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Seed Production of Wild *Brassica juncea* on Riversides in Japan

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Abstract

Genetically modified (GM) *Brassica napus* derived from spilled seeds have been found over the years along roads around the oilseed import ports in Japan. Large populations of *B. juncea*, a relative species of *B. napus*, are often observed along riversides in Japan. If gene flow occurs from GM *B. napus* to wild *B. juncea*, and if the transgene enhances its fitness, this hybrid with improved fitness traits can raise concerns about it possibly becoming invasive and displacing native vegetation. Although seed production is essential to evaluate fitness, the availability of information regarding such ecological knowledge as the seed production of wild *B. juncea* is limited. Therefore, we conducted quantitative research on *B. juncea* seed production at six natural habitats along riversides in Japan to obtain baseline yield data for assessing the risk of GM *B. napus*. The seed production of *B. juncea* varied largely among individual plants within and across habitats. The average total seed production number was 3,800 per plant, which was two to eight times greater than that of neighboring *B. rapa*.

Discipline: Weed control
Additional key words: *Brassica napus*, Cartagena Protocol, fitness, genetically modified

Introduction

Japan imported 2.4 million tons of canola as a raw material such as oilseed from Canada in 2012 (Ministry of Agriculture, Forestry and Fisheries 2012). As the world's largest producer of canola, Canada cultivates genetically modified (GM) *Brassica napus* in 97.5% of its canola growing areas (James 2012). By multiplying the cultivation ratio (97.5%) and the volume imported (2.4 million tons) together, approximately 2.2 million tons of GM *B. napus* are apparently being imported to Japan each year. GM *B. napus* derived from spilled seeds have been found over the years along roads around the oilseed import ports in Japan (Katsuta et al. 2015).

Abiotic stress-tolerant GM crops (e.g. those with drought or chilling injury tolerance) have recently been developed and are expected to become next-generation crops that will bring stable yields against population growth and climate change due to global warming (Yoshimura & Matsuo 2012). However, there are concerns about possible gene flow from abiotic stress-tolerant GM crops to related weed species, and about the transgene possibly enhancing the invasiveness of such related weeds.

Twenty-three *Brassica* species are related and can be crossed with GM *B. napus* (Bing et al. 1996, Jørgensen et al. 1998, FitzJohn et al. 2007). Among closely related species relatives, *B. rapa* and *B. juncea* are the major candidate recipients of introgression from *B. napus* (Tsuda et al. 2014). Both species are not considered native species affected by GM *B. napus* in the Cartagena Protocol on Biosafety to the Convention on Biological Diversity, as both have been labeled alien species derived from crops in Japan (Yogo 2005). However, if gene flow occurs from GM *B. napus* to either of *B. juncea* and *B. rapa*, and if the transgene enhances their fitness, this hybrid with improved fitness traits can raise concerns about it possibly becoming

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invasive and displacing native vegetation. Meanwhile, much remains unknown about the ecological characteristics of wild *B. juncea*, that is, the baseline data needed to assess its invasiveness is not available.

B. juncea is distributed in 45 of Japan's 47 prefectures, accounting for more than 95% of all local governments (Konta et al. 2006, Hokkaido 2010, Ministry of Land, Infrastructure, Transport and Tourism 2013). In fact, *B. juncea* is seen virtually throughout Japan, and large populations are often observed along riversides (Takematsu & Ichizen 1993). In contrast, *B. rapa* is only distributed in 11 prefectures in Japan, including that in our study (Konta et al. 2006, Tachikake & Nakamura 2007, Ministry of Land, Infrastructure, Transport and Tourism 2012, Shizuoka 2014). *B. juncea* is also found in all provinces and territories of Canada, being most abundant in the western provinces and with *B. rapa* being less common (Frankton & Mulligan 1987). This distribution tendency suggests that *B. juncea* has better fitness than *B. rapa*.

