REPRODUCTIVE BARRIERS IN SUGAR BEET AND ITS WILD RELATIVES OF THE SECTION *VULGARES*, THE GENUS *BETA*

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Introduction

Wild relatives of sugar beet (*Beta vulgaris* L.) in the section *Vulgares* are classified into five species, *B. maritima*, *B. atriplicifolia*, *B. macrocarpa*, *B. patula* and *B. adanensis*[3,4]. They cross readily with sugar beet and with one another, and produce vigorous F_1 hybrids. Segregating populations obtained from crosses between sugar beet and these wild species, however, are extremely variable with respect to morphological traits such as root shape, foliar type and seed size, indicating the genetic divergence between them[8]. Also, these species hybrids show some phenomena associated with reproductive isolation, i. e., pollen sterility of F_1 hybrids,[1,9] and F_2 segregations of chlorotic[1] and sterile plants.[9] In this paper, we elucidate the reproductive isolation among the species of the section *Vulgares* in detail, and discuss their species relationships.

Materials and Methods

The materials used were 12 strains belonging to five species, *B. vulgaris*, *B. maritima*, *B. atriplicifolia*, *B. macrocarpa* and *B. patula*. Interspecific F_1 and F_2 hybrids were obtained from the crosses in which *B. vulgaris* Red beet (SP561001-0), *B. macrocarpa* and *B. patula* were used as testers. In addition, progenies of some B_1F_1's and a test cross were examined.

The characters examined are the pollen and seed fertility of F_1 hybrids and F_2 segregations of chlorosis, weakness and pollen sterility. The pollen fertility was evaluated with a mean percentage of two replicates of the stainable pollen grains with cotton blue. The seed fertility was given by the percentage of fertilized seeds to total flowers of 15 flower clusters. All

the strains used had multigerm seeds. The pollen and seed fertilities of the F_1 were examined on at least five and three plants, respectively.

Results

1. F pollen sterility

All the F_1 hybrids exhibited vigorous growth. As reported previously,[1] partial-sterile plants with red pigmented anthers segregated in the F_1 of *B. patula* × Red beet. Their pollen fertilities ranged from 2.3 to 38.8%, whereas plants with normal anthers showed high fertilities (mean 85.6%). This sterility seems to be not gametic but sporophytic since it accompanied the abnormality of anthers.

TABLE 1. F_1 pollen fertility in interspecific hybrids

		Pollen fertility (%) of F_1 with		
		Red beet	*B. patula*	*B. macrocarpa*
B. vulgaris	Red beet		85.4♯	65.8
	Seiyo Natsuna*	95<	89.6	75.0
B. maritima	W35b (A)	95<	86.8	73.9
	WB37 (A)	94.5	86.1	41.9
	SP581103-0 (P)	95<	88.7	74.7
	SP581105-0 (P)	95<	91.3	83.4
	SP673000-0 (P)	93.7	88.0	69.7
	SP733050-01 (P)	95<	77.9	75.5
B. atriplicifolia		95<	89.1	77.7

A and P show annual and perennial strains, respectively.
*: var. cicla
♯: pollen fertility of plants with normal anthers.

Table 1 shows the pollen fertility of F_1 hybrids. The F_1's with Red beet and *B. patula* showed almost normal fertilities, although some crosses with the latter gave slightly low fertilities. In contrast, the F_1's with *B. macrocarpa* were partially sterile. The F_1 of *B. macricaroa* × *B. maritima* WB37 showed the lowest fertility (41.9%), whereas pollen fertilities of the others ranged from 65.8 to 83.4% (mean 74.5%).

2. Seed abortion due to zygotic lethal

Table 2 shows the seed fertility of the F_1 hybrids with *B. patula* or *B. macrocarpa*. All the F_1's with *B. patula* showed high seed fertilities, whereas seed fertilities of those with *B. macrocarpa* were lowered, and

TABLE 2. F_1 seed fertility in interspecific hybrids

		Seed fertility (%) of F_1 with	
		B. patula	*B. macrocarpa*
B. vulgaris	Red beet	92.0	49.5
	Seiyo Natsuna	92.0	49.5
B. maritima	SP581103-0	91.5	45.5
	SP673000-0	—	52.9
Mean		89.7	49.0

