.4% of type 4. In Japanese wild radishes, the prominent genetic diversity clearly showed that 11 populations out of 13 observed maintained more than two types. The cultivated radishes were classified into four types including 1/2, 4, 7, and 8, and a large number of European varieties was of type 4 (66.7%), the Chinese and Japanese varieties were of type 7 (42.4%), type 4 (36.4%), type 8 (18.2%), and 1/2 type (3%), respectively. On the other hand, Yamane et al. (2005) investigated the nucleotide sequence of the 5′-*mat*K region of chloroplast DNA and five regions (*apt*A–*apt*I, *ndh*D–*ndh*H, *rpo*A–*rps*3, *rpo*C3–*trn*C and *psb*B–*psb*H) by PCR-RFLP analysis for 17 accessions including the cultivated radish, *R. raphanistrum*, and East Asian wild radish. They suggested that *R. raphanistrum* was not the maternal ancestor of the cultivated radish, and the East Asian wild radish has contributed to the establishment of the East Asian cultivated radish. Furthermore, Yamane et al. (2009) also studied the origin of cultivated radish by single sequence repeat (cpSS) variation in chloroplast DNA using 59 cultivated radishes and 23 strains of three wild *Raphanus* species. Seven of the 25 cpSSRs studied were polymorphic (two to four alleles), and a total of 20 haplotypes were designated as A to T. Haplotype E is widely distributed in Europe and East Asia. Haplotype N is geographically restricted in Asia. A total of 13 haplotyes were detected in wild *Raphanus*, and seven of them (A, B, G, J, Q, S, and T) were distributed only in them. By minimum-spanning network (MSN) analysis, it was found that there were at least three independent domestication events, relating black Spanish radish and those grouped with two distinct cpSSR haplotypes. Yamane et al. (2009) presumed that the Asian cultivated radish was not originated from the diffused descendants of European cultivated radish, but probably originated from a wild species that is distinct from the wild ancestor of European cultivated radish.

As a result, the cultivated radishes were suggested to be developed through multiple origin, a part of which cytoplasm might inherit from wild relatives of the genus *Raphanus*, plants maintaining differentiated cytoplasm were independently domesticated at the districtive area. A few types of cytoplasm from *R. raphanistrum* were transmitted to the Japanese wild radish. The cultivated radish developed in the Eurasian continent might have produced a large number of varieties around the world. In Japan, the ancestors with the distinct two types of cytoplasm might be firstly introduced, and then new domestic cultigens have evolved through hybridization between types. The wild radishes distributed widely in the Eurasian continent, Japanese wild radish originated the wild radishes adapted to East Asia, and then, the native varieties have been bred by the hybridization among Japanese wild radish (Yamagishi 2006).

Genetic approaches for wild and cultivated radish of Eurasian Continent would provide some potential information to understand the evolutional relationships of radishes around the world.

11.7 Characteristics of *R. raphanistrum* as a Weed

R. raphanistrum grows vigorously as a weed in all continents except for Antarctica (Hinata 1995; Holm et al. 1997). Two species of *R. raphanistrum* and *R. sativus* grow in the wild in North America (Warwick 1993), and the authors observed both these species in Paraguay and South America also (Fig. 11.2). In Australia, three species have been identified:

Fig. 11.2 The wild radishes in Paraguay, South America

R. raphanistrum, *R. maritimus,* and *R. sativus* (Cheam and Code 1995). Lefol et al. (1997) indicated that *R. raphanistrum* was a major weed in Weed Seeds Order of the Canadian Seed Act. This species is distributed in the provinces on the Atlantic seaboard, the Canadian Pacific coast, but is infrequent in the prairie region of western Canada.

Wild radish not only has colonized new regions, but also it has caused yield losses in a variety of crops (Sahli et al. 2008). In Australia, the weedy *R. raphanistrum* is estimated to bring about an average yield loss of 2 ton ha^{-1} and A$ 30 ha^{-1} for herbicide in rainfall areas (Streibig et al. 1989). At Rutherglen and Victoria, 10% and 50% of yield losses were confirmed in the field with only seven wild plants m^{-2} and 200 wild plants m^{-2}, respectively (Cheam and Code 1995). Webster and MacDonald (2001) surveyed 83 troublesome weeds in Georgia and wild radish was found to be the most troublesome one both in the fields of cereals and vegetables. Wild radish is one of the most troublesome weed for the production of spring-sown wheat and barley in Sweden (Bostrom 2003).

