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Dominance of *Brassica* and No Effects of *Raphanus* in Mature Seed Production in Intergeneric Hybrid between *Brassica rapa* ssp. *Pekinensis* and *Raphanus*

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Abstract

We succeeded in producing mature seed from a line of *Brassica rapa* ssp. *pekinensis* that had been hybridized with *Raphanus sativus* var. major. Our focus was on dominance of *B. rapa* ssp. *pekinensis*; radish (*R. sativus* var. *major*) had no influence. Marker tests for similarity showed that the original CR291M-64 x HwiM-2 hybrid was an inbred CR291M-64, rather than a genuine cross; this appears to have resulted from weak self-incompatibility in this strain. The plants from the mature seed bloomed with reddish flowers differently shown up to present. The intergeneric hybrid between *Brassica* inbred and *Raphanus* hybrid was very weak in strength compared to the *Brassica* inbred which was self-pollinated even though the cause of the weak was not identified. The hybrids between *Brassica* hybrid, dominant and elite recessive, and *Raphanus* can be developed in large quantities using mature hybrid seed without resorting to ovule culture techniques.

Keywords

Intergeneric Hybrid, Brassica Dominance, No Raphanus Effect, Mature Seed

1. Introduction

For variety improvement, research regarding interspecific hybridization in cruciferous plants has focused on crosses between diploid plants or between diploids and tetraploids in the triangle of U [1] [2] [3] [4] [5]. Intergeneric crosses between cultivated varieties and wild species have also been used to enhance the

genetic base [6] [7] [8]. Concerning the genus *Raphanus*, two hybrids with *Brassica* have been available: a cross between *R. sativus* and *B. oleracea*, and a cross between *B. rapa* and *R. sativus* [9] [10] [11]. The first crossbreeding in a cruciferous crop was between *R. sativus* and *B. oleracea*, by Augustin Sageret ([12] cited from [10]). Karpechenko succeeded in obtaining F_2 intergeneric hybrid seeds [13]. Intensive research was carried out by McNaughton [14] [15]. Chen and Wu published details of the world's first stable intergeneric hybrid [16], which was later utilized to improve *B. napus* trait [17]. All hybridization efforts were performed using radish (*Raphanus sativus* L.) as a female parent.

Some subspecies of *B. rapa* have different morphologies and display distinct features in their intergeneric hybridization with *Raphanus*. The known subspecies include *pekinensis* (heading Chinese cabbage), *chinensis* (pakchoi), *rapifera* (turnip), *narinosa* (rosette pakchoi), *parachinensis* (flowering pakchoi), *japonica* (mizuna), and *oleifera* (turnip rape) [18]. The world's first *Brassica* x *Raphanus* hybrid seed was obtained by Terasawa [19]. Subsequently, the seed was used to transfer nematode-resistance traits from the wild radish to inter-cropped Chinese cabbage [20] [21]. Dolstra (1982) collected varieties of turnip, pakchoi, turnip rape, and heading Chinese cabbage worldwide, then hybridized them with radish varieties. No seed was produced by heading Chinese cabbage (ssp. *Pekinensis*), but seed was obtained from the other three subspecies. Although young hybrid ovules were not obtained in the cross between ssp. *pekinensis* and *Raphanus* [22], this cross has been used subsequently to develop intergeneric hybrids. A culture system of ovules between ssp. *pekinensis* x *Raphanus* was established during plant acquisition [23] and was improved in a subsequent study [24]. Many hybrids can potentially be obtained using these techniques [25] [26] [27], and good stabilization has been achieved when the hybrid was used [28] [29] [30]. The stabilized intergeneric hybrid has been used for analysis of constituent components [31] [32] [33].

To date, hybrid seeds or plants have been obtained with *Brassica* as a maternal line [23] [34]. Young hybrid ovules should be cultured to generate intergeneric hybrids. A combination of ssp. *pekinensis*, **CR291M-64 x HwiM-2**—identified as an inbred variety of **CR291M-64** according to markers analysis later—produced mature seeds as a dominant in hybridization with *R. sativus* var. *major*, and *R. sativus* were not effective on this mature seed production. Therefore, intergeneric hybrids between *Brassica* hybrid with CR291M-64 which was originated from a line of ECD-4, and *Raphanus* can be developed using mature hybrid seed in the future. It does mean that resorting to ovule culture techniques is not necessary. These results are expected to support major advances in intergeneric breeding.