TABLE 3. Seed fertility (%) of *B. maritima* SP673000-0 and WB37, crossed as female to F_1's of *B. macrocarpa* with the other species as male parents

F_1 of *macrocarpa* with		Seed fertility of *B. maritima*	
		SP673000-0	WB37
B. vulgaris	Red beet	70.0	70.0
	Seiyo Natsuna	73.1	57.0
B. maritima	SP581103-0	70.2	52.8
	SP673000-0	68.8	66.5
Mean		70.8	61.7
Open-pollinated plants		88.2±4.6	81.0±6.5

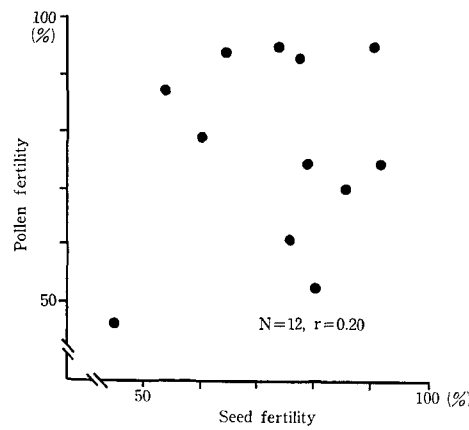

Fig. 1. Association between the pollen and seed fertilities in randomly selected B_1F_1 plants of the cross, *B. vulgaris* TK80-0×F_1 (*B. vulgaris* Red beet×*B. macrocarpa*).

ranged from 45.5 to 52%. Furthermore, when the F_1's with *B. macrocarpa* were backcrossed as pollen parents to *B. maritima*, seed fertilities of backcrossed plants decreased from 6 to 20% in comparison with those of open-pollinated ones (Table 3). This indicates seed abortion due to zygotic lethal after fertilization.

Fig. 1 shows an association between the pollen and seed fertilities in randomly selected B_1F_1 segregants of *B. vulgaris* TK80-0 × F_1 (Red beet × *B. macrocarpa*). The seed fertilities of the B_1F_1 plants were not related directly to their pollen fertilities. Thus the sterility of F_1 hybrids with *B. macrocarpa* disrupted only male gametogenesis, and their low seed fertilities may also be caused by the seed abortion, although female sterility can not be completely precluded as a causal factor.

3. F_2 chlorosis and weakness

The chlorotic plants, which died at the cotyledon stage without any leaf expansion, segregated in *B. maritima* W35b × Red beet, and the crosses with *B. macrocarpa* (Table 4). The frequencies of chlorotic plants were less than 1% in the crosses between *B. macrocarpa*, and *B. maritima* SP581103-0 or SP673000-0, whereas they ranged from 3.5 to 8.5% in the other crosses, suggesting 15 : 1 ratio for normal and chlorotic plants (Table 5). It was then assumed that there were duplicate recessive chlorosis genes, ch_1 and ch_2, double recessive homozygotes expressing a chlorosis lethal. The observed frequencies fitted the expectation in four crosses except *B. atriplicifolia* × *B. macrocarpa*.

TABLE 4. F_2 segregations of chlorotic plants in the crosses in which Red beet, *B. patula* and *B. macrocarpa* each was used as a tester

		Red beet	*B. patula*	*B. macrocarpa*
B. vulgaris	Red beet		−	+
	Seiyo Natsuna		−	+
B. maritima	W35b	+	−	
	WB37	−	−	+
	SP581103-0	−	−	+
	SP581105-0	−	−	−
	SP673000-0	−	−	+
	SP733050-01	−	−	
B. atriplicifolia		−	−	+

− : Not observed. + : Observed. Brank : Not examied.

TABLE 5. Observed frequencies of chlorotic plants in some F_2 populations and χ^2 tests for fittness to the segregation ratio of duplicate ressecive genes

Cross combination		No. of plants			Goodness of fit	
		Total	Chlorotic		χ^2 (15 : 1)	p
B. macrocarpa × *B. vulgaris*						
	Red beet	276	22	8.0%	2.60	10% < p < 25%
	Seiyo Natsuna	240	18	7.5%	1.60	10% < p < 25%
B. macrocarpa × *B. maritima*						
	WB37	352	19	5.4%	3.48	5% < p < 10%
	SP581103-0	467	4	0.9%	23.67	p < 0.1%
	SP673000-0	321	1	0.3%	19.41	p < 0.1%
B. atriplicifolia × *B. macrocarpa*		912	31	3.4%	12.65	p < 0.1%
B. maritima W35 × Red beet		375	31	8.3%	2.46	10% < p < 25%

