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Linkage among the *R, Y* and *Bl* loci in table beet

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Abstract The primary pigments in table beet are the betalains, which are comprised of the red-violet betacyanins and the yellow betaxanthins. The presence of dominant alleles at two linked loci (*R* and *Y*) condition the qualitative production of betalain pigment in the beet plant. Red-pigmented roots are observed only in the presence of dominant alleles at both the *R* and *Y* loci, while white roots are conditioned by recessive alleles at the *Y* locus, and yellow roots by the genotype *rrY-*. A newly described gene 'blotchy' (*bl)* conditions a blotchy or irregular pigment patterning in either red or yellow roots. The objective of the present investigation was to characterize the linkage relationships between the *R* and *Y* loci and the *bl* gene by evaluating segregating progenies developed from a series of matings of colored and white table beets. Due to epistatic interactions among the *R*, *Y*, and *bl* loci, algorithms for estimating linkage were developed using maximum-likelihood estimators for each cross. The two-point linkage estimate between the *R* and *Y* loci pooled over eight crosses was 7.4±1.7 cM. Segregation data indicated the *bl* gene is linked to the *R* and *Y* loci. The recombination fraction between *R* and *bl* was estimated from a pooled sample of four crosses at 16.7±10.8 cM. The most-likely gene order was *R-Y-bl*. These data demonstrate that the *bl* gene is a third locus conditioning betalain pigment production in table beet. The *R-Y-bl* genomic region is therefore important in the genetic control of betalain biosynthesis in table beet.

Key words Betalain · Betacyanin · Betaxanthin · Table beet · Linkage

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Introduction

Table beet (*Beta vulgaris* subsp. *vulgaris*) is an important vegetable crop in Europe and parts of Asia. The table beet was originally selected for its use as a leaf vegetable in the Mediterranean region and then later for use as a fresh or stored root vegetable (Campbell 1976). Traits such as the swollen red root and petiole have also been desired since the middle ages in Europe (Pink 1992). The root, hypocotyl, and petiole color of most table beet cultivars throughout the world is red. However, variations in color patterning in these organs are present in certain cultivars and genetic stocks. For example, yellow- and white-rooted cultivars have existed for at least several hundred years; however, their origin and patterns of distribution have not been reported.

The pigments in table beet responsible for the colors mentioned above are the betalains, a class of pigments unique to the order Caryophyllales. Betalain pigment, comprised of the red-violet betacyanins (BC) and the yellow betaxanthins (BX), is derived from betalamic acid following the cleavage of L-DOPA between the 4- and 5- positions (Clement et al. 1992). The presence of various alleles at two linked loci (*R* and *Y*) conditions the production of betalain pigment in the beet plant (Keller 1936). Some workers have used the symbol *G* as a synonym for *Y* (Linde-Laursen 1972). The *R* and *Y* loci are linked at a distance of 7.5 cM (Keller 1936) and the *R* locus has been mapped to various linkage groups in different investigations. Schumacher et al. (1997) combined three different linkage maps from different sources into a single molecular-marker linkage map for sugar beet. In the consensus map, the *R* locus was present on the linkage group that Barzen et al. (1995) designated as III and Pillen et al. (1992) designated as I. Based on the trisomic series of Butterfass (1964), Butterfass (1968) reported the presence of *R* on linkage group II. Schondelmaier and Jung (1997) mapped a number of marker loci to the trisomic series of Butterfass (1964) and determined that the *R* locus was on trisomic II.

Red roots are observed only in the presence of dominant alleles at both the *R* and *Y* loci, while white roots are condi-

tioned by recessive alleles at the *Y* locus. Two white-rooted phenotypes can occur: *R-yy,* which has red petioles, and *rryy,* which is associated with the production of BX in the petioles, although the petiole color is perhaps best described as yellow-green. The *rryy* genotype is typical for most sugarbeet cultivars. The *rrYY* genotype is associated with yellow roots and yellow-green petioles. Wolyn and Gabelman (1989) demonstrated that three alleles at the *R* locus determine the ratio of betacyanin to betaxanthin in the beet root and shoot. These workers observed incomplete dominance for pigment ratio in *R^{t}'*- and *R'*-genotypes.