Fitness is measured by population growth rate, namely, the dominant eigenvalue lambda (λ) of the population projection matrix (Caswell 2001). Though both reproduction and survival are important parts of fitness, the maximization of fitness is equivalent to maximizing reproductive value when the life cycle is as simple as that of mustards. The seed production number can thus be used as a rough measure of fitness, and although not exact it offers a first order approximation. Hauser et al. (1998a, 1998b, 2003) used seed production per plant as a measure of fitness. Their experiments confirmed the importance of seed production for overall fitness, where seed production per plant explained 93% of the variation in estimated fitness among plant types. Thus, the seed production numbers of *B. juncea* and *B. rapa* found in natural habitats in Japan are needed in order to determine fitness, both as a rough index of their fitness and as a life cycle parameter to calculate their population growth rate as an exact measure of fitness.

Some yield data is available on *B. juncea* as a traditional crop with edible leaves and seeds in Japan (OECD 2012). Conversely, very little information is available on the yield performance of wild *B. juncea* in natural habitats. Stevens (1932, 1957) reported the seed production of wild *B. juncea* collected at natural habitats in North Dakota. The samples were expected to have the maximum number of mature seeds, as well grown plants with comparatively little competition were intentionally selected. However, in natural habitats, many individual plants suffer under competition, unfavorable soil conditions, insufficient sunshine, or other negatives that adversely affect fertility.

The primary objective of this paper is to show the seed production number of wild *B. juncea* in a natural habitat. In Japan, this species is the most abundant wild mustard, and the major candidate recipient of introgression from *B. napus*.

Materials and methods

1. Plant sampling

Six sampling sites with abundant mustard were chosen around Tsukuba in Ibaraki, Japan (Fig. 1). Table 1 lists the geographical information, infested area, and density of *B. juncea* and *B. rapa*. These six sites are located along rivers that flow through rice paddy areas on the Joso Tableland. And the entire area is within 12 km in diameter. All sites are thus expected to have the same land features, amounts of sunshine, amounts of rainfall, air temperatures, and soil types.

B. juncea samples were collected on May 29, June 6 and 7, 2012 at sites A, B, C, D, E and F. *B. rapa* samples were collected on May 17 and 29, 2012 at sites A, B and C. *B. rapa* was not found at the other three sites (D, E and F) where we only collected *B. juncea*. The whole shoots for both species of plants were randomly collected, just prior to seed dispersal. There were ten samples for each species and site.

The plant materials sampled were dried by natural airflow in mesh bags. After having been dried, the pods of each plant were counted, and then, the seeds were gathered. Seeds were visually inspected to remove immature or damaged seeds that would not germinate. A seed counter IC-VA manufactured by Aidex Co. Ltd. was used to count the seeds.

2. Sampling and analysis of primary macronutrients of soil

Soil materials were randomly collected at the six sites. There were five samples for each site. Every sample included nearly 500 g of soil down to a depth of 5 cm from the surface. These materials were dried in a glass house before chemical analysis. A glass electrode pH meter was used for measuring pH levels. The ammonia nitrogen was determined by indophenol blue absorptiometry (Japan Soil Association 2001). The soil nitrate nitrogen was determined by using the sulfanilamide - naphthyl ethylene diamine method (Japan Soil Association 2001). The effective phosphoric acid was determined by molybdenum blue spectrophotometry (Japan Soil Association 2001). Inductively coupled plasma atomic emission spectrometry (ICP-AES) was used for measuring the exchangeable potassium (Japan Soil Association 2001).

3. Statistical analysis

All analyses were conducted with R (R Core Team 2013). ANOVA tests and Tukey tests were used to check 5% significant differences between the sites for both plants and soil. Data skewness was checked, and then, logarithmic

Table 1. Locations, areas and density of wild mustard infestation around Tsukuba, Japan.

River	Sampling site [a]				Infested area		Density (plants/m^2)	
		Town	Latitude	Longitude	Width × Length (m)	Side of river	*B. juncea*	*B. rapa*
Hanamuro [b]								
	A	Hanamuro	36°5′14″N	140°7′33″E	3×400	Both sides	24	6.1
Kokai [b]								
	B	Oosakimachi	36°2′34″N	140°1′24″E	1×20	One side	<1.0	<1.0
Hakkenbori [b]								
	C	Nakayamamachi	36°2′22″N	140°0′13″E	5×1,300	One side	6.8	7.9
Inari [c]								
	D	Teshirogi	36°3′13″N	140°6′06″E	5×180	Both sides	9.8	–
	E	Minaminakazuma	36°1′51″N	140°6′46″E	1×400	Both sides	7.2	–
	F	Kannondai	36°1′37″N	140°6′43″E	1×150	One side	<1.0	–

[a] Figure 1 shows the site locations. A: Hanamuro on the Hanamuro River, B: Oosakimachi on the Kokai River, C: Nakayamamachi on the Hakkenbori River, D: Teshirogi on the Inari River, E: Minaminakazuma on the Inari River, and F: Kannondai on the Inari River.

