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GENETIC ANALYSIS FOR ANNUAL AND EARLY-FLOWERING HABIT OF *BETA MACROCARPA* GUSS., A RELATED SPECIES OF *B. VULGARIS* L.: AN ANALYSIS WITH ENZYME-CODING LOCI AS CHROMOSOME MARKERS

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Introduction

The genetic basis of species differences is less amenable to analysis because hybridization between species is often difficult and, even if sufficient hybrid progenies are avairable, anomalous segregation associated with reproductive barriers preclude the conventional gene analysis based on segregating ratios. In such cases, analyses with marker genes for effects of chromosomes on phenotypes provide useful clues for the genetic studies (MANGERSDORF 1974, TANKSLEY *et al.* 1982, VAN DIJK 1984).

Beta macrocarpa Guss., a wild relative of sugar beet (*B. vulgaris* L), is an annual and self-fertile species, which is reproductively isolated from sugar beet and other species of the section *Vulgares* (OLDEMEYER 1957, ABE *et al.* 1987). F_1 hybrids between *B. macrocarpa* and the other species are vigorous but partially sterile, and hybrid breakdown usually causes the production of chlorotic, weak and/or sterile segregants in the F_2 progenies. In addition, the normal transfer of alleles for some enzyme-coding loci was found to be strongly disturbed in the hybrid progenies (ABE and TSUDA 1987).

In this paper, we report the results of the genetic analysis for annual and early-flowering habits of *B. macrocarpa*. Based on the analysis with enzyme-coding loci as chromosome markers, these habits were found to be controlled by a few major genes proximal to the enzyme markers used.

Materials and Methods

The materials used in this study consisted of *B. macrocarpa* (an accession

from the Imperial Valley of California), *B. vulgaris* SP 561001-0 and *B. maritima* SP 581103-0. The latter two strains exhibited the perennial habit which requires a low temperature (vernalization) for floral initiation. F_1 and F_2 hybrids were obtained from the crosses between *B. macrocarpa* and the two perennial species.

F_2 seeds were sown in paper pots in a green house. Six weeks later, when they were at the three to four leaf stage, these plants along with the parents and F_1 hybrids were transplanted in an experimental field, and examined for their bolting and flowering characteristics. In addition, records were taken on the lowest node with a seed ball on a main stem.

F_2 progenies were also scored for five independently inherited enzyme-coding loci, *Got-2*, *Lap*, *Gdh-2*, *Aph-1*, and *Px-2*. Of these, *Got-2* is linked with the gene R for red hypocotyl-color (ABE and TSUDA 1987). For the five loci, *B. macrocarpa* had the alleles which were not detected or at least infrequently observed in *B. vulgaris* and *B. maritima*, $Got\text{-}2^1$, Lap^1, $Gdh\text{-}2^1$, $Aph\text{-}1^1$, and $Px\text{-}2^1$, while $Got\text{-}2^2$, Lap^2 and Lap^3, $Gdh\text{-}2^2$ and $Gdh\text{-}2^3$, and $Aph\text{-}1^2$ and $Aph\text{-}1^3$ were predominant in the latter two species (ABE and TSUDA 1987). *Px-2* was not expressed in *B. vulgaris* and *B. maritima*. Methods of electrophoresis have been described elsewhere (ABE and TSUDA 1987).

Results and Discussion

Reproductive barriers and distorted segregation for enzyme-coding loci

The F_1 hybrids of *B. macrocarpa* with *B. vulgaris* and *B. maritima* were vigorous but partially sterile, as previously reported (OLDEMEYER 1957, ABE *et al.* 1987). Approximately one-quater the pollen and half the seed were aborted. Chlorotic plants which died at the cotyledon stage segregated in the F_2; the frequency was 8.2% in the cross with *B. vulgaris* SP 561001-0, and 0.9% in the cross with *B. maritima* SP 581103-0. No zygotic lethals except for the chlorosis were detected in the seedling and subsequent growing stages.

Table 1 shows the chi-square values obtained to determine whether F_2 segregation for each enzyme-coding locus can be expected from the Mendelian ratio. Three of the five loci assayed yielded ratios that significantly deviated from the expectation. The segregation of *Got-2* showed a significant excess of the *B. macrocarpa* allele, whereas a significant excess of the *B. vulgaris* or *B. maritima* allele was observed for *Lap* and *Gdh-2*. The distorted segregation may be due to the linkage with the genes responsible for reproductive barriers found in the hybrids. Our preliminary test suggested that

TABLE 1. F_2 segregation for five enzyme-coding loci

Loci	M/M	M/+	+/+	χ^2 values (1:2:1 or 3:1)
B. macrocarpa×*B. vulgaris* S P561001-0				
Got-2	52	76	24	11.84**
Lap	10	69	73	55.51**
Gdh-2	10	90	46	25.67**
Aph-1	48	67	37	3.72
Px-2	114[1)]		38	0.00
B. macrocarpa×*B. maritima* SP 581103-0				
Got-2	77	126	29	21.59**
Lap	36	125	71	11.96**
Gdh-2	9	134	89	60.76**
Aph-1	67	117	48	3.13
Px-2	179		53	0.57

M and + show the alleles derived from *B. macrocarpa*, and *B. vulgaris* or *B. maritima*, respectively.