A third gene, *p,* has been identified as a color-supressor in roots of mangel (*B. vulgaris* subsp. *vulgaris*) carrying dominant alleles at *R* and *Y* (Linde-Laursen 1972). Colored beet strains carry the dominant allele *P,* which, along with dominant alleles at *R* and *Y,* are necessary for pigment production. Certain cultivars of mangel such as Gartons White Knight and Gartons White Chief carry dominant alleles at *R* and *Y* but exhibit no pigment production in their roots because they are homozygous recessive at the *P* locus. The *P* locus is linked to the *R* and *Y* loci at a distance of 6.3 cM (Linde-Laursen 1972). The *p* allele has not been introduced into table beet and therefore no information is available on the phenotype of table beet strains carrying recessive alleles at *P* and dominant alleles at *R* and *Y.*

Watson and Goldman (1997) described 'blotchy' (*bl*), a gene conditioning irregular sectors of blotchy white root patterning on both the exterior and interior of the table beet root. The *bl* gene disrupts betalain biosynthesis in the root of red table beet plants. Prior to this study, the phenotype of *bl* homozygotes in a yellow background was unknown. Similarly, linkage and/or allelism of the *bl* gene have not been reported. In plants with dominant alleles at the *R* and *Y* loci, *bl* homozygotes have a very different phenotype from mangel plants carrying *pp.* The former exhibit irregular patches of pigment while the latter exhibit no pigment production whatsoever. No attempt has yet been made to develop stocks carrying both *p* and *bl* in the homozygous recessive condition.

Linkage relationships among these genes are not easily evaluated due to epistatic interactions among the *R, Y,* and *bl* loci. For example, the disruption of pigment biosynthesis by *bl* renders the identification of *bl* homozygotes in a non-pigmented background impossible. Epistatic interactions among these genes precluded the use of standard linkage mapping software programs for estimation of recombination frequencies. For this reason, separate algorithms based on maximum-likelihood estimators were developed for each cross. We sought to characterize the linkage relationships among the *R, Y,* and *bl* loci affecting pigment production and distribution in the table beet plant by evaluating segregating progenies developed from matings of blotchy beets with colored and white table beets.

Materials and methods

Genetic Material and Methods

The source of the red-blotchy phenotype (conditioned by *blbl*) was segregating progeny from self-pollination of an F_1 plant derived from a mating between W411B and W440B, two inbred lines developed in the University of Wisconsin Table Beet Breeding Program. W411B was described by Goldman (1996). W440B is an unreleased inbred line from the University of Wisconsin Table Beet Breeding Program. The genotype of the blotchy plant was determined to be *RRYYblbl* following the evaluation of selfed progeny. A blotchy phenotype that arose from the inbred line W336B has been described elsewhere (Watson and Goldman 1997). The two blotchy sources exhibit similar phenotypes, although their genetic relationship is unknown.

Linkage estimation among *R, Y,* and *bl* loci and the estimation of gene order was accomplished by matings between red-blotchy plants with red petioles (*RRYYblbl)* and white-rooted plants with yellow-green petioles (*rryyBlBl).* Four crosses were made using the *RRYYblbl* plants as females, and four crosses were made using the *RRYYblbl* plants as males, giving a total of eight crosses. Linkage estimation between *R* and *bl* was made using matings between yellow-rooted plants (*rrYYBlBl)* and red-blotchy plants (*RRYYblbl).* Four crosses were made using the *rrYYBlBl* plants as females. The *rryyBlBl* genotype (white root, yellow-green hypocotyl and petiole) was produced from the sugar beet (*B. vulgaris* subsp. *vulgaris*) breeding line 52–208 obtained from the American Crystal Sugar Company (Moorehead, Minnesota). The *rrYYBlBl* genotype (yellow root, yellow-green hypocotyl, yellow-green petiole) was produced from yellow-rooted beet breeding lines W399B and W400B, inbred lines extracted from the open pollinated cultivar Burpee's Goldman in the University of Wisconsin Table Beet Breeding Program. Descriptions of genotypes and phenotypes evaluated in this investigation are presented in Table 1 and phenotypes of selected genotypes are pictured in Fig. 1.

Fig. 1 Interior root-color phenotypes and genotypes studied in this investigation. From right to left: white root with yellow-green hypocotyl (*rryyBlBl),* red-blotchy (*RRYYblbl*) used in this investigation, miminal red blotchy (genotype unknown, not used in this investigation), red root and red hypocotyl (*RRYYBlBl*), yellow with yellow-green hypocotyl (*rrYYBlBl)* ▶

Fig. 2 Exterior root-color phenotypes and genotypes studied in this investigation. Clockwise from upper left: white with red hypocotyl/petiole (*RryyBlBl),* red with red hypocotyl/petiole (*RRYYBlBl*), yellow with yellow-green hypocotyl/petiole (*rrYYBlBl),* white with yellow-green hypocotyl/petiole (*rryyBlBl),* red-blotchy with red hypocotyl/petiole (*Rryyblbl*) yellow-blotchy with yellow-green hypocotyl/petiole (*rrYYblbl*)

Table 1 Genotypes and phenotypes of root, hypocotyl, and petiole pigmentation in table beet studied in this investigation.

