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# Inheritance of annual habit in celery: cosegregation with isozyme and anthocyanin markers

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**Summary.** Vernalization response was determined in an annual and two biennial celery strains, *Apium graveolens* L. and their $F_2$ hybrids. Although the annual strain did not require vernalization to bolt, plants exposed to 10 °C for 7 days bolted 2 weeks earlier than non-treated plants. Inheritance studies based on $F_2$ and backcross segregations demonstrate that annual habit in celery is partially dominant over biennial and determined by a single gene designated *Hb*. Cosegregation studies of this trait with nine isozyme loci and a gene determining petiole anthocyanin pigmentation disclosed the following linkage relationships: *Adh-1–Sdh-1–Mdh-1*, and *Got-1–Mdh-2–Hb–A*. The recombination frequency observed for *Hb* and *Mdh-2* was too large to use the latter as a useful marker for annual habit.

**Key words:** *Apium graveolens* – bolting – flowering – isoenzymes – vernalization – biennial

## Introduction

Vernalization response in celery, *Apium graveolens* L. is quite variable. It ranges from annual strains which bolt without the need of vernalization, to strongly biennial strains which require prolonged periods of cold temperature to bolt (Bouwkamp et al. 1970; Pressman and Sachs 1985; Suiter et al. 1983). Emsweller (1934) in his early studies in celery, suggested that slow bolting was a quantitative and recessive trait. Honma (1959) and Bouwkamp and Honma (1970) reported the inheritance of easy bolting plants, those requiring 2 weeks of cold exposure, and hard-to-bolt plants, those failing to bolt after 7 weeks of vernalizing temperatures. They found that easy bolting was dominant and determined by a major gene, designated *Vr* and that the degree of bolting resistance was determined by modifiers.

The relationships between easy bolting biennial and annual habits in celery has not been established. The purpose of this paper is to report the inheritance of annual habit in celery, its response to vernalization and its linkage relationships to various traits cosegregating in some of the progenies. These include nine enzyme coding loci and one for petiole anthocyanin pigmentation.

## Materials and methods

### *Plant materials*

These were (1) PI1257228, an annual celery accession from Thailand (Orton 1983) which does not require vernalization to flower, hereon designated A143; (2) PI169001, a biennial celeriac accession from Turkey PI169001, hereon designated A112 and (3) the biennial celery cv 'Tall Utah 52-70R', hereon designated R.

### *Vernalization response*

To determine the vernalization response of the accessions A143, A112 and $F_2$ population, a replicated trial was established in 1984 at Harden-Crisp Farms in Salinas. The cultivar R was used as control. Plants from each line were subjected to the following treatments: no vernalization, 7 day vernalization and 21 day vernalization. A complete block randomized design with eight replications; 20 plants per replication were used. For the vernalization treatments, the 10-week-old seedlings were transfered to a refrigerated trailer set at 10 °C and 12 h light. After vernalization, the plants were transplanted into the field. Seedlings for the no-vernalization and 7 day vernalization treatments were maintained in the greenhouse until the plants from the 21 day vernalization treatment completed their cold exposure.