Lehtila and Brann (2007) performed a selection for flower size of *R. raphanistrum* concerning to both reproductive and vegetative traits through two generations. The lines having large flowers produced smaller seeds and flowered later than the lines with small flowers. The lines selected for large flower size had more flowers and a larger plant size than those selected for small flowers. The authors also showed that flower size had a positive genetic correlation with flowering and plant height. As to the pollination systems and pollinators for propagation of weedy *R. raphanistrum*, Conner et al. (1995) surveyed the effect of flower morphology of wild radish on pollination using four pollinators: honey bees, small native bees, butterflies, and syrphid flies. The nectar-feeding butterflies had higher potentials for pollination than both the nectar- and pollen-feeding honey bees. Flowers with intermediate stigma exertion had the highest number of pollen grains deposited on their stigma by butterflies, but stigma exertion had no effect on the deposition by honey bees. For both butterflies and honey bees, pollen deposition on the recipient flower increased with the amount of pollen removed from the donor. Furthermore, Conner and Rush (1996) also observed the effects of flower number and the size on visitation of syrphid flies and small bees to wild radish for 3 years could clarify the positive correlation between both flower number and corolla size and the number of flower-visiting syrphid flies. Increase in flower size might cause a little increase for small bee. Additionally, they observed that the 12 small bees of three families (Anthophoridae, Colletidae, and Halictidae) and syrphid flies of nine genera visited to *R. raphanistrum* in 2 years. Sahli and Conner (2007) inspected the pollinators to *R. raphanistrum* and showed that sweat bees of the genus *Dialictus* visited more frequently than the syrphid flies *Toxomerus*, *Syritta*, and *Eristalis*. These four genera accounted for 81% of visitors to *R. raphanistrum,* although 14 visitors of 15 genera were effective pollinators. On the other hand, effects on seed setting widely varied from 0.10 seeds a visit of *Sphaeorphoria* to 1.66 seeds a visit of *Apis*. Larger pollinators were more effective than smaller ones for seed setting. Concerning to the differentiation of quantitative traits in *R. raphanistrum*, they concluded that the no-rosette and the early flowering traits might be the significant adaptations enabling *R. raphanistrum* to be a major agricultural weed.

11.8 Introgression Between the Cultivated Radish and Wild Radish in California

Both cultivated radish and *R. raphanistrum* were introduced to the San Francisco Bay area from Europe in the middle of 1800s. *R. raphanistrum* might be introduced as a weed contaminant together with cereals (Robbins 1940).

From a study on the wild *R. sativus* and *R. raphanistrum* by Panetsos and Baker (1967), the differentiation of two species was confirmed. The wild *R. sativus* has the distinctive traits including the white or partially purple flower on a white background, and tender and slightly thick pod made of spongy parenchyma. On the other hand, *R. raphanistrum* has yellow flower, and slender and hardy pod breaking into pieces when ripened. In California, the above two species were growing with their intermediate types that would be bred from natural hybridization between them. The F$_1$ plants induced by artificial crossing between the two species showed intermediate characteristics, chromosome configurations of 1IV + 7II at first metaphase (MI) of PMCs and moderate fertility of 50% in both pollen formation and seed setting. From these facts, it was suggested that the F_1 plants were carrying some reciprocal translocations in one pair of chromosomes. When the wild *R. sativus* type was naturally backcrossed to cultivated radish, some progenies did not form the quadrivalent chromosomes, and had showed high seed setting. These facts suggest that the introgression might occur from *R. raphanistrum* to cultivated radishes in the course of acclimatization of radishes. Using eight morphological traits and 10 allozyme loci, Hegde et al. (2006) analyzed the genetic features of more than 50 wild radish populations collected from Cannon Beach, Oregon, through California and south of Santo Tomas, northern Baja California, Mexico, including both the coastal and inland valley areas. They concluded that California wild radishes were intermediate in specific combination of traits, and confirmed the introgression among populations in California. Today, the genetically pure populations of *R. raphanistrum* indicated by Panetsos and Baker (1967) have been replaced by the hybrid populations in these 40 years.

Campbell et al. (2006) investigated the genetic changes using four *R. raphanistrum* collected from Michigan and hybrid populations (*R. raphanistrum* × *R. sativus*). Hybrid and wild populations showed similar growth for four generations, and the pollen fertility of hybrid ones was enhanced progressively. The advanced progenies showed lower fertility than *R. raphanistrum* exhibiting ca. 270% greater lifetime fecundity and ca. 22% greater survival rate than California wild radish. They proposed a hypothesis that crop–wild hybridization might generate new genotypes with potentials enough to surpass the parental species under new environments.