Genotype	Root Phenotype	Hypocotyl phenotype	Petiole phenotype
R-Y-BL-	Red	Red	Red
rrY-BL-	Yellow	Yellow-green	Yellow-green
R-Y-blbl	Red-blotchy	Red	Red
rrY-blbl	Yellow-blotchy	Yellow-green	Yellow-green
R-yyBL-	White	Red	Red
R-yyblbl	White-blotchy[a]	Red	Red
rryyBL-	White	Green	Yellow-green
rryyblbl[a]	White-blotchy[a]	Green	Yellow-green

[a] Not detectable because *bl* conditions white blotchy sectors on a colored background. The root phenotype is white.

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Seed of all genotypes used in this investigation was planted in the table beet-breeding nursery at the University of Wisconsin in Madison during May 1996. At harvest (August 1996), roots were washed, trimmed, and vernalized for 8 weeks. Vernalized roots were planted in the greenhouse in November and used in crosses according to the protocols described by Wolyn and Gabelman (1989). F_1 seed was harvested in April and planted in the breeding nursery in May 1997. All matings were made between fertile plants. Due to the possibility of some self-pollination during the formation of F_1 populations, care was taken to identify obvious hybrids, based on heterosis for size and expression of known dominant color phenotypes, during the production of F_1 plants in 1997. F_1 roots were harvested in August, vernalized as described above, and planted in the greenhouse in November for crossing. Although most red beet germplasm is self-incompatible, the blotchy mutant was detected in self-compatible breeding material in *N* cytoplasm and was therefore self-fertile. F_1 plants were self-pollinated during the winter of 1997–1998. F_2 populations were planted in May 1998 in the breeding nursery. Plants were harvested in August and scored for color phenotypes.

Statistical Methods

Several software packages are available to estimate linkage and gene order for controlled crosses. However, for this study software was not available to estimate *R-Y-bl* linkage simultaneously or to estimate gene order because of epistasis between the *Y* and *bl* loci. Therefore maximum-likelihood methods using log likelihood were used to estimate all of the recombination fractions and gene order. The log likelihood for a cross is $\sum_i f_i \log p_i$ where f_i is the frequency of the genotype and p_i is the expected genotype frequency in terms of the recombination fraction(s). Estimates of linkage (θ) were made using the EM algorithm. The EM algorithm involves: (1) an initial guess, termed θ^{old}; (2) an expectation step (E) using θ^{old} as if it were the true recombination fraction to compute the expected number of recombinant progeny; (3) a maximization (M) step using the expected progeny counts to compute the new recombination fraction, θ^{new}; (4) iterating the E and M steps until the likelihood reaches its maximum or the estimate converges, that is until $|\theta^{new}-\theta^{old}|<tolerance$. For these crosses, the maximum-likelihood estimate is given by $\theta^{new}=\frac{1}{2}\sum f_{iP_i}(R|G)$ where f_i is the observed frequency of each phenotype, $P_i(R|G)$ is the conditional probability that a plant is a recombinant given the genotype, and *N* is the total number of plants in the cross. The tolerance for this study was set at 10^{-6}.

The expected genotypic frequencies (p_i) and the conditional probability of recombination given the genotype [$P_i(R|G)$] for dominant genes linked in coupling phase (Liu 1998) were used to estimate recombination between the *R* and *Y* loci (see Table 2). Similarly, p_i and $P_i(R|G)$ for dominant genes linked in repulsion phase (Liu 1998) were used to estimate recombination for the *R* and *bl* loci (see Table 4). To estimate gene order and to estimate both recombination fractions simultaneously, all three possible gene orders were examined: *R-Y-bl*, *Y-R-bl*, and *R-bl-Y*. Expected frequencies of non-recombinant, single-recombinant, and double-recombinant gametes (assuming no interference) for the three possible gene orders were calculated, and expected frequencies (p_i) were determined for each phenotype. Conditional frequencies of recombination [$P_i(R_i|G)$] were calculated for each recombination fraction and used in the EM algorithm.