2. Materials and Methods

2.1. The CR291M-64 Line Produced Mature Seeds of Dominant Character and Radish Has No Effects

The letter "M" in the names of material lines refers to the origin of a particular

microspore culture. For example, the line **HwiM-2** is the second strain derived from the microspore culture of a leading cultivar **Hwiparam**. The line **CR291M** was selected for resistance against clubroot and a virus, following microspore culture of a hybrid with **BR079 x ECD-4** (IT number: **04-33-3**) to target clubroot and with **3M-291** to induce virus resistance. All lines incorporating **CR291M** in these experiments, such as **CR291M-64**, therefore have resistance to two diseases.

Three F_1 *Brassica*, **C218M-13 x HagamM-50, CC507 x BulM-68** and **CR291-7M-64 x HwiM-2** had sown for this trial with **KB-68 x WY-25** *Raphanus* first. Female parent plants were *B. rapa* ssp. *pekinensis*. Because inbred intergeneric crosses may be sterile [27], F_1 crosses were sown to produce offspring similar **BB#12**. **BB#12** was developed using both female and male F_1 hybrids. The seed of the combinations including **CR291M-64 x HwiM-2** was produced in mutual crossing by bees in small net cages that were 2 m long × 1 m wide × 2 m high. Two cultivars of ssp. *rapifera* (turnip) were included in dominant investigation later.

The **KB-68 x WY-25** radish has round, red, fleshy roots and purple leaf veins. It was employed to understand the segregation pattern of the flesh color in the intergeneric hybrid, because both parents have red flesh, although the female Chinese cabbage are F_1 hybrids. Ovule culture was conducted 10 days after hybridization between *Brassica* and *Raphanus*. However, a flower branch of **CR291M-64 x HwiM-2** was unexpectedly maintained for approximately 25 days, and the seed pods appeared to grow well. Because many of the ovules had already been cultured at that time, ovule culture was stopped to assess mature seed production. Eventually, the **CR291M-64 x HwiM-2** hybrid produced mature seed from its cross with *Raphanus*. To our knowledge, this is the first report of mature seed production in an F_1 *Brassica*. Thirty-six plants (from 64 hybrid seeds) appeared to have similar morphology, including purple veins and leaves in the growing point area, regardless of their production from the hybrid **CR291M-64 x HwiM-2**, with different traits in *Brassica*. A marker test for similarity was requested from Seoul National University.

The combination of **CR291M-64 x HwiM-2** and its parental inbred, together with crosses of reciprocal hybrids of both parents and two accessions of *Raphanus*, **KB-68 x WY-25** and **locally inbred**, were sown to investigate the dominance relationships and the effect of radish in mature seed. Because dominance and no effects were recognized in this experiment, we established 11 further plantings to confirm the dominance relationships and no influence of radish already observed; we also obtained additional information concerning the production of mature seed. These experiments deployed two crosses from each of the **CR291M-64** and **CR291M-96** strains, five fraternal lineages (**CR291M-2, CR291M-5, CR291M-10, CR291M-66**, and **CR291M-96), Shogoin** turnip (introduced from Japan), and **Kangwha** turnip (our line). Two F_1 combinations, **KB-68 x WY-25** and **TBM-48 x BDM-7**, were sown together with an inbred

Shogoin radish to investigate radish effects. The synthesis of **TBM-48 x BDM-7** has white flesh and short, fat roots. The inbred **Shogoin** radish had completely white skin and flesh, as well as a slender root. This appearance was therefore quite different from the appearance of the **KB-68 x WY-25** hybrid. Thirty-one radish varieties were sown on August 20 for an autumn test. These varieties were secured and hybridized with **CR291M-64** to investigate their ability to produce mature seed.

In total, 432 hybrid non-dry seeds were sown by the end of December. Of these, 328 germinated plants grew by the end of the following June and were able to self-pollinate. The 305 grains of dry seed obtained from **CR291M-64 x HwiM-2** were sown to examine the differences between non-dried and dry seeds. Some large seeds were observed during sowing, but they were excluded from analysis because non-dried seeds could not be distinguished by seed size and every plant exhibited purple central veins and leaves. Some green plants germinated from these large seeds primarily; because they were all similar in appearance (distinct from hybrids crossed with radish at the five-leaf stage), a marker test was requested from Seoul National University.