To test whether *B. maritima* W35b and *B. macrocarpa*, both of which gave chlorotic plants in the crosses with Red beet, have the same genes responsible for the chlorosis or not, the segregation of chlorotic plants was examined in the cross of F_1 (*B. maritima* × Red beet) × F_1 (*B. macrocarpa* × Red beet). If the F_2 chlorosis observed is controlled by the duplicate recessive genes as assumed above, and both *B. macrocarpa* and *B. maritima* W35b have the same genotype, the segregation of the chlorosis in the progeny would be expected to be 15 : 1 for normal and chlorotic plants. As a result, three chlorotic plants segregated in a total of 70 plants, and the frequency fitted the expectation ($X^2=0.48$, df=1, 25% < p < 50%). Thus F_2 chlorosis could be partly explained by the duplicate recessive genes, ch_1 and ch_2, and the genotype of *B. macrocarpa* and *B. maritima* W35b was assumed to be $+_1/+_1$ ch_2/ch_2, and that of Red beet to be ch_1/ch_1 $+_2/+_2$. On the other hand, genotypes of the other species could be estimated from the crosses with these three strains (Table 4). *B. vulgaris* var. *cicla* Seiyo Natsuna and *B. maritima* WB37 were ch_1/ch_2 $+_2/+_2$, and *B. patula* and *B. maritima* SP581105-0 were $+_1/+_1$ $+_2/+_2$. *B. atriplicifolia* appears to have the same ch_1 gene as *B. vulgaris*, although the chlorosis frequency did not fit the expected one in the cross with *B. macrocarpa*.

In addition to the chlorosis, weak plants which exhibited the dwarf stature segregated in the crosses with *B. patula* or *B. macrocarpa* (Table 6). The frequencies of weak plants ranged from 0.3 to 7.6%. Their pollen

TABLE 6. F_2 segregations of weak plants in interspecific hybrids with *B. patula* and *B. macrocarpa*

		B. patula			*B. macrocarpa*		
		Total	Weak (%)		Total	Weak (%)	
B. vulgaris	Red beet		—			—	
	Seiyo Natsuna		—			—	
B. maritima	W35b	249	19	7.6%			
	WB37				215	7	3.3%
	South Itary	260	3	1.2%			
	SP581103-0	433	3	0.7%	376	1	0.3%
	SP673000-0		—				
	SP733050-01					—	
B. atriplicifolia			—		302	16	5.3%

—: Not observed. Brank: Not examined.

TABLE 7. Frequency distribution of pollen fertility in F_2's of the crosses of four species with *B. vulgaris* Red beet

Species	Pollen fertility (%)											No. of plants
	<1	10	20	30	40	50	60	70	80	90	100	
B. maritima W35b								1	1	7	51	60
B. atriplicifolia									3	8	71	82
B. macrocarpa	6	3	6	8	7	10	16	29	26	19	8	139
B. patula	6	3	3	4	5	7	3	9	19	31	39	129

fertilities varied from less than 10% to more than 90%, and did not show any association between segregations of the weakness and the pollen sterility.

4. F_2 pollen sterility

Table 7 shows F_2 segregations of pollen fertility in the crosses between some annual species and Red beet. The pollen fertility was examined only for plants which flowered in the first year. Most of F_2 plants in the crosses with *B. maritima* W35b and *B. atriplicifolia* had high pollen fertilities (above 70%), whereas the pollen fertility showed a continuous variation, and complete-sterile plants segregated at a frequency of 4.7% in *B. macrocarpa* × Red beet, and 4.2% in *B. patula* × Red beet. The latter two species also gave partial-and complete-sterile segregants in the crosses with *B. maritima* or *B. atriplicifolia* (Table 8).