The tests for linkage used a generalized likelihood ratio statistic $G=2[L(\theta)-L(0.5)]$, where L(θ) is the log likelihood of the data from a specific cross using the estimated value of θ and L(0.5) is the log likelihood of the data using 0.5 as the value of θ. The G statistic has an approximate chi-square distribution. Parametric standard errors and 95% confidence intervals were estimated using the average information content per observation according to Liu (1998).

Results and discussion

Linkage between *R* and *Y*

Linkage between *R* and *Y* has been noted previously (Keller 1936) and will not be discussed in detail herein. Matings between red-blotchy plants with red petioles (*RRYYblbl)* and white-rooted plants with yellow-green petioles (*rryyBlBl)*, in both directions of the cross, revealed F_1 plants with red roots, hypocotyls, and petioles as expected based on previously noted dominance relations at these loci (Keller 1936; Wolyn and Gabelman 1989; Watson and Goldman 1997). Segregation in the F_2 generation resulted in five phenotypic classes (Table 2). A yellow root with blotchy patterning was a possible phenotype but was not detected.

In each of the eight crosses, *R* and *Y* were linked in coupling phase and *bl* was linked to *R* and *Y* in repulsion phase. Initially, a two-point estimate of linkage between *R* and *Y* was made by combining progeny over the *bl* locus. Although all three genes were segregating in these progenies, epistasis confounded the expected progeny types for *bl* in association with the genotypes at *R* and *Y* that produce white-rooted progeny (i.e., *R-yy* and *rryy*). Because the irregular patterning caused by *bl* in a homo-

Table 2 Estimate of the *R–Y* recombination, standard error, and test for linkage for eight crosses. The expected phenotype frequency (p_i) and probability of recombination given the phenotype [$P_i(R|G)$] for an F_2 with dominant genes linked in coupling are also given (Liu 1998)

Cross	Progeny				$\hat{\theta}$	$SE(\hat{\theta})$	G	$P(\chi^2>G)$
	Red and red-blotchy (*R-Y-*)	White/red (*R-yy*)	Yellow (*rrY-*)	White/green (*rryy*)				
1	90	9	4	23	0.112	0.030	39.43	<0.0001
2	70	5	3	25	0.077	0.028	43.16	<0.0001
3	77	9	3	23	0.112	0.032	35.70	<0.0001
4	99	4	4	25	0.066	0.022	57.07	<0.0001
5	105	4	3	45	0.042	0.016	89.10	<0.0001
6	106	4	8	28	0.088	0.025	54.06	<0.0001
7	69	2	2	20	0.045	0.022	47.99	<0.0001
8	73	2	5	32	0.059	0.023	55.92	<0.0001
P_i	$0.25(3-2\theta+\theta^2)$	$0.25\theta(2-\theta)$	$0.25\theta(2-\theta)$	$0.25(1-\theta)^2$				
$P_I(R\|G)$	$2\theta/(3-2\theta+\theta^2)$	$1/(2-\theta)$	$1/(2-\theta)$	0.0				

Table 3 Tests for heterogeneity and pooled estimates of *R–Y* recombination for crosses 1–8

Pooled crosses	$\hat{\theta}$	SE($\hat{\theta}$)	95% CI	G_{total}	G_{pooled}	$G_{heterogeneity}$	P(χ^2 >G)[a]
1–4	0.091	0.014	0.064–0.119	175.36	173.20	2.16	0.540
5–8	0.059	0.011	0.037–0.080	247.07	244.20	2.87	0.412
1–8 (all)	0.074	0.009	0.057–0.091	422.43	414.07	8.37	0.301

[a] *df*=3 for crosses 1–4 and crosses 5–8; *df*=7 for crosses 1–8

Table 4 Estimate of the recombination fraction, standard error, and test for linkage for four crosses and for all four crosses pooled. The expected phenotype frequency (f_i) and probability of recombination given the phenotype [$P_i(R|P)$] for an F_2 with dominant genes linked in repulsion are also given (Liu 1998)

Cross	Progeny				$\hat{\theta}$	SE($\hat{\theta}$)	G	P(χ^2>G)
	Red	Red blotchy	Yellow	Yellow blotchy				
9	36	28	20	1	0.177	0.104	16.76	<0.0001
10[a]	35	16	23	0	0	0.116	14.19	0.0002
11	10	61	19	3	0.196	0.099	36.53	<0.0001
12[a]	29	12	14	0	0	0.135	8.13	0.0045
Pooled	110	117	76	4	0.167	0.055	75.53	<0.0001
p_i	$0.25(2+\theta^2)$	$0.25(1-\theta^2)$	$0.25(1-\theta^2)$	$0.25\theta^2$				
$P_i(R\|G)$	$\theta(2+\theta)/(2+\theta^2)$	$\theta/(1+\theta)$	$\theta/(1+\theta)$	1.0				