[b] Infested area and density measurements were conducted in April 2011.

[c] Infested area and density measurements were conducted in April 2013.

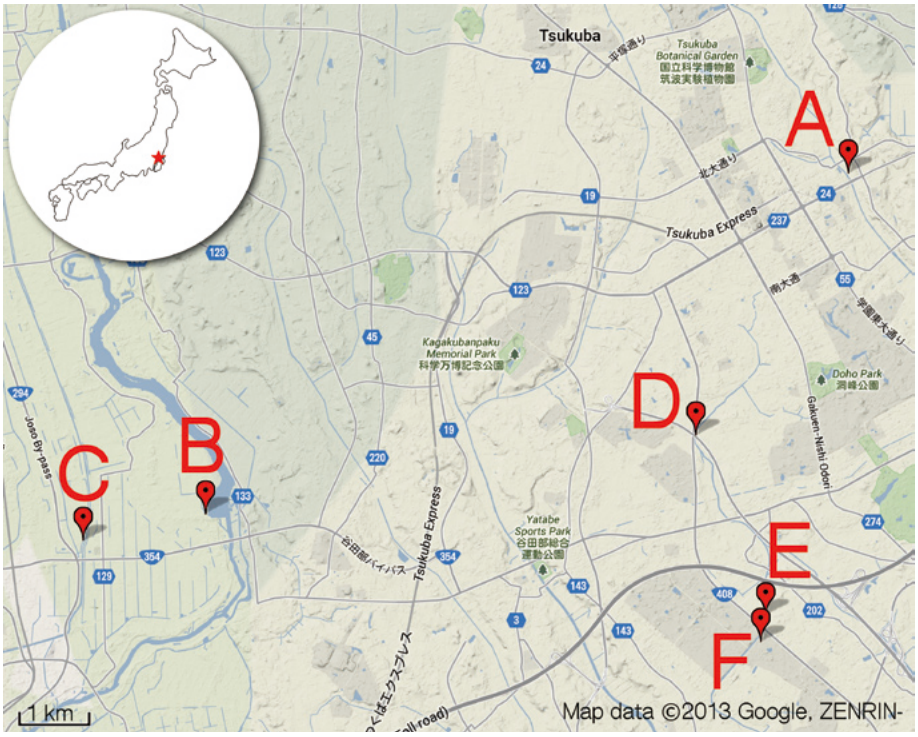

Fig. 1. Locations of natural habitats of mustards around Tsukuba, Japan.
A: Hanamuro on the Hanamuro River (36°5′14″N, 140°7′33″E), B: Oosakimachi on the Kokai River (36°2′34″N, 140°1′24″E), C: Nakayamamachi on the Hakkenbori River (36°2′22″N, 140°0′13″E), D: Teshirogi on the Inari River (36°3′13″N, 140°6′06″E), E: Minaminakazuma on the Inari River (36°1′51″N, 140°6′46″E), and F: Kannondai on the Inari River (36°1′37″N, 140°6′43″E). The location of the entire area is marked by a star on the upper-left national map. The largest distance between sites is 12 km (from A to C).

data transformation was applied to three components: pods per plant, seeds per plant, and yield per plant (Table 2). To calculate the correlations between the plants and soil, plant data and soil data were merged together at each site by the mean. *B. juncea* data were used for the plant components of correlation analysis. Pearson's correlations were calculated and 95% confidences were checked. Double box plot charts were used to visualize the correlations between soil components and plant materials (Tomizono 2013).