1): Including M/M and M/+ because *Px-2* is not expressed in *B. vulgaris* and *B. maritima*.

**: Significant at 1% level.

the distortion for *Lap* may result from the linkage with a sterility gene causing partial abortion of pollen (unpublished data).

Genetic basis of annual habit

The F_1 hybrids flowered without vernalization, indicating that the annual habit of *B. macrocarpa* was dominant over the perennial one. The F_2 progenies exhibited various growth forms (Table 2). Bolting did not necessarily lead to flowering, and the presence of a main stem was controlled by a genetic system different from that for the floral initiation; a few plants which had lateral stems with flowers instead of the main stem segregated in the cross with *B. maritima* SP 581103-0. Frequency of flowering plants was higher in the cross with *B. vulgaris* SP 561001-0 than in the cross with *B. maritima* SP 581103-0, the difference being highly significant ($G=30.3$, $p \ll 0.01$).

Heterogeneity test for the segregation of flowering plants among enzyme genotypes was carried out to detect linkages between a enzyme locus and the gene affecting the annual habit. In total, *Got-2*, *Lap* and *Px-2* showed a significant effect on the segregation (Table 3). In all of the cases, the frequency of flowering plants was the highest in the homozygote of the *B.*

TABLE 2. The segregation of various growth forms and the frequency of flowering plants in the F_2 progenies

Crosses of *B. macrocarpa* with	Main stem					Frequency of flowering plants
	Presence		Absence			
	F	B	F	B	R	
B. vulgaris SP 561001-0	142	4	0	0	6	93.4%
B. maritima SP 581103-0	152	3	15	22	40	72.0%

F: Flowering, B: Bolting only, R: Rosette.

TABLE 3. G test of heterogeneity for the segregation of flowering plants among genotypes for five enzyme-coding loci

Loci	Enzyme genotypes						G values for heterogeneity
	M/M (M/−)		M/+		+/+		
	F[1)]	B+R	F	B+R	F	B+R	
B. macrocarpa×*B. vulgaris* SP 561001-0							
Got-2	52	0	69	7	21	3	8.23*
Lap	10	0	66	3	66	7	2.55
Gdh-2	10	0	86	4	40	6	3.96
Aph-1	46	2	63	4	33	4	1.38
Px-2[2)]	111	3			31	7	9.05**
B. macrocarpa×*B. maritima* SP 581103-0							
Got-2	58	19	89	37	20	9	0.68
Lap	29	7	95	30	43	28	6.64*
Gdh-2	6	3	97	37	64	25	0.13
Aph-1	50	17	83	34	34	14	0.33
Px-2	137	42			30	23	8.52**

1): See Table 2.
2): See Table 1.
*, **: Significant at 5% and 1% levels, respectively.

macrocarpa allele or the class including the homo- and heterozygote of that allele, suggesting that the annual habit was controlled by at least two or three pairs of genes proximal to these enzyme-coding loci. However, the data obtained were not sufficient to analyze their intra- and interlocus interactions

in detail because the detection of chromosomal effects itself depends on not only the effects of the genes noted but their proximity to the enzyme-coding loci used as markers (TANKSLEY *et al.* 1982). Of these loci, the significances of *Got-2* and *Lap* were detected only in one or the other of the two crosses examined, suggesting the existance of a gene interaction with the genetic background.

The annual habit in sugar beet is determined by a single dominant gene (*B*), which is linked to the gene *R* for red hypocotyl-color with a recombination value of 15.5% (ABEGG 1936). The fact that, of the three loci affecting the annual habit, *Got-2* belongs to the same linkage group as the gene *R* (ABE and TSUDA 1987) suggests that *B. macrocarpa* might have a gene at a homeologous locus similar to the gene *B*. Since the gene *B* was detected in a commercial variety (MUNERATI 1931), it is probable that these genes occurred independently through a parallel mutation.

The results of the analyses for isozyme variation and reproductive barriers among the species of the section *Vulgares* (ABE and TSUDA 1987, ABE *et al.* 1987) suggested that the differentiation to annuality in the section might not be monophyletic. It would be of special interst in this connection to determine whether the other annual taxa would carry the same or similar genes for the annual habit as *B. macrocarpa* did. Such a comparative study between species of different phyletic lines can provide useful clues to determine what kinds of genetic changes underlie species differences.

Genetic basis of difference in position of seed balls

B. macrocarpa is also characterized by an early-flowering bahit (COONS 1954, BUTTLER 1977). Under favorable environments, flowering starts from the lower nodes in contrast to the perennial species which usually flower from the upper nodes. In our field experiment, mean number of nodes from the basal region to the lowest node with a seed ball was 2.4 in *B. macrocarpa*, 11.4 in *B. vulgaris* SP 561001-0 and 12.5 in *B. maritima* SP 581103-0.