[a] There were no yellow-blotchy beets (the double-recessive class) produced in crosses 10 and 12. When this occurs the MLE is 0 regardless of the frequencies of the other genotypes (Liu 1998)

zygous recessive condition can be visualized only in a colored (e.g., red or yellow) background, the identification of *bl* recessive homozygotes in a white-rooted plant was not possible using color phenotypes. As described previously, the EM algorithm was used to estimate recombination between the *R* and *Y* loci.

Linkage between *R* and *Y* was detected (*P*<0.0001) in all eight crosses (Table 2). The linkage estimates ranged from 6.6 cM to 11.2 cM for crosses 1–4 and from 4.2 cM to 8.8 cM for crosses 5–8. Tests for heterogeneity for crosses 1–4, crosses 5–8, and all eight crosses were not significant (Table 3). In addition, a test was made to determine whether the pooled estimate from crosses 1–4 and the pooled estimate from crosses 5–8 differed from the overall pooled estimate for all eight crosses. This test was not significant (G=3.34, *P*=0.068). Therefore, the pooled estimate of 7.4 cM with a 95% confidence interval of 5.7–9.1 cM is the linkage distance between the *R* and *Y* loci. This estimate is highly consistent with a previously published linkage estimate between *R* and *Y* of 7.5 cM (Keller 1936).

Linkage between *R* and *bl*

Linkage estimation between *R* and *bl* was determined from matings between yellow-rooted plants (*rrYYBlBl)* and red-blotchy plants (*RRYYblbl).* Four crosses were made using the *rrYYBlBl* plants as females. In these matings, *R* and *bl* were linked in repulsion phase. All F_1 plants had red roots and red petioles as expected, since both the *R* and *bl* loci were heterozygous and all plants carried dominant alleles at *Y.* Four types of progeny were observed in the F_2 generation: red root and red petiole, yellow root, red-blotchy root, and yellow-blotchy root. These crosses produced the first examples of the yellow-blotchy phenotype, but only a total of four yellow-blotchy plants were observed in the four F_2 populations.

Assuming no linkage, the expected frequencies of the four progeny classes: red root and red petiole, yellow root, red-blotchy root, and yellow-blotchy root, would be 9:3:3:1. As discussed previously, the EM algorithm was used to estimate the recombination fraction θ.

All of the tests for linkage were significant (*P*<0.005, Table 4). A non-significant heterogeneity test ($G_{heterogeneity}$= G_{total}–G_{pooled}=75.61–75.53=0.08, *P*=0.89) allowed for pooling the four crosses. The pooled estimate of linkage between *R* and *bl* was 16.7 cM with a 95% confidence interval of 5.9–27.4 cM.

Linkage Order Among *R, Y,* and *bl*

Three possible orders for these loci exist: *R-Y-bl, Y-R-bl,* and *R-bl-Y.* As described previously, expected frequencies of non-recombinant, single-recombinant, and double-recombinant gametes (assuming no interference) for the three possible gene orders were calculated, and expected frequencies (p_i) were determined for each phenotype. Conditional frequencies of recombination [$P_i(R_i|G)$] were calculated for each recombination fraction for use in the EM algorithm. *R-Y* and *Y-bl* recombination was calculated for the gene order *R-Y-bl*; *R-Y* (or *Y-R*), *R-bl* recombination was calculated for the gene or-

Table 5 Linkage estimates, standard errors, log likelihood and tests for heterogeneity for three gene orders

Gene order	$\hat{\theta}_{ry}$ (SE)	$\hat{\theta}_{rbl}$ (SE)	$\hat{\theta}_{rbl}$ (SE)	Log likelihood		G	$P(\chi^2>G)$
				Total	Pooled		
R-Y-bl	0.074 (0.009)	0.922 (0.009)	–	–406.3	–413.7	14.8	0.40
Y-R-bl	0.074 (0.009)	–	0.844 (0.013)	–426.8	–433.6	13.6	0.48
R-bl-Y	–	0.947 (0.007)	0.900 (0.010)	–436.0	–444.5	17.0	0.25