Results

1. Plant height, seed production, and 1,000 seed weight

The plant height of *B. juncea* at the six sites was 160 cm as an overall mean, and ranged from 67 to 238 cm (Table 3). There was a high degree of variance among individual plants both within and across sites. Among the means by sites, the maximum (190 cm at site B) was 1.5 times larger than the minimum (126 cm at site E). The plant height of *B. rapa* at all three sites was 109.4 cm as an overall mean, and ranged from 53.0 to 147.0 cm. There was also a high degree of variance among individual plants within each site, but with less variance across sites.

The mean seed production per plant of *B. juncea* at the six sites was 3,800, with a 95% confidence interval from 2,400 to 6,000 (Table 3). The large variance in seed production per plant was attributed to a range from less than 100 to 92,100. Among the means of *B. juncea* by sites, the maximum (19,700 at site B) was 20 times larger than the minimum (1,000 at site E). Therefore, the variance by sites was also large. In contrast, the mean seed production per plant of *B. rapa* at the three sites was 2,800. The seed production ranged from 100 to 34,800. Among the means of *B. rapa* by sites, the maximum (4,500 at site A) was 2.4 times larger than the minimum (1,900 at site C).

Table 4 lists the results of ANOVA tests on the seed production differences between *B. juncea* and *B. rapa*. Before testing for differences between the means of *B. juncea* and *B. rapa*, we excluded the data on sites D, E and F where *B. rapa* was not found. Both the means of seed production per plant and the thousand seed weight were different with a 5% significance level between *B. juncea* and *B. rapa*. The means of seed production per plant of *B. juncea* and *B. rapa* at the three sites were 8,400 and 2,800, respectively. The means of thousand seed weight of *B. juncea* and *B. rapa* at the three sites were 1.50 g and 2.29 g, respectively. The test concluded that *B. juncea* produced a greater number of seeds with smaller weight as compared to *B. rapa*.

ANOVA tests were also conducted for each site (Table 4). These tests concluded that there were no differences with a 5% significance level between the seed production numbers of *B. juncea* and *B. rapa* at sites A and C. The seed production number at site B was significantly different. The thousand seed weight was significantly different at all three sites.

Table 2. Data symmetry of wild *B. juncea* and *B. rapa* samples around Tsukuba, Japan.

Component		Skewness	
		B. juncea	*B. rapa*
Parent plant			
Height		−0.49	−0.82
Number of production			
Pods per plant	*	2.47	4.38
Seeds per plant	*	2.80	3.41
Seeds per pod		0.70	0.34
Seed weight			
1,000 grain		0.64	−0.02
Yield per plant	*	3.11	3.98

All skewness was calculated with raw data.

* After this table, logarithmic data transformations were applied to components marked by *.

2. Correlations between plant yield components and soil primary macronutrients

The mean soil pH of the six wild mustard habitats ranged from 6.06 to 7.14 (Table 5). The sites excluding D (7.14) have the pH recommended for mustards, that is, between 5.5 and 6.8 (Duke 1983), so these habitats have roughly favorable pH levels. Ammonia nitrogen was appropriate in all habitats given its recommendation of 1-5 mg/100 g (Fujiwara et al. 1996). Moreover, 5-15 mg/100 g of soil nitrate nitrogen was also recommended (Fujiwara et al. 1996). Thus, sites C, D and E were appropriate, while the other sites were deficient. The effective phosphoric acid recommended for crops is 20-50 mg/100 g (Fujiwara et al. 1996). Thus, the site B was sufficient and the other sites were deficient.

Two pairs were found to have significant correlations (Table 6). The soil nitrate nitrogen had a significant positive correlation with the thousand seed weight. The effective phosphoric acid had a significant positive correlation with the seed production per pod.

Discussion

This study revealed both the plant height and seed production capability of wild *B. juncea*. Our efforts have obtained baseline data that can be used in future studies. In particular, this data represents essential key factor data

Table 3. Plant height and yield components of wild *B. juncea* and *B. rapa* sampled around Tsukuba, Japan.