Seeds of Chinese cabbage and radish were sown in a fall crop on August 10 and 20, respectively. Some plants from this sowing were chosen for seed production at the adult stage. For analysis of absolute seed production only, seeds were sown and vernalized under natural conditions from September to February of the following year. If seeding was requested from March to June, the seedling tray was incubated in a refrigerator at 4˚C - 5˚C for at least 45 days to prevent de-vernalization in summer. Sowing in July and August was postponed until September seeding time.

Temperatures rose to 35˚C on some days in July and August, but generally fell below 25˚C at night. Beginning in mid-November, heating facilities were used to prevent exposure of plant materials to nighttime temperatures below 5˚C. The plants were therefore grown at temperatures of 5˚C - 25˚C from October until the following February, with minimum temperatures of about 15˚C from March to June. If vernalization was needed, nursery-stage plants were moved to unheated plastic greenhouses and maintained at minimum temperatures of 2.0˚C - 4.0˚C and a maximum temperature of 12.0˚C by ventilation. All stamens of *Brassica* were removed within 1 day of flowering. They were then hybridized immediately with radish pollen and covered with an oiled paper bag. The pollinated branches were usually harvested at 35 days after mating; however, they were harvested 45 days after mating during winter, regardless of heating system usage. All other management protocols followed the standard practices of the BioBreeding Institute.

2.2. Marker Test

The numbering systems of chromosomes 1 and 5 were used for marker investigation in radish. Thirty plants were collected from the BioBreeding Institute for

marker investigation. Single nucleotide polymorphisms of **KB68 (KB)** and **Wonyeon25 (WY)** radish were discovered using the Genome Analysis Toolkit (version 3.6-0) HaplotypeCaller [35]. Genomic sequences of **WK10039** in radish [36] were used as reference genomes. Variant filtration was performed manually based on read depth. For CAPS marker design, digestibility of flanking regions from filtered single nucleotide polymorphism sites of two cultivars was examined to identify candidate regions for CAPS markers. For each pair of sequences, if only one sequence was the target site of the restriction enzyme Hind III (AAGCTT), primer sets were designed around the target region. Two primer sets were designed to distinguish hybrids from **KB68** and **WY25** strains. Polymerase chain reaction was performed using 20-μl reactions containing 200 ng of genomic DNA, 4 units of Taq polymerase (Takara), 0.2 mM dNTPs, and 0.2 mM primers, combined with 1× polymerase chain reaction buffer (Takara). Polymerase chain reactions were performed under the following conditions: denaturation at 95˚C for 5 min followed by 30 cycles of amplification (95˚C for 30 sec, 60˚C for 30 sec, and 72˚C for 1 min) and a final extension at 72˚C for 10 min. Amplicons were digested with Hind III (New England Biolabs) and visualized with 2% agarose gel electrophoresis. This procedure was generally similar to the protocol used in the radish marker test on dry-seed-derived green plants of Chinese cabbage **CR291M-64** and **HwiM-2**, although it used standards **CR (CR291M-64)**, **Hwi (Hwi-M2)**, **BF1 (CR x Hwi)**, **KB (KB-68)**, **WY (WY-25)**, and **RF1 (KB x WY)**. The line **Chiifu-401** [37] was used as the reference genome. Four primer sets were designed to distinguish **CR291M-64** and **HwiM-2**, using 3 purple and 9 green plants.

3. Results

3.1. The CR291M-64 Line Produced Mature Seeds of Dominant Character and Radish Has No Effects

CR291M-64 x HwiM-2 hybrids produced pods that were 3.5 - 5.3 cm in length by day 37 and the others not produced seeds (Figure 1). Sixty-four seeds from 11 pods were placed on filter paper in Petri dishes (Table 1). Seeds sown on filter paper generally germinate within 2 - 3 days at 25˚C; in this trial, germination began on day 9 and continued for 33 days (Supplementary Table S1). In addition, the germinated plants appeared fragile. They were therefore kept in the Petri dish for several more days before transplantation into soil. Retardation and extension of germination were presumably caused by seed dormancy that was found in the sowing of the dry seed.