Ridley et al. (2008) studied the evolutionary processes in four cultivars of radish, six populations of European *R. raphanistrum* and 11 populations of Californian wild radish analyzing the *trn*L–*rpl*132 intergenic region of chloroplast DNA. Eight haplotypes were identified in cpDNA of the genus *Raphanus*. Cultivated radishes had B and D types and *R. raphanistrum* had five haplotypes (A, C, E, F, and H). No haplotype was commonly shared between *R. raphanistrum* and cultivated radishes. On the other hand, the Californian wild radish possessed six haplotypes, B and D of which were similar to *R. sativus*, and A, E and F were to *R. raphanistrum*, respectively. Only haplotype G was independently unique. In the geographical distribution, little genic inclination was observed either on North–South axis or West–East (coastal–inland) axis. Genetic diversity among populations of Californian wild radish was 0.412, whereas that of *R. raphanistrum* was 0.742. It was confirmed that the Californian wild radishes originated through hybridizations between *R. sativus* and *R. raphanistrum*. Ridley and Ellstrand (2009) investigated on the Californian wild radish populations using the reproductive traits including days to flowering, length of the longest leaf at flowering, root crown diameter at flowering, number of pods per plant, average number of seeds per pod, and total seeds per plant. Survival rates for both Californian wild radish and *R. raphanistrum* were higher over years and growing sites. Californian wild radish showed intermediate phenotypes to its progenitors in many traits at the beginning of flowering. For potentials of pod and seed production, a significant correlation could be identified between genotype and environment. Californian wild radish has become more fertile than parental progenitors through ca. 150 generations since the original interspecific hybridization. Ridley and Ellstrand (2009) suggested that the progressive hybridization might contribute to enhancement of fertility contributing to higher survival rate and reproductive potential.

Eber et al. (1994, 1998) also confirmed the introgression from *R. raphanistrum* into cultivated radish by the analysis of hybrids between the native *R. raphanistrum* and cultivated radish in France. On the other hand, Kato and Fukuyama (1982) observed normal chromosome configuration of 9II in meiosis and high seed setting in F_1 hybrids between the cultivated radish and *R. raphanistrum* L. ssp. *landra*. From these results, it could be assumed that the chromosomal reconstruction might occur even in *R. raphanistrum*.

11.9 Intergeneric Hybridization Between Wild Radishes and *B. napus* and Its Implications on Gene Flow from Transgenic Rapeseeds

The rapeseed (*B. napus*, $2n = 38$, AACC) is known as incompletely self-fertilized crop with the outcrossing rate of 30% (Rahow and Woods 1987; Rieger et al.

2001). It is feared that the transgene of transgenic rapeseeds would be introduced into wild relatives through hybridization, and the risk of gene dispersal into crops was assumed to be high. The successive steps to assess the probability of transgene transfer into wild relatives are (1) the crops and wild relatives grow side by side and come simultaneously into flower, (2) pollen scatter and seed dispersal, (3) production of viable and fertile F_1 hybrids, (4) stable introgression of the transgene into wild relatives, and (5) persistence of introgressed gene in natural populations (Prieto et al. 2005; Devos et al. 2007).

As to the crossability to *B. napus*, Devos et al. (2007) pointed out that *R. raphanistrum* in Belgium was a member of rank 3. *B. rapa* and *Hirschfeldia incana* were classified to rank 1 and rank 2 in hybridizing potentials for introgression. Kelran et al. (1992) investigated pollen germination and hybrid production in vitro for risk assessment of outcrossing between transgenic rapeseed and *R. raphanistrum*. In the case of *B. napus* × *R. raphanistrum*, no pollen tube penetrated into the style of *B. napus*. In the reciprocal cross, on the other hand, the germination percentage of rapeseed pollen grains was significantly lower (12%) than the former, although the pollen tubes of rapeseed did not penetrate into stigma of *R. raphanistrum*. As a result, they obtained a few hybrids ($2n = 28$, ACR and RAC) in reciprocal crossings by ovary culture.

Prieto et al. (2005) developed cross-specific transposable element (SINE) markers for screening of introgression of rapeseed genes into *R. raphanistrum*. In 47 markers observed, 30 revealed polymorphism in which 17 markers were dominant and 13 were codominant. Polymorphic markers were mapped on 10 linkage groups of *B. napus*. They presumed that the SINE markers might provide efficient tools to analyze the introgression from transgenic rapeseeds to *R. raphanistrum*.

Based on these information, we could survey the transgenic hybridization in reciprocal crossings between *B. napus* and *R. raphanistrum* under natural conditions as shown in Table 11.4.