Species	Parent plant		Number of production						Seed weight			
Site [i]	Height (cm)		Pods per plant		Seeds per plant		Seeds per pod		1,000 grain (g)		Yield per plant (g)	
Total [j]												
B. juncea												
	160	(67-238)	470	(10-8,430)	3,800	(<100-92,100)	8.36	(3.83-16.21)	1.567	(1.080-2.270)	5.8	(0.1-151.1)
		[150-171]		[310-710]		[2,400-6,000]		[7.69-9.04]		[1.500-1.634]		[3.7-9.1]
B. rapa												
	109.4	(53.0-147.0)	350	(20-6,980)	2,800	(100-34,800)	8.8	(2.6-17.0)	2.29	(1.29-3.40)	6.3	(0.2-92.5)
		[101.5-117.4]		[220-550]		[1,800-4,500]		[7.5-10.1]		[2.10-2.49]		[3.9-10.0]
Site [k]												
B. juncea												
A	156^{abc}	(119-193)	1,080^{a}	(250-8,430)	8,300^{a}	(1,200-92,100)	8.00^{b}	(4.45-10.92)	1.376^{b}	(1.140-1.640)	11.4^{ab}	(1.9-151.1)
B	190^{a}	(127-237)	1,730^{a}	(630-5,330)	19,700^{a}	(8,400-62,700)	11.78^{a}	(7.48-16.21)	1.418^{ab}	(1.080-2.070)	27.6^{a}	(13.1-85.0)
C	171^{abc}	(103-201)	490^{ab}	(30-1,700)	4,000^{ab}	(200-15,900)	8.25^{b}	(4.80-10.26)	1.709^{a}	(1.430-2.130)	6.8^{ab}	(0.4-25.9)
D	183^{ab}	(152-238)	730^{ab}	(210-5,000)	5,000^{ab}	(1,200-29,900)	6.89^{b}	(4.90-8.96)	1.720^{a}	(1.340-2.270)	8.4^{ab}	(2.0-40.9)
E	126^{c}	(67-198)	130^{b}	(20-2,090)	1,000^{b}	(100-18,800)	8.07^{b}	(3.95-13.01)	1.660^{ab}	(1.360-1.920)	1.6^{b}	(0.1-28.4)
F	139^{bc}	(75-202)	150^{b}	(10-1,870)	1,100^{b}	(<100-20,300)	7.54^{b}	(3.83-10.81)	1.504^{ab}	(1.220-1.740)	1.6^{b}	(0.1-30.9)
B. rapa												
A	107.7^{z}	(53.0-133.0)	420^{z}	(30-1,240)	4,500^{z}	(300-11,700)	11.2^{z}	(6.3-17.0)	1.88^{y}	(1.29-2.46)	8.4^{z}	(0.6-24.0)
B	111.0^{z}	(71.0-147.0)	420^{z}	(30-6,980)	2,600^{z}	(200-34,800)	6.5^{y}	(4.0-8.4)	2.43^{z}	(1.52-3.40)	6.1^{z}	(0.3-92.5)
C	109.8^{z}	(63.0-140.0)	240^{z}	(20-1,270)	1,900^{z}	(100-7,800)	8.6^{zy}	(2.6-14.8)	2.58^{z}	(2.20-3.16)	4.8^{z}	(0.2-21.3)

Values indicate means with the range in round brackets. Square brackets indicate 95% confidence limits of total mean. Values by site in each column followed by the same lowercase letter do not differ significantly at the 5% level in the ANOVA-Tukey test. Because ANOVA was conducted only by site within species, the significance of differences across species is not shown. [i] Figure 1 shows the site locations; A: Hanamuro on the Hanamuro River, B: Oosakimachi on the Kokai River, C: Nakayamamachi on the Hakkenbori River, D: Teshirogi on the Inari River, E: Minaminakazuma on the Inari River, and F: Kannondai on the Inari River. [j] Statistic values are based on 59 *B. juncea* samples and 29 *B. rapa* samples, respectively. [k] Statistic values are based on 10 samples each except for site B where 9 samples were collected for each of the two plants.

Table 4. Seed production comparison between *B. juncea* and *B. rapa* in wild habitats.