Individuals subjected to marker analysis at Seoul National University were genetically differentiated and became morphologically dissimilar as they grew into adult plants. In a dominance trial, the combination of **CR291M-64 x HwiM-2** generated mature seeds. The parent **CR291M-64** plants also produced seeds, but the cross counterpart **HwiM-2** did not produce seed. Mature seeds from the **CR291M-64** line were definitively dominant. Because the line **HwiM-2**

Figure 1. Morphology of pods of three F_1 *Brassica* hybrids C218M-13 x HagamM-50, CC507 x BulM-68 and CR291-7M-64 x HwiM-2 from left crossed with same *Raphanus* hybrid KB-68 x WY-25 at 5 buds prior to 37 days.

Table 1. Number of pollinated buds, pod length, hybrid seeds sown and color of pedigree plant of (CR291M-64 x HwiM-2) x (KB-68 x WY-25) hybrid.

Maternal combination	Number of Pollinated buds	Length of pods (cm)	Number of seeds sown	Color of plants
(CR291M-64 x HwiM-2) x (KB-68 x WY-25)	6 + 5 = 11	3.5 - 5.3 long	64 grains	purple

was recessive, a **CR291M-40 x HwiM-2** hybrid was used to generate dominant seeds. The strain **CR291M-180** was uncertain because it was crossed with the dominant line **CR291-64**. The **HwiM-2 x CR68M-107** hybrid failed to produce seeds; so, the **CR68M-107** line was recessive, which derived from **BulM-68** through microspore culture for virus resistant line after hybridization with **3M-291** (Table 2). Four combinations and five inbred lines, including two turnip strains were mated with one, two, or all three radish cultivars; they produced mature, dominant seed. Since the line of **C218M-13** is recessive, **CR291M-96** is a dominance stain: that is as the line test (Table 3). On the other hand, the **CR291M-64** line produced intergeneric mature seed without exception when hybridized with 31 radish strains cultured for fall cropping (Supplementary Table S2). It has known that radish is no influence on mature seed production of intergeneric hybrid between Chinese cabbage and radish.

Two hundred and fifty-four of 305 dry seeds germinated without delay and continued growing for an extended duration. Among the germinated plants, 25 that originated from larger seeds were green and grew more vigorously than did purple plants that sprouted concurrently (Figure 2). The results of marker examination indicated that the green plants were induced from self-pollination or apomixis of the **CR291M-64** strain. Three purple individuals were true hybrids

Table 2. Seed yield of parents and reciprocal combinations by parents of CR291M-64 x HwiM-2 with two radish cultivars of intergeneric hybrids between *Brassica* and *Raphanus.*

Maternal parents	The number of seeds by paternal parents	
	KB-68 x WY-25	Local inbred (05-80-14B)
CR291M-64 x HwiM-2	2	6
CR291M-64	212	12
HwiM-2	0	0
CR291-64 x CR291-96	14	10
CR291M-180 x CR291M-64	102	13
CR291M-40 x HwiM-2	1	3
HwiM-2 x CR68M-107	0	0

*(05-80-14B): Introduction number.

Table 3. Seed yield of each cross and cultivars by radish parents in intergeneric hybrids between *Brassica* and *Raphanus.*

Line code	The number of seeds by paternal radish		
	KB-68 x WY-25	TBM-48 x BDM-7	Shogoin radish
CR291M-64 x C218M-13	7	12	no experiments
CR291M-64 x GreenM-2	no experiments	1	no experiments
(CR291M-96 x C218M-13)-2	7	no experiments	no experiments
CR291M-96 x CR291M-180	no experiments	1	no experiments
CR291M-2	18	no experiments	no experiments
CR291M-5	18	11	3
CR291M-10	13	no experiments	no experiments
CR291M-66	1	no experiments	no experiments
CR291M-96	no experiments	1	no experiments
Shogoin turnip	no experiments	no experiments	1
Kangwha turnip	3	1	8

Figure 2. Appearance of green (vigorous) and purple (weak) individuals sown the dry seed between *Brassica* CR291M-64 x HwiM-2 and *Raphanus* KB-68 x WY-25. *Green plants were identified as an inbred of CR291M-64 from marker test and purple plants were hybrid between the inbred CR291M-64 and radish hybrid KB-68 x WY-25.