Eber et al. (1994) analyzed 7,018 seeds obtained from the crossing between *B. napus* and *R. raphanistrum*. All plants obtained from large seeds (longer than 2 mm in diameter) were not hybrid. On the other hand, 3,734 small seeds (shorter than 1.6 mm) developed into 188 hybrids were $2n = 28$ with ACRr genome. One plant was ascertained to be amphidiploid ($2n = 56$, AACCRrRr). The $2n = 28$ plants formed trivalents and quadrivalents in 9.2% of the PMCs, suggesting the possibility of crossing-over between AC and Rr. In the isolated field where the intergeneric hybrid plants were surrounded with *R. raphanistrum*, 8.5% of the 281 F_1 plants were confirmed to produce pods. From 234 pods obtained, 20 seeds were larger than the F_1 seeds. In the case of rapeseed field, 17 plants out of 20 F_1 plants developed pods, and 567 seeds were obtained. From this result, they assumed that fertile progenies could be maintained by backcross with parental donor. Baranger et al. (1995) examined the spontaneous outcrossing of six male-sterile (CMS) rapeseed lines and *R. raphanistrum*. The 42.4% of 651,400 seeds were large (longer than 1.6 mm in diameter) and 57.6% were small (less than 1.6). All the 244 plants obtained from large seeds were true rapeseed. Among 240 plants from small seeds, however, many triploid hybrids ($2n = 28$, ACRr) were bred, in addition to AACCRrRr amphidiploids with $2n = 56$, diploidal AACC ($2n = 38$) and haploid AC ($2n = 19$) plants. It was suggested that the frequent genomic reconstitution was proceeding in those plants with $2n = 28$ from small seeds. Confirming the production of BC_1 plants, they suggested that close relative between Rr genome and the A or C genome might explain the wide hybridization with *B. napus*. Chevre et al. (1996) obtained from 2.8 seeds per 100 pollinated flowers to 23.8 seeds in the pollination of CMS rapeseeds with *R. raphanistrum* under field conditions for 3 years. By the cytogenetical analysis of the 427 F_1 plants, 423 were ACRr with $2n = 28$ and four plants were AACCRrRr with $2n = 56$, respectively. The BC_1 seeds were produced from F_1 hybrids with ACRr genome through open pollination. They observed multivalents at MI of PMCs of BC_1 plants with $2n = 37$ (AACCRrRr) and $2n = 56$. From these facts, they suggested that the chromosome rearrangement between transgenic rapeseeds and *R. raphanistrum* might occur in their following progenies. Lefol et al. (1997) performed hybridizations of *R. raphanistrum* with *B. napus*, *B. rapa* and *B. juncea* in the reciprocal crossings. Only two seeds (0.4 seeds per 100 pollinated flowers) were obtained from the crossing of *B. napus* with wild radish, three and 13 seeds were obtained from *B. rapa* and *B. juncea*, respectively. In these crossings, only two plants generated in the cross between *B. napus* and wild radish were confirmed to be true hybrid. The F_1 plants produced four seeds by the backcrossing with *B. napus*. In the

Table 11.4 Production of F_1 hybrid and their progenies between *B. napus* and wild radish under natural pollinations

Combination	Researchers and years published	Production of F_1 hybrid[a]	Production of progeny from F_1 hybrid[b]
B. napus × wild radish	Eber et al. (1994)	○	○
	Baranger et al. (1995)	○	○
	Chevre et al. (1996)	○	○
	Lefol et al. (1997)	○	○
	Darmency et al. (1998)	○	○
	Chevre et al. (2000)	○	?
	Rieger et al. (2001)	○	○
	Chevre et al. (2007)	○	○
Wild radish × *B. napus*	Lefol et al. (1997)	×	–
	Darmency et al. (1998)	○	○
	Chevre et al. (2000)	○	?
	Rieger et al. (2001)	×	–
	Thalmann et al. (2001)	×	–
	Warwick et al. (2003)	○	?
	Halfhill et al. (2004)	×	–
	Daniels et al. (2005)	×	–

[a] ○ More than one hybrid was obtained successful in hybridization, × no hybrid, ? Unknown