(a) Total of sites A, B and C

	Three-site total (A, B and C) [a]		
	B. juncea	*B. rapa*	Significance of difference
Seeds per plant	8,400 (5,100-13,900) [b]	2,800 (1,700-4,600)	** [c]
1,000 seed weight (g)	1.50 (1.35-1.66)	2.29 (2.14-2.45)	**
Number of samples	29	29	58

(b) Each of sites A, B and C

	Significance of difference at each sampling site		
	A [a]	B	C
Seeds per plant	NS [c]	**	NS
1,000 seed weight	**	**	**
Number of samples	20	18	20

[a] Figure 1 shows the site locations. A: Hanamuro on the Hanamuro River, B: Oosakimachi on the Kokai River, and C: Nakayamamachi on the Hakkenbori River. Both *B. juncea* and *B. rapa* were found at these three sites. [b] Values indicate the means with a 95% confidence interval in parentheses. [c] Significance judged by ANOVA. * P = 0.05; ** P = 0.01; NS = not significant.

to estimate the λ of population dynamics and the impact as prescribed in the Cartagena Protocol on Biosafety to the Convention on Biological Diversity. Literally, this data is essential for assessing the environmental risk of GM *B. napus* in the approval process. Despite the low possibility of introgression from GM *B. napus* into wild *B. juncea* (Tsuda et al. 2014), this study found *B. juncea* to have a high capability for seed fecundity. True risk must be calculated by using both the introgression rate and fecundity, along with the introduction of enhanced fitness, in order to determine whether the hybrid may adversely affect surrounding biota as prescribed in the Cartagena Protocol.

The plant height of *B. juncea* that we obtained was higher than that documented in Japanese literature. In Japanese plant dictionaries, the plant height of *B. juncea* is described as being 30-80 cm (Shimizu et al. 2001), 50-100 cm (Takematsu & Ichizen 1993), and 30-100 cm (Nakai 2003). These values are evidently lower than our resultant mean (and range) of plant height, namely, 160 cm (67-238 cm) (Table 3). In Canada, wild mature *B. juncea* plants grow to a height of 100 to 200 cm (CFIA 2007). In contrast, the plant height of cultivated *B. juncea* was about 100 cm in both Japan and Canada (Ishikawa 1990, May et al. 2010), while three Indian cultivars were 158-173 cm in height (Singh et al. 2001). This difference between the plant dictionaries and our data could have been caused by confusion over crop and weed varieties in the dictionaries. Another possibility is that differences in varieties, locations or years may have resulted in the different heights reported.

This is the first study to show the accurate seed production capability of wild *B. juncea*. Specifically, the mean number was 3,800, the 95% confidence limit was 2,400-6,000, and the range was 100-92,100. This study also showed a high degree of variance among individual plants within each site and also across sites. Stevens (1932, 1957) reported that the seed production per plant of *B. juncea* totaled 4,950 and 4,780 in a low competitive environment. According to an Indian experiment using cultivated *B. juncea* (L.) Czern. 'Varuna', the seed production per plant was 7,075 (Ramana & Ghildiyal 1997). Another Indian cultivation study reported seed production of 1,842-3,301 from a two-year experiment using three cultivars (Singh et al. 2001). Our resultant wild *B. juncea* seed production number was 3,800, which was within the range of previous studies for cultivated *B. juncea*.

The mean thousand seed weight obtained from our results was definitely smaller than those from previous studies of cultivated *B. juncea*. We reported a mean thousand seed weight of 1.57 g (Table 3), whereas previous studies reported 4.64 g (Ramana & Ghildiyal 1997), 3.49-4.49 g (Singh et al. 2001), and 3.47 g and 5.75 g (Razaq et al. 2011). Stevens (1932, 1957) reported 2.63 g and 1.84 g, as obtained in natural habitats, which were similar to our result. Therefore, the difference between the larger (> 3 g) and smaller (< 3 g) values in the thousand seed weight may be due to differences in crop fields and natural habitats. Wild *B. juncea* living in a natural habitat suffers serious competition with other *B. juncea* plants and other species

Table 5. Soil chemical properties of wild *B. juncea* and *B. rapa* habitats around Tsukuba, Japan.