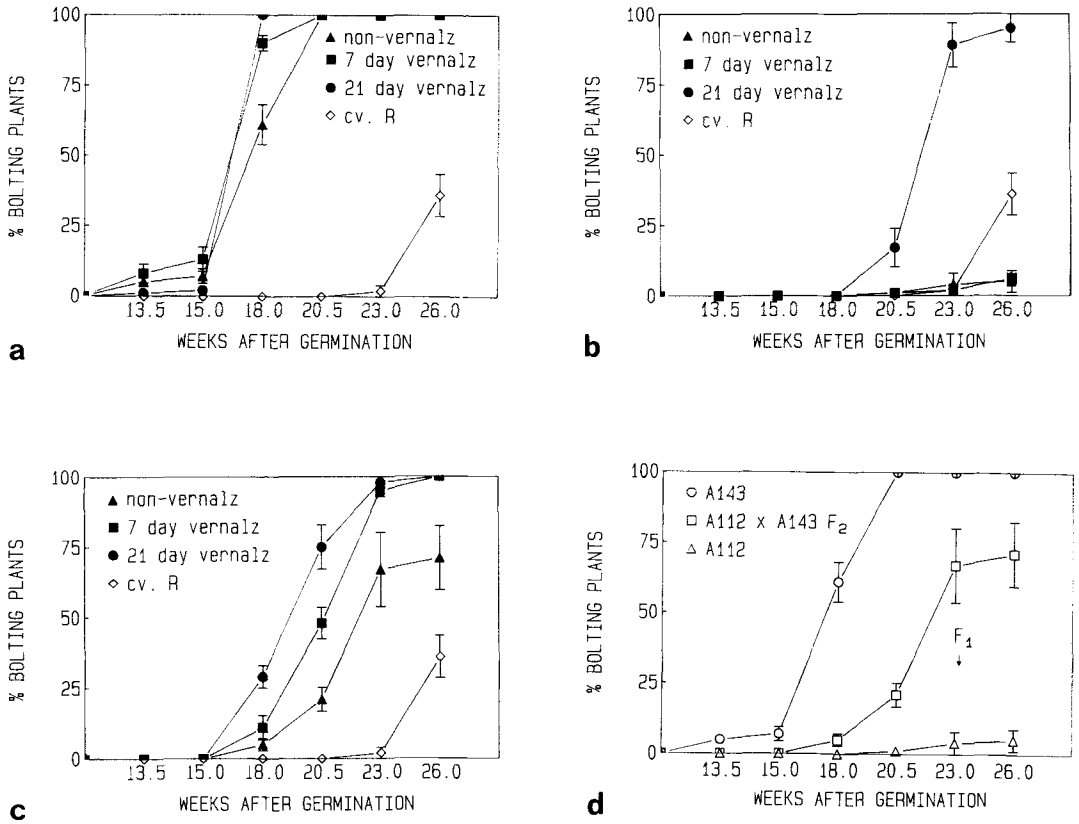


**Fig. 1a–d.** Bolting response for the two celery strains and their hybrid after three vernalization treatments. **a** Annual strain A143. **b** biennial strain A112. **c** $F_2$ population A143×A112. Response of cv 'T.U.52-70R' to 21 day vernalization at 10 °C is included as reference (diamond). **d** Bolting response for non-vernalized parental strain and the $F_2$ hybrids. Arrow indicates bolting time when 100% of the $F_1$ hybrid plants bolted. Bars represent standard errors

*Inheritance studies*

The accessions A143 and A112 were hybridized in both directions following the technique of Ochoa et al. (1986). The resulting $F_1$ hybrids were selfed and backcrossed to both parents, A143 and A112, and to the cultivar R. These progenies were grown in 1985–86 at the USDA Salinas Station.

Two $F_2$ progenies derived from crossing R×A143, segregating for nine isozyme loci, were scored for annual habit and petiole anthocyanin pigmentation. The data from both progenies was pooled after $X^2$ testing for homogeneity. The inheritance of three of these enzyme coding genes, *Pgi-2*, *Pgm-2* and *Adh-1*, and for anthocyanin pigmentation locus *A* has been reported by Arus and Orton (1984). Linkage data were analysed with the computer program Linkage-1, developed by Suiter et al. 1983. Non-vernalized plants were used for the inheritance studies.

*Electrophoresis*

The technique of Arus and Orton (1984) was employed. The following enzyme systems were assayed: malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), 6 phosphogluconate dehydrogenase (6PGD), triose phosphate isomerase (TPI), phosphoglucomutase (PGM), alcohol dehydrogenase (ADH), glutamate oxaloacetate transaminase (GOT) and shikimic acid dehydrogenase (SDH). The recipes for these assays have been described by Vallejos (1983).

## Results

*Vernalization response in the parental accessions and hybrids*

This determination was done for the R, A143, A112 strains and A112×A143 $F_2$ population. Although plants of the annual strain A143 did not require vernalization to bolt, they did respond to the cold treatment (Fig. 1a). Vernalization induced several of these plants to bolt as early as 13.5 weeks after germination. The number of bolting plants increased as the vernalization period was extended. By week 18, most of the treated plants but only 60% of the untreated ones had bolted. All bolted 20 weeks after seed germination, regardless of vernalization treatment.

On the other hand, in the biennial strain A112, very few plants which were not vernalized or had only 7 days of cold treatment bolted 26 weeks after germination (Fig. 1b). The 21 day vernalization treatment started to show its effects by week 20.5, when about 20% of these plants bolted and the number of bolting plants increased to almost 90% by the 23rd week.

The $F_2$ progeny derived from the hybridization of the two strains has an intermediate response (Fig. 1 c, d). About 75% of the plants bolted at the 26th week without vernalization. Vernalization treatments of 7 or 21 days induced almost all the plants to bolt by the 23rd week (Fig. 1 c). Figure 1 d summarizes the bolting behavior without vernalization for A143, A112, $F_2$ progeny. The response of strain R to vernalization was significantly less than that observed for the other biennial strain, A112. Only 50% of the R plants had bolted by the 26th week after 21 days of vernalization (Fig. 1 a, b and c). Practically none of the R plants bolted without vernalization or with vernalization for 7 days. Although the $F_1$ hybrids of A143×A112 and A143×R were not included in this study, field and greenhouse observations showed that they had the annual habit of the A143 parent. However, bolting in non-vernalized plants was delayed by about 3 weeks and 6 weeks for the A143×A112 and A143×R $F_1$ hybrids, respectively (Fig. 1 d).