[b] ○ Production of progenies by backcrossing with parents or by self-pollination, ? Unknown

reciprocal cross, no hybrid plant could be obtained in the crosses between wild radish and *Brassica* species. Darmency et al. (1998) investigated the spontaneous hybridization between rapeseed and wild radish by growing them in cages and field. When CMS rapeseed and wild radish were grown in the field in a ratio of 1:1 and 1:2, 40% and 80% of the germinated seeds developed into true hybrid, respectively. On the other hand, only two spontaneous hybrid plants were obtained from 147,671 seeds when plant materials were grown in cage. The chromosome number of these plants were $2n = 28$ suggesting to be the genome constitution of RrAC. Furthermore, hybrids were fertile in the following progenies. Chevre et al. (2000) obtained 23 hybrids from 73,847 seedlings in the cross between CMS rapeseeds and *R. raphanistrum*, hybridization ratios of which ranged from 2×10^{-5} to 5×10^{-4}. The chromosome number of hybrids were shown to be $2n = 28$, $2n = 37$ and $2n = 56$ for 18 triploids (ACRr), one tetraploid (ACRrRr), and four amphidiploids (AACCRrRr), respectively. In contrary, only one hybrid (RrRrAC, $2n = 37$) was developed from 189,084 seedlings in the reciprocal cross. Most of the hybrids were obtained from small seeds shorter than 1.6 mm in diameter. The chromosome configuration at MI of PMCs in ACRr and RrRrAC hybrids showed trivalents and/or quadrivalents suggesting the allosyndetic chromosome pairing between Rr genome and A or C genome. Rieger et al. (2001) tried the natural hybridization in vivo, where one or four plants of *R. raphanistrum* were planted randomly in each 100 m^2 field of rapeseeds. Two hybrids with $2n = 56$ (AACCRrRr) were obtained from 52,000,000 seedlings via rapeseeds in 2 years. These hybrids were fertile in selfing and backcrossing with *R. raphanistrum*. On the other hand, no hybrid was produced from 25,000 seedlings via *R. raphanistrum*. Thalmann et al. (2001) studied on the spontaneous hybridization between *R. raphanistrum* collected from three regions on Swiss Plateau and rapeseeds under agricultural conditions. A total of 754 plants developed from *R. raphanistrum* were confirmed not to be hybrid not only by the flow cytometry but also by the RAPD analysis. These facts could suggest that higher hybridization barrier might operate between *R. raphanistrum* as recipient and rapeseeds as pollen donor, and gene flow may be extremely rare. Warwick et al. (2003) studied on the natural cross of *R. raphanistrum* with rapeseeds using seven Canadian populations and six European ones in greenhouse and using three Canadian populations and one population from France under field conditions. No hybrid developed from the experiment in the former. On the other hand, in field conditions, only one hybrid was found from 32,821 individuals of four populations, showing 0.003% of hybridization ratios. The chromosome number of this hybrid was $2n = 34$ or 35 with genomic constitution of RrRrAC. From this and previous data (Chevre et al. 1998, 2000), Warwick et al. (2003)

concluded that hybridization between *R. raphanistrum* and *B. napus* was extremely rare. Halfhill et al. (2004) studied on hybridization between 11 transformed rapeseed lines and *R. raphanistrum* under a higher crop ratio (600:1) and a lower one (180:1) to wild radish. No natural hybridization was confirmed in 19,274 seedlings screened with green fluorescent protein (GFP) phenotype. By contrast, 9.7% of hybridization rate was observed between *B. rapa* and *B. napus* in the same condition. Daniels et al. (2005) observed the intergeneric hybridization between *R. raphanistrum* or *R. sativus* and six related species in the cultivated conditions. Seedlings grown from 1,793 seeds via *R. raphanistrum* in 3 years and 117 seedlings via *R. sativus* in 1 year developed no herbicide tolerant plants. Chevre et al. (2007) studied on the evolution of hybrid genome during the successive generations followed by the formation of intergeneric hybrid of *B. napus* × *R. raphanistrum*, in order to set up a framework for gene flow modeling between these two species. They used seven CMS genetically modified (GM)-rapeseed lines that had different insertion sites of the same transgene carrying the same genetic background. More than 500 plants were the true intergeneric F_1 hybrids of ACRr genomic construction with $2n = 28$. A majority of BC_1 plants were expected to have $2n = 37$ chromosomes, but the chromosome number showed a wide range from 24 to 80. When the BC_1 plants with $2n = 28$ to 64 were successively backcrossed with *R. raphanistrum*, the chromosome number decreased progressively from BC_2 to BC_4 generations.

As described above, using the CMS rapeseeds as recipient in hybridization production between *R. raphanistrum* and *B. napus*, a large number of plants really showed hybridity. *B. napus* forming normal pollen was not bred by hybridization but by self-pollination within themselves. As a seed parent, *R. raphanistrum* is so incompatible to the related species that the risk of gene introgression from GM rapeseed to *R. raphanistrum* was extremely low.

11.10 Prospects for Wild Radish

Wild radish has not provided the agronomic traits except for CMS for hybrid seed production system. However, it may offer useful information for genetics and breeding of cultivated radishes in near future.

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