Sampling site [i]	pH		Ammonia nitrogen (mg/100g)		Soil nitrate nitrogen (mg/100g)		Effective phosphoric acid (mg/100g)		Exchangeable potassium (mg/100g)	
Total [j]										
	6.66	(6.00-7.60)	2.89	(0.65-7.33)	6.9	(1.4-21.5)	14.2	(1.0-68.2)	84	(27-231)
		[6.51-6.81]		[2.36-3.42]		[5.0-8.8]		[9.1-19.3]		[63-105]
Site [k]										
A	6.32^{bc}	(6.10-6.50)	2.96^{ab}	(1.61-4.36)	3.4^{c}	(1.4-6.5)	9.0^{b}	(8.2-9.7)	54^{b}	(40-78)
B	6.76^{a}	(6.50-7.00)	2.45^{ab}	(1.86-3.11)	2.5^{c}	(1.4-3.5)	42.0^{a}	(27.4-68.2)	68^{b}	(30-124)
C	6.72^{ab}	(6.60-6.90)	4.28^{a}	(2.63-5.80)	14.8^{a}	(7.3-21.5)	5.9^{b}	(4.0-8.0)	201^{a}	(159-231)
D	7.14^{a}	(6.80-7.60)	2.46^{ab}	(1.80-2.89)	7.1^{bc}	(4.6-11.3)	13.8^{b}	(10.0-17.0)	77^{b}	(60-101)
E	6.06^{c}	(6.00-6.20)	4.07^{a}	(2.78-7.33)	10.3^{ab}	(8.1-14.4)	9.0^{b}	(5.7-13.2)	60^{b}	(44-82)
F	6.96^{a}	(6.50-7.30)	1.12^{b}	(0.65-2.04)	3.0^{c}	(1.9-4.8)	5.5^{b}	(1.0-8.5)	45^{b}	(27-67)

Values indicate the means with the range in round brackets. Square brackets indicate 95% confidence limits of the total mean. Values by site in each column followed by the same lowercase letter do not differ significantly at the 5% level in the ANOVA-Tukey test. [i] Figure 1 shows the site locations; A: Hanamuro on the Hanamuro River, B: Oosakimachi on the Kokai River, C: Nakayamamachi on the Hakkenbori River, D: Teshirogi on the Inari River, E: Minaminakazuma on the Inari River, and F: Kannondai on the Inari River. [j] Statistic values are based on 30 samples. [k] Statistic values are based on 5 samples each.

in struggling for daylight, water and nutrients. However, cultivated *B. juncea* in a crop field typically experiences much less competition; moreover, the plants are blessed with human care, such as the application of fertilizer and pest control. These differences would increase the seed weight of cultivated *B. juncea*. In fact, the soil primary macronutrients of our six habitats were not sufficiently favorable for *B. juncea* as shown in Table 5. Although ammonia nitrogen was appropriate, soil nitrate nitrogen was deficient at sites A, B and F. Moreover, effective phosphoric acid was deficient at every site except B. Therefore, our results regarding *B. juncea* yields were far from being an optimized yield under human care. We believe that these deficiencies in nutrients are common in natural habitats.

The effective phosphoric acid positively correlated with the seed production number per pod (r = 0.90, Table 6). Coefficients with seed number (and weight) per plant were also given as positive values, though these correlations were not significant (Table 6). These weak positive correlations are reasonable, because the effective phosphoric acid contributes to cellular energy metabolism and is an essential nutrient for plant growth in general, and because the phosphate requirement is not very high for *B. juncea* as reported by Booth and Gunstone (2004). No significant correlations were found between the soil nitrate nitrogen and the seed weight (or number) per plant. However, a strong positive correlation (r = 0.82) was found between the soil nitrate nitrogen and the thousand seed weight (Table 6). Though nitrogen is important for increasing seed yield (Duke 1983, Pouzet 1995), the relationship between the soil nitrogen and the *B. juncea* yield was ambiguous in our results. Nitrogen was not a rate-determining factor at these six habitats. This phenomenon could arise from a deficiency or an excess of other soil nutrients including micronutrients. The two strong correlations (r = 0.90 and 0.82) above were unexpected, and the reason for these correlations is unknown. And given the fact that there were only six sites with large variance within the sites, these strong correlations still have some uncertainty regardless of their significance by p-values (Fig. 2).

Another possibility is that the difference in seed production arises from differences in varieties; e.g. a difference between wild and crop varieties. This question can be solved by conducting a pot experiment with several fertilizer levels and several varieties. However such an effort is beyond the scope of this study.