crossed between inbred **CR291M-64** and a **KB-68 x WY-25** radish cross. Therefore, every item mentioned so far has turned out to be false except that the inbred **CR291M-64** produced an intergeneric hybrid seed with dominance. The hybrid is thought to have resulted from the two being in the same net cage with bees. Weak self-incompatibility may have been responsible. It was identified that another experiment carried out after the marker test (Supplementary **Table S3**). The 328 plants sown from non-dried seeds and 254 plants grown from dried seed were all purple and produced purplish flowers, a result not previously reported in cruciferous intergeneric hybrid crops (**Figure 3**). Unusually, they did not produce seeds within more than about 100 days of pollination after blooming. Some of the 36 plants initially seeded produced microspore-derived embryos; the offspring of approximately 150 plants did not generate seeds in self- or cross-pollination involving more than 100 flowers each [27].

3.2. Marker Test

Seoul National University prepared two CAPS markers of radish and tested 30 of 36 hybrids. Two markers of **KB-68** were 562 and 786 base pairs long. In **WY-25**, markers were 414:148 and 261:525 base pairs long when cut with Hind III. The individual numbers of plants 1, 2, 6, and 8 of the two markers were KB (KB-68): WY (WY-25), WY: KB, KB: KB, and WY: WY, respectively (**Table 4**). These plants clearly all differed from each other. Seoul National University also investigated four CAPS markers prepared on chromosomes 4, 6, 8, and 10 in the maternal parent **CR291M-64**; these markers were analyzed in 25 green plants. The line of **HwiM-2** was cut off at the marker of chromosome 6, and strain **CR291M-64** was cut off at all other markers of three chromosomes (**Table 5**). Nine green and three purple plants had the same **CR291M-64** genotype, including **CR291M-64** and **BF1** (**CR291M-64** x **HwiM-2**) (**Figure 4**). However, **HwiM-2** had different genotypes of the **CR291M-64 x HwiM-2** hybrid. Two

Figure 3. Reddish flowers from mature seeds of intergeneric hybrid between *Brassica* strain CR291M-64 and *Raphanus* KB-68 x WY-25.

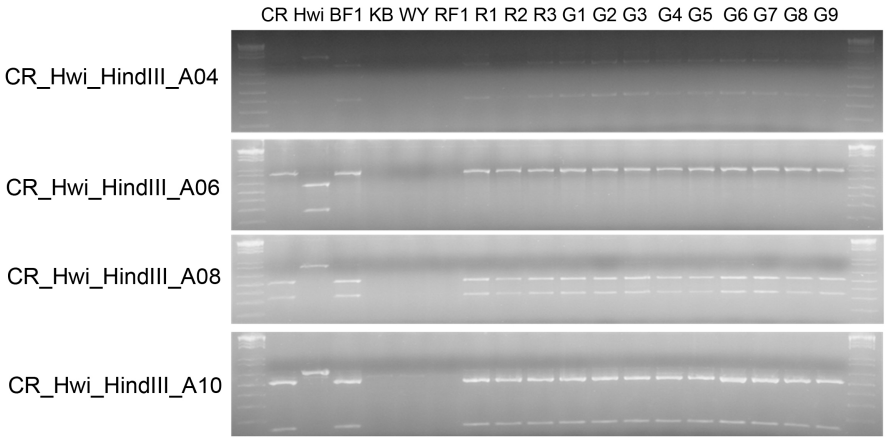

Figure 4. Nine of green (G1 to G9) and 3 (R1 to R3) of purple plants presented the same specific bands in marker test. *A = chromosome numbers R = purple plant G = green plant CR = CR291M-64, Hwi = Hwi-M2 BF1 = CR x Hwi KB = KB-68 WY = WY-25 RF1 = HB x WY.

Table 4. Sequences of 2 CAPS markers and results applied the markers to 30 individuals in the mature seeds in intergeneric hybrids between *Brassica* and *Raphanus*.