der *Y-R-bl*; *R-bl*, and *Y-bl* (or *bl-Y*) recombination was calculated for the gene order *R-bl-Y* (Table 5). The tests for heterogeneity were non-significant for all three gene orders, allowing all eight crosses to be pooled (Table 5). The pooled estimates of *R-Y* recombination were the same for the *R-Y-bl* and *Y-R-bl* gene orders (7.4 cM) and were very consistent with the two-point linkage estimate calculated for these loci. The pooled estimates of *R-bl* recombination (from the orders *Y-R-bl* and *R-bl-Y*) and *Y-bl* recombination (from the orders *R-Y-bl* and *R-bl-Y*) were all greater than 0.5, ranging from 0.844 to 0.900 for *R-bl* and from 0.922 to 0.947 for *Y-bl*. Calculation of a two-point recombination fraction for *Y-bl* by pooling over *R* locus genotypes gave similar results. There are several possible reasons for the large recombination fraction estimate. First, no yellow-blotchy plants were observed in these crosses, which may reflect a difference in the segregation of the *bl* locus when *Y* is also segregating. As noted previously, difficulties in estimating recombination between *Y* and *bl* are also caused by the impossibility of distinguishing between *Bl-* and *blbl* genotypes when the root is white. Furthermore, *bl* is linked in repulsion to both *R* and *Y* in these matings, and there is a very low information content in such matings.

The log-likelihood estimate for the gene order *R-Y-bl* was greater than the likelihood for the gene orders *Y-R-bl* and *R-bl-Y* for each cross and for the pooled crosses. Therefore, the most likely order for these genes is *R-Y-bl*.

Chromosome Assignment

In recent years, several molecular-marker linkage maps have been constructed using restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), and amplified fragment length polymorphisms (AFLPs) (Barzen et al. 1995; Hallden et al. 1996; Schondelmaier et al. 1996; Schumacher et al. 1997). Schumacher et al. (1997) combined three different linkage maps from different sources into a single molecular-marker linkage map for sugar beet. Population A was from Kiel and was used by both Pillen et al. (1992) and Schondelmaier et al. (1996). The population designated B, used by Barzen et al. (1995), was from Köln and and segregated at the *R* locus. The population designated C was also from Köln, but differed in structure from population B. Linkage group 1 from the A population was very colinear with linkage group III from the Koln population. Thus, even though the *R* locus was mapped to these two different linkage groups by Pillen et al. (1992) and by Barzen et al. (1995), the two linkage groups were functionally identical.

Schondelmaier and Jung (1997) assigned marker loci from each of the nine linkage groups of sugarbeet to the nine primary trisomics previously identified by Butterfass (1964) and assigned a standard numbering system to the sugarbeet chromosomes. Thus, the *R* locus, which had previously been assigned to linkage group 1 (Pillen et al. 1992; Uphoff and Wricke 1995) and to linkage group III (Barzen et al. 1995), was placed on trisomic II by Schondelmaier and Jung (1997). Based on the trisomic series of Butterfass (1964), Butterfass (1968) had also reported the presence of *R* on linkage group II. Neither the *Y* nor the *bl* loci have been mapped on the combined molecular-marker linkage map of Schumacher et al. (1997).

Conclusions

Although the *rryyblbl* and *R-yyblbl* genotypes theoretically condition a white-blotchy phenotype, it was not possible to detect this class of progeny because the blotchy trait is characterized by irregular white sectors in a colored background. With the use of molecular markers linked to each of these loci, it would theoretically be possible to detect *rryyblbl* and *R-yyblbl* genotypes in an F_2 population such as the one reported here. Barzen et al. (1995), Schumacher et al. (1997), and Weber et al. (1999) have all reported RFLP and RAPD markers linked to the *R* locus. Because these workers have generated saturated molecular-marker linkage maps, it is likely that flanking markers are linked to *Y* and *bl*. Detailed investigation of marker-tagged loci in this region may provide insight into the genetic control of betalain pigment production. Further molecular characterization of the gene products from these loci may reveal important uses for this triple-recessive genotype as a standard for comparative study.

Epistatic interactions between *R, Y,* and *bl* make the estimation of linkage among these genes difficult and precludes the use of standard linkage-mapping software for an estimation of recombination frequencies with these genes. The development of maximum-likelihood estimators for each cross in this investigation allowed for the detection of linkage among *R, Y,* and *bl*. The close linkage between these loci suggests that this genomic region plays a critical

role in the genetic control of betalain biosynthesis. Efforts are currently underway to further characterize these genes in an effort to gain a better understanding of the production and distribution of these pigments in the beet plant.

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