The seed production number per plant was larger for *B. juncea* than for *B. rapa* in the same habitat. Conversely, the thousand seed weight was significantly smaller for *B. juncea* than for *B. rapa* in the same habitat (Table 4). Although usually a trade-off exists between seed size and the number of seeds produced, the variance in seed size for a species is rather small because there would be an optimized seed size that could maximize the fitness of the parent plant (Seiwa 2003). Both mustards have probably developed their seed production characteristics (e.g. large seed number, large seed size) as a result of selection to optimize their reproduction as a variety. Currently *B. juncea* has larger populations than *B. rapa* along riversides in Japan. This fact may suggest why the strategy of producing a larger number of seeds has proven successful for *B. juncea*.

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Table 6. Correlation coefficients of pairwise comparison between wild *B. juncea* yield components and soil chemical properties of six habitats around Tsukuba, Japan.

	Parent plant	Number of production			Seed weight	
Soil chemical property	Height	Pods per plant	Seeds per plant	Seeds per pod	1,000 grain	Yield per plant
pH	0.60	0.23	0.20	−0.09	0.19	0.22
Ammonia nitrogen	−0.08	−0.02	−0.01	0.01	0.46	0.03
Soil nitrate nitrogen	−0.10	−0.31	−0.32	−0.30	0.82 *	−0.26
Effective phosphoric acid	0.63	0.65	0.73	0.90 *	−0.41	0.72
Exchangeable potassium	0.32	0.11	0.11	−0.01	0.54	0.16

* 5% significant correlation.

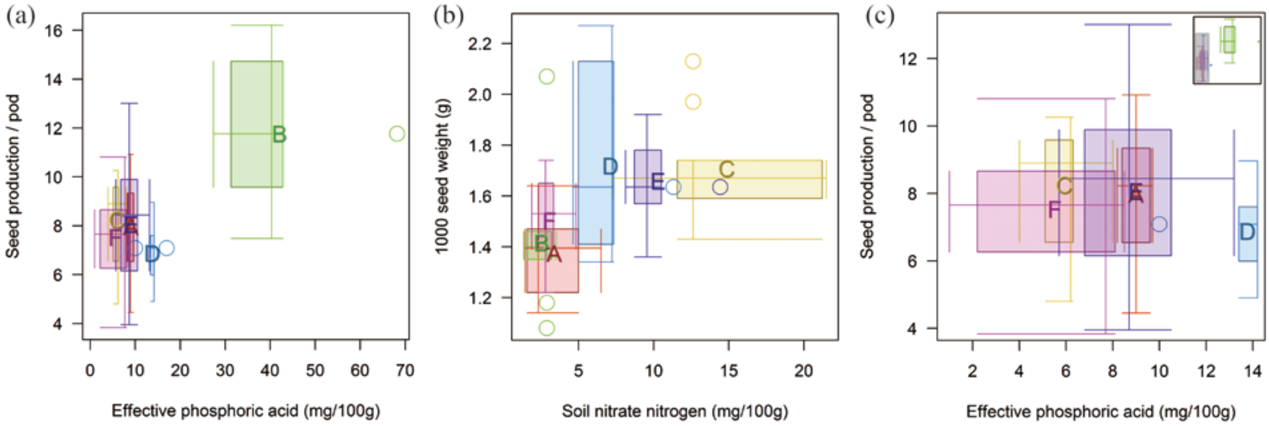

Fig. 2. Relationship between soil and plant components of wild *B. juncea* around Tsukuba, Japan.
(a) Box plot correlation diagram between effective phosphoric acid and number of pods per plant (r = 0.90). **(b)** Box plot correlation diagram between soil nitrate nitrogen and thousand seed weight (r = 0.82). These two pairs were found to have significant correlations in Table 6. **(c)** Zoomed chart of **(a)**. The bottom-left complicated area of **(a)** is zoomed. The area of zooming is indicated by a gray rectangle in a top-right square thumbnail. Site labels (A, B, C, D, E and F) are placed at means by site, in the same manner as in a scatter chart. Figure 1 shows the site locations; A: Hanamuro on the Hanamuro River, B: Oosakimachi on the Kokai River, C: Nakayamamachi on the Hakkenbori River, D: Teshirogi on the Inari River, E: Minaminakazuma on the Inari River, and F: Kannondai on the Inari River.

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