*Inheritance of annual habit*

The experiments on the bolting response indicated that annual habit is dominant over biennial. To further determine the inheritance of this trait, a series of $F_1$, $F_2$ and backcross progenies were studied. All the $F_1$, about 75% of the $F_2$ and about 50% of the plants from backcross to the biennial parent displayed annual habit (Tables 1 and 2). All plants from the backcross to the annual parent were annual. No maternal effect was observed in the reciprocal crosses on the expression of bolting. These data indicate that annual habit is determined by a single gene, hereon designated *Hb* for habit type. Two out of eight $F_2$ progenies tested deviated significantly from the expected ratio. One had an excess of biennial plants (79 annual: 56 biennial) and the other had an excess of annual plants (79 annual: 13 biennial). The delay in flowering observed in the non-vernalized $F_1$ and $F_2$ hybrids shows that the expression of this gene is partially dominant. This delay was also evident in the backcross progenies. The backcross to the stronger biennal strain R extended the bolting range of the annual plants in the progeny by 6 weeks when compared to the A112×A143 $F_1$ and $F_2$ hybrids and backcrosses to A112. Furthermore, in the backcross to R, and excess of biennial plants was observed in the pooled data resulting in a 0.07 probability for 1:1 segregation (Table 2). Testing the two families independently, however, yielded probabilities of 0.23 and 0.29. Plants from backcrosses to the annual A143 initiated bolting as early as the A143 parent.

**Table 1.** Data on segregations for annual vs. biennial habit in $F_2$ progenies of celery crosses

| Cross[a] | No progenies | Annual | Biennial | $X^2$ | $P$ |
|---|---|---|---|---|---|
| A112(b)×A143(b) | 3 | 188 | 62 | 0.0 | 1.0 |
| A143×A112 | 1 | 111 | 29 | 1.15 | 0.30 |
| R(b)×143(a) | 2 | 162 | 56 | 0.05 | 0.81 |

[a] (a) = annual; (b) = biennial

**Table 2.** Data on segregations for annual vs. biennial habit in backcross progenies of celery

| Cross[a] | No progenies | Annual | Biennial | $X^2$ | $P$ |
|---|---|---|---|---|---|
| (A112×A143) ×A143 | 4 | 153 | 0 | – | – |
| (A112×A143) ×A112 | 4 | 21 | 20 | 0.0 | 1.0 |
| R×(A112×A143) | 2 | 45 | 65 | 3.3 | 0.07 |

[a] A112 and R = biennial; A143 = annual

*Cosegregations of habit, anthocyanin pigmentation and isozymes*

The two R×A143 $F_2$ progenies segregated for habit, petiole anthocyanin pigmentation and nine isozyme loci. The data from the two progenies was pooled except when deviations from expected ratios were observed. In addition to the four enzyme coding loci, *Pgi-3* (Fig. 2 c), *Pgm-2*, *Adh-1* and *Sdh-1* previously reported by Arus and Orton (1984), we studied segregations for the following five loci coding for dimeric enzymes: malate dehydrogenase *Mdh-1* and *Mdh-2* (Fig. 2 a), 6 phosphogluconate dehydrogenase, *6pdg-1* (Fig. 2 d), triose phosphate isomerase *Tri-2* (Fig. 2 b) and glutamate oxaloacetate transaminase, *Got-1* (Table 3). In most instances, the isozyme and anthocyanin loci tested followed expected mendelian segregations.

**Table 3.** Segregation ratios for nine enzyme coding loci and anthocyanin pigmentation. Based on two R×A143 $F_2$ progenies

| Parental genotypes | Observed ratio | $X^2$ | $P$ |
|---|---|---|---|
| *Mdh-1* (SS)×(FF) | 25(FF):50(FS):20(SS) | 0.90 | 0.56 |
| *Mdh-2* (FF)×(SS) | 69(FF):118(FS):50(SS) | 3.05 | 0.22 |
| *Pgi-3* (11)×(22) | 33(11):48(12):30(22) | 2.50 | 0.32 |
| *Tpi-2* (11)×(22) | 65(11):134(12):54(22) | 1.84 | 0.40 |
| *Pgm-2* (11)×(22) | 32(11):58(12):20(22) | 2.95 | 0.23 |
| *Adh-1* (11)×(22)* | 37(11):106(12):63(22) | 6.73 | 0.03* |
| *Sdh-1* (22)×(11) | 73(11):126(12):53(22) | 3.17 | 0.20 |
| *Got-1* (22)×(11) | 55(11):119(12):73(22) | 2.95 | 0.23 |
| *6pgd-1* (1 –)**×(22) | 187(1 –):61(22) | 0.02 | 0.88 |
| (*A* –)*×(aa) | 174(A –):51(aa) | 0.65 | 0.41 |

* Non significant deviations when families tested independently

** Tested for 3:1 ratio