Primer	Sequence (5' to 3')	Tm	bp
KB_WY_HindIII_1F	GAGGCTTGCACATCAGTATGG	60.7	562 KB
KB_WY_HindIII_1R	CTTTTTGGGGATCTTTAGAGG	59.0	148 WY 414
KB_WY_HindIII_5F	ACCACATCCAGAACTCATTCAC	58.9	786 KB
KB_WY_HindIII_5R	GCTTCGGCGTAAAACTCAAC	59.9	525 WY 261

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Marker 1	KB	WY	KB	KB	KB	KB	WY	WY	KB	KB	WY	WY	WY	WY	KB
Marker 2	WY	KB	WY	WY	WY	KB	KB	WY	WY	WY	WY	KB	WY	KB	KB
Sample	16	17	19	20	21	22	23	24	25	26	28	29	30	31	32
Marker 1	WY	WY	WY	WY	WY	WY	KB	WY	KB	KB	KB	WY	WY	WY	KB
Marker 2	KB	WY	KB	WY	KB	WY	KB	WY	KB	KB	WY	KB	KB	KB	KB

*KB: KB-68, WY: WY-25, 0ne and 5: number of chromosomes, F: forward, R: reverse. *Two markers of KB-68 were 562 and 786 base pairs long. In WY-25, markers were 414:148 and 261:525 base pairs long when cut with Hind III.

previously identified radish markers were not present in the nine green plants, although they were expressed in the three purple individuals containing **RF1** (**KB-68 x WY-25**) without BF1 (**CR291M-64 x HwiM-2**) (Figure 5).

4. Discussion

Mature seeds of an intergeneric hybrid between *Brassica* and *Raphanus* were first created by Terasawa in 1933 [19]. These first seeds were derived from ssp. *chinensis*, rather than ssp. *pekinensis*. Dolstra [20] also produced mature seed in

Table 5. Sequences of 4 CAPS markers used for identification of purple and green plants of dried mature seeds in intergeneric hybrids between kimchi cabbage and radish.

Primer	Sequence (5' to 3')	Tm	bp	
CR-Hwi-HindIII-A04-F	ACATCTCCCCTCGTGTTTCG	59.8	1351	971, 380
CR-Hwi-HindIII-A04-R	TCGCTTGTCTGGACAGTGTC	60.0		CR291cut
CR-Hwi-HindIII-A06-F	ACACATATTGGACCAGCCCC	60.0	902	617, 285
CR-Hwi-HindIII-A06-R	AGCTCAGACAACTAGTTAAGCC	57.8		HwiM2cut
CR-Hwi-HindIII-A08-F	ACTTTCTAGTGCCGGTCCTG	59.3	874	526, 348
CR-Hwi-HindIII-A08-R	GTACGTCAGATGTCCAATCGC	59.1		CR291cut
CR-Hwi-HindIII-A10-F	AGGTTGGCCAGACGTTATGG	60.0	692	533, 159
CR-Hwi-HindIII-A10-R	CTTCTGGTGAAACACGCAGC	60.0		CR291cut

*CR = CR291 = CR291M-64. Hwi = HwiM2 = Hwi-M2. A = chromosome numbers. F: forward, R: reverse. *The line of HwiM-2 was cut off at the marker of chromosome 6, 617:285 and strain CR291M-64 was cut off at all other markers of three chromosomes 971:380, 526:348 and 533:159 base pairs long when cut with Hind III.

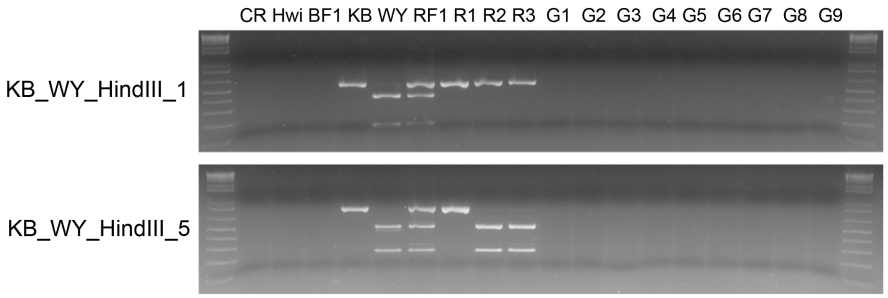

Figure 5. The 3 purple plants presented the specific band of 2 markers, but no band on the 9 green plants. R = purple plant. G = green plant. One and 5 = number of chromosomes. CR = CR291M-64. Hwi = Hwi-M2. BF1 = BB#12. KB = KB-68. WY = WY-25. BF1 = CR x Hwi. RF1 = KB x WY.