TABLE 8. Frequency distribution of pollen fertility in F_2's of the crosses of *B. macrocarpa* and *B. patula* with *B. maritima* and *B. atriplicifolia*

Cross combination	Pollen fertility (%)											No of plants
	<1	10	20	30	40	50	60	70	80	90	100	
The crosses of *B. macrocarpa* with												
B. maritima WB37	10	1	2	7	7	9	20	12	31	36	21	156
B. atriplicifolia	14	5	8	10	19	16	23	22	30	26	4	177
The crosses of *B. patula* with												
B. maritima W35b	11	6	4	1	5	4	8	9	15	16	15	94
B. maritima SP733050-01	7	4	2	2	3	3	1	1	11	17	22	74

As mentioned above, partial-sterile plants with red pigmented anthers segregated in the F_1 of *B. patula* × Red beet. In the F_2 obtained from sibmating of normal F_1 plants, both partial-and complete-sterile plants segregated irrespective of the anther pigmentation. The segregation of pollen fertility did not differ significantly between phenotypes at the R locus (KOLMOGORV-SMILNOV test, $D=0.09<D_{0.05}=0.27$). Thus the anther pigmentation appears to be a byproduct of the sterility. A similar association between the pollen sterility and the anther pigmentation is also reported in table beet[10].

Discussion

Five knids of reproductive barriers were found in sugar beet and its wild relatives of the section *vulgares*, i. e., the partial sterility of pollen and the seed abortion in the F_1, and F_2 segregations of the chlorosis, weakness and sterility. These species have been considered to be closely related because of high cross affinity and morphological similarity[7]. However, reproductive isolation has developed among them as well as among species of different sections[2].

All the barriers observed existed between *B. macrocarpa*, and *B. vulgaris*, *B. maritima* and *B. atriplicifolia*. The extent of hybrid breakdown between species generally increases as their genetic constitutions differentiate[5,6]. Thus *B. macrocarpa* may be the most divergent species of this section. This is also in general agreement with the genetic difference at several loci coding enzymes between *B. macrocarpa* and the other species (unpublished data). *B. macrocarpa* is partly sympatric with *B. maritima* and *B. atriplicifolia* along the coasts of the Mediterranian area[3,4]. The extensive reproductive barriers between these species, however, suggest that

they are not a monophyletic assemblage, but rather *B. macrocarpa* and the others may have independently differentiated.

In addition, *B. patula* gave weak and sterile segregants in the crosses with *B. vulgaris, B. maritima* and *B. atriplicifolia,* indicating that it differs slightly from the latter three species. On the other hand, no reproductive barriers were detected among *B. vulgaris, B. maritima* and *B. atriplicifolia* except for the chlorosis found in a cross between the former two species.

The classification in the section *Vulgares* have not been well established. Some taxa treated as species in this study according to COONS[4] have been given the rank of subspecies or variety by different authors. On the basis of morphological similarity, FORD-LLOYD and WILLIAMS[7] classified *B. vulgaris, B. maritima* and *B. patula* into subspecies of *B. vulgaris,* and *B. macrocarpa* and *B. atriplicifolia* into variety of spp. *maritima.* Based on the reproductive isolation, however, *B. macrocarpa* and *B. patula* may be treated as species, and the remaining taxa as subspecies of a different species from the formers.

Out of the reproductive barriers observed, the F_2 chlorosis could be partly explained by the duplicated recessive genes, and the genotypes varied not only among the species but within *B. maritima.* Particularly, *B. macrocarpa* and *B. patula* differed in their genotypes from each other, and also from *B. atriplicifolia* and the annual strains (W35b and WB37) of *B. maritima,* although *B. maritima* W35b had the same genotype as *B. macrocarpa.* Such a polymorphism in the chlorosis genotype may furnish another circumstantial evidence for the polyphyletic origin of these annuals.

Summary

The reproductive barrier was examined in the interspecific F_1 and F_2 hybrids in the section *Vulgares.* The barriers observed were the partial pollen sterility and partial seed abortion in the F_1, and the segregation of chlorosis, weakness and sterility in the F_2. All the barriers existed between *B. macrocarpa,* and *B. vulgaris, B. maritima* and *B. atriplicifolia,* suggesting that *B. macrocarpa* is the most divergent species in this section. *B. patula* gave the weak and sterile segregants in the crosses with *B. vulgaris, B. maritima* and *B. atriplicifolia,* whereas no reproductive barriers were recognized among the latter three species, except for F_2 segregation of the chlorosis in a cross between *B. vulgaris* and *B. maritima.* Out of these barriers, the F_2 chlorosis was partly controlled by duplicate recessive genes, and its genotype was polymorphic among the species and also within *B. maritima.*

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