The plant type of the F_1 hybrids was nearly intermediate between that of the parental species. The average of the lowest node with a seed ball was 6.5 in the cross with *B. vulgaris* SP 561001-0, and 7.3 in the cross with *B. maritima* SP 581103-0, which closely approximated to the mid-parent values, 6.7 and 7.1, respectively. In the F_2 the trait exhibited a continuous variation typical for quantitative characters, although the distribution was highly skewed toward *B. macrocarpa*.

Table 4 shows the results of the analysis for the chromosomal effects on the position of seed balls. Tests of significance among the enzyme genotypes were performed by a one-way multiple analysis of variance in which

TABLE 4. Test of significance for mean values of the lowest node with seed ball[1] among genotypes for five enzyme-coding loci

Loci	Genotypes	*B. macrocarpa*×*B. vulgaris* SP 561001-0				*B. macrocarpa*× *B. maritima* SP 581103-0			
		N	Mean	SD	F[2]	N	Mean	SD	F
Got-2	M/M	51	4.6	2.2		52	5.9	2.9	
	M/+	67	5.4	3.0	5.41**	77	6.2	3.7	4.95**
	+/+	21	7.1	7.1		18	9.5	4.9	
Lap	M/M	10	3.9	2.0		27	7 5	5.1	
	M/+	66	5.3	2.5	2.61	83	6.5	3.5	0.53
	+/+	63	5.7	3.6		37	6.1	2.6	
Gdh-2	M/M	10	4.5	2.2		5	6.2	4.1	
	M/+	84	5.4	3.3	2.12	86	6.5	3.5	0.07
	+/+	40	5.8	2.6		56	6.5	4.1	
Aph-1	M/M	45	6.2	3.6		39	6.8	3.9	
	M/+	61	5.1	2.9	1.26	78	6.1	3.4	0 96
	+/+	33	4.7	2.1		30	7.2	4.2	
Px-2	M/−	109	5.3	3.0		123	6.5	3.8	
	+/+	30	5.6	3.2	1.47	24	6.7	3.4	0.02

1): Presented by number of nodes.
2): Tested against the log-transformed data.
N: Number of plants.
**: Significant at 1% level.

the data were log-transformed. Of the five loci assayed, *Got-2* showed a significant effect in both crosses, suggesting the presence of a genetic factor affecting the flowering habit proximal to the locus. The effect of *Got-2* was not a by-product of the gene responsible for the annual habit on the same chromosome because the effect of the latter gene was different between the crosses examined (Table 3). The early-flowering habit of *B. macrocarpa* was partly controlled by a major gene closely linked to one of the genes affecting the annual habit.

Usefulness of enzyme-coding loci for genetic studies between species.

As shown in Table 1, of the five enzyme-coding loci used as chromosome markers, *Got-2*, *Lap* and *Gdh-2* yielded the F_2 ratio that significantly deviated from the expected Mendelian ratio. Although further studies should

be carried out on the causal factors, the distorted segregation of *Got-2* and *Lap* and their effects on the annual and flowering habits may provide an example of linkages between genes affecting morphology and viability (M-V linkages) as reported in several plant species (GRANT 1967).

Unequal transmission of chromosomes and M-V linkages may be main factors to preclude genetic studies between species. Enzyme-coding loci, unlike morphological markers, are usually codominant and nonepistatic even in a hybrid background where different genomes are combined (TANKSLEY 1983). Such inherited properties of enzyme markers make it possible to readily analyze the genetic behavior of alien chromosomes in hybrid progenies, and to determine the genetic basis of species differences through their effects on phenotypes.

Summary

The genetic basis of the annual and early-flowering habit of *B. macrocarpa*, a related species of sugar beet, was examined in the F_2 progenies of the crosses with sugar beet and *B. maritima*. The five independently inherited enzyme-coding loci, *Got-2*, *Lap*, *Gdh-2*, *Aph-1* and *Px-2*, were used as chromosome markers. Of these, *Got-2*, *Lap* and *Gdh-2* showed F_2 ratios that significantly deviated from the expected Mendelian ratio. Based on the results of the analysis for chromosomal effects on phenotypes, it was indicated that the annual habit of *B. macrocarpa* was controlled by at least two or three pairs of genes proximal to *Got-2*, *Lap* and *Px-2*. The effects of *Got-2* and *Lap* differed between the crosses examined, suggesting the existance of a gene interaction with the genetic background. Since of the three loci, *Got-2* belonged to the same linkage group as the gene *B* controlling the annual habit in sugar beet, it was suggested that *B. macrocarpa* might have a gene at a homoeologous locus similar to the gene *B*. Furthermore, the major factor affecting the early-flowering habit of *B. macrocarpa* was associated with the chromosome marked by *Got-2*.

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