ssp. *chinensis* and discovered it in ssp. *rapifera* (turnip). The genes associated with mature seed production have been traced because "**Shogoin**" turnip has produced seeds with radish varieties [38]. Recent publications have reported the cultivation of intergeneric hybrid seeds with turnip cultivars [39] [40]. Mature seed has been obtained from ssp. *pekinensis* and *Raphanus* crosses, and successful seed production has been associated with the dominance of the **CR291M-64** line. If a line has produced mature seed in the strain test, it can be used to generate seeds with every possible genotype. In other trials, strains of **Chiifu** and **Gaeseong** as shown in Supplementary **Table S4** and **Table S5** and five strains of the fraternal lines of **CR291M-64** produced mature seed when crossed with radish. Pakchoi [19] [20] and turnip strains [20] [38] [39] [40] have produced mature seeds. Therefore, hybrids of dominant and recessive *Brassica* varieties can be prepared within and between subspecies. Large quantities of mature seeds can presumably be obtained from *Brassica* and *Raphanus* in subsequent studies. The microspore mutation technique could be applied to develop stable strains of intergeneric hybrids [28] [30].

Radish appears to have a minimal effect on the formation of mature seed in the intergeneric hybrid between ssp. *pekinensis* and *Raphanus*. Two distinct accessions, an F_1 hybrid **KB-68 x WY-25** with red flesh and an open-pollinated cultivar cultivated in the northern part of Korea with white flesh (IT no. **05-80-14B-1-1**) generated the same result in terms of mature seeds. Three radish varieties (**KB-68 x WY-25, TBM-48 x BDM-7**, and **Shogoin radish**) formed mature seed in hybridization with one, two or three of four crosses and five inbred lines of Chinese cabbages and two turnips. The 31 diverse radish varieties were hybridized with the line **CR291M-64** and provided seeds without any rupture. This production of mature seed from intergeneric crosses with radish varieties was recorded for the first time in this study. Therefore, *Raphanus* has no effect on mature seed production in intergeneric hybrid between Chinese cabbage and radish.

The combination of Chinese cabbage with **CR291M-64 x HwiM-2** produced mature seeds from the intergeneric cross with radish, **KB-68 x WY-25**. To our knowledge, this is the first report of mature seed generation following hybridization with *Brassica*. Mature seed produced from hybrids tends to raise questions. First, all 582 F_1 plants sown from non-dry seeds (328 plants) and dry seeds (254 plants) were purple individuals with similar early-stage morphologies, although the *Brassica* was a hybrid between two morphologically distinct inbred strains. Second, some of the cultivated plants produced F_2 seeds upon self-fertilization (e.g., **BB#12**) [28]. However, no seed was obtained from the F_1 hybrid or from the microspore-derived progeny of the F_1 cross. Marker tests resolved these unclear aspects, indicating that the combination of **CR291M-64 x HwiM-2** was an inbred strain of **CR291M-64**, rather than a true F_1 hybrid. The purple color of the intergeneric hybrid was affected by the radish in an inbred line of *Brassica*. The inbred *Brassica* line **CR291M-64** did not produce F_2 seeds in the intergeneric hybrid [27]. The matroclinal (apomictic) plants that originated from **CR291M-64 x** (**KB-68 x WY-25**) have not been useful because they were produced from somatic tissue around the egg, rather than from egg cells [41].

Intergeneric hybrid plants germinated from non-dry seeds were fragile and required additional incubation in Petri dishes for several days prior to transplantation into soil. Hybrid plants from dry seeds were also weaker at the early stage, compared with simultaneously generated matromorphic individuals. Developmental turning points between weak and strong growth should be identified in the future because the hybrid grew more vigorously during later stages than did non-hybrid plants [42].

Mature seeds regardless of non-dry and dry ones bloomed reddish flowers. An experience on fixed yellow color flowers as an unstable line at BioBreeding Institute was carried out some years ago (no report). Different colors of flowers therefore could be developed using intergeneric hybrids [43]. As such the mature seed production technique in intergeneric hybrids between *Brassica* hybrid, dominant and elite recessive, and *Raphanus* can be used diversely in the future.

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Conflicts of Interest

We hereby declare that authors have no pecuniary or other personal interest, direct or indirect, in any matter that raises or may raise a conflict.

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Supplementary

Table S1. Days for germination of non-dried mature seeds sowing on filter paper in Petri-dish of the hybrid of (CR291M-64 x HwiM-2) x (KB-68 x WY-25).

Number of sown seeds	Number of seeds germinated by date							Total germination
	9th	13th	14th	15th	16th	20th	33rd	
64	19	28	29	36	40	47	51	51 (80%)

Table S2. The number of seeds obtained in inter-generic hybridization between one kimchi cabbage and 31 radish lines.

Sowing No.	Line code	No. of seeds
18MO-201x18R-8	CR291M-64xWY-28	7
18MO-201x18R-10	CR291M-64xSanga W-97	12
18MO-201x18R-13	CR291M-64x40 Days 8	4
18MO-201x18R-15	CR291M-64x07-80-200	2
18MO-201x18R-17	CR291M-64x07-80-202	9
18MO-201x18R-29	CR291M-64x(KB68xWY-25)	214
18MO-201x18R-1	CR291M-64x16-80-101	8
18MO-201x18R-26	CR291M-64xTaebackM-35	11
18MO-201x18R-27	CR291M-64x12-80-60	6
18MO-201x18R-34	CR291M-64x16-80-102	14
18MO-201x18R-36	CR291M-64x16-80-104	32
18MO-201x18R-41	CR291M-64xKB-68	4
18MO-201x18R-46	CR291M-64xKB68(S6)	7
18MO-201x18R-49	CR291M-64xLocalKim-1	1
18MO-201x18R-50	CR291M-64xLocalKim-2	3
18MO-201x18R-51	CR291M-64x13-80-67-1	7
18MO-201x18R-52	CR291M-64x13-80-67-2	17
18MO-201x18R-62	CR291M-64xYuheon-5	4
18MO-201x18R-65	CR291M-64xWY(25-2x-1)	20
18MO-201x18R-77	CR291M-64x14-80-71	2
18MO-201x18R-79	CR291M-64x11-80-27	3
18MO-201x18R-81	CR291M-64x11-80-24	5
18MO-201x18R-83	CR291M-64xWY 28	3
18MO-201x18R-92	CR291M-64xR-25	4
18MO-201x18MO-204	CR291M-64x07-80-209	299
18MO-34x18MO-31	CR291M-64xKB-68-5	11
18MO-34x18MO-32	CR291M-64xWY-25	27
18MO-34x18MO-66	CR291M-64xWG-39	5
18MO-34x18MO-67	CR291M-64xDD-2	86
18MO-34x18MO-69	CR291M-64xCHT-1	38
18MO-34x18MO-70	CR291M-64x06-80-62	7
Total	Radish 31 lines	872

Table S3. Marker test results of 3 plants with remanded and differently produced seeds at the same year of 2016 (seeding in 2019.03.07).

Seeding number	Line number	Manufacture number	Marker test	Production amount	Seed production techniques
19 projects −355	HwiM-2x CR291M-64	16NC-163	All self of HwiM-2	1ml	Net cage with bees
−358	CR291M-64x HwiM-2	16SP-164	Two hybrids and one CR291M-64	235ml	Net cage with bees
−356	CR291M-64x HwiM-2	16SP-473	Two hybrids and one CR291M-64	2ml	Flower cross without emasculation
−357	CR291M-64x HwiM-2	16SP-474	All hybrids	79	Flower cross with emasculation
−359	HwiM-2Ⓧ	16SP-307	All HwiM-2	14	Bud-self
−360	CR291M-64Ⓧ	1SPP-335	All CR291M-64	250	Bud-self

Table S4. Production and germination of the mature seed between *B. rapa* ssp. *pekinensis* cv. Chibu and *R. sativus* var. *major* cv. WK-39 in 2006 and 2007.

Pollination	No. of pods	No. of seeds			Germinated seeds	Alive plants
		Normal	Blasted	Total		
431 buds	331	37	284	321	116	82

Table S5. Seeds obtained in cross between *B. rapa* ssp. *pekinensis* cv. Gaeseong and *R. sativus* var. *major* cv. Twenty-day in 2006.

Variety	Species	Number of Branches	Pollination tech.	Number of seeds
Gaeseong x Twenty days	*Brassica* x *Raphanus*	2	BC	4
		3	BC	16
		4	BC	15
		1	BC	6 grains

*Introduction number: Gaeseong (04-33-84) x Twenty day (02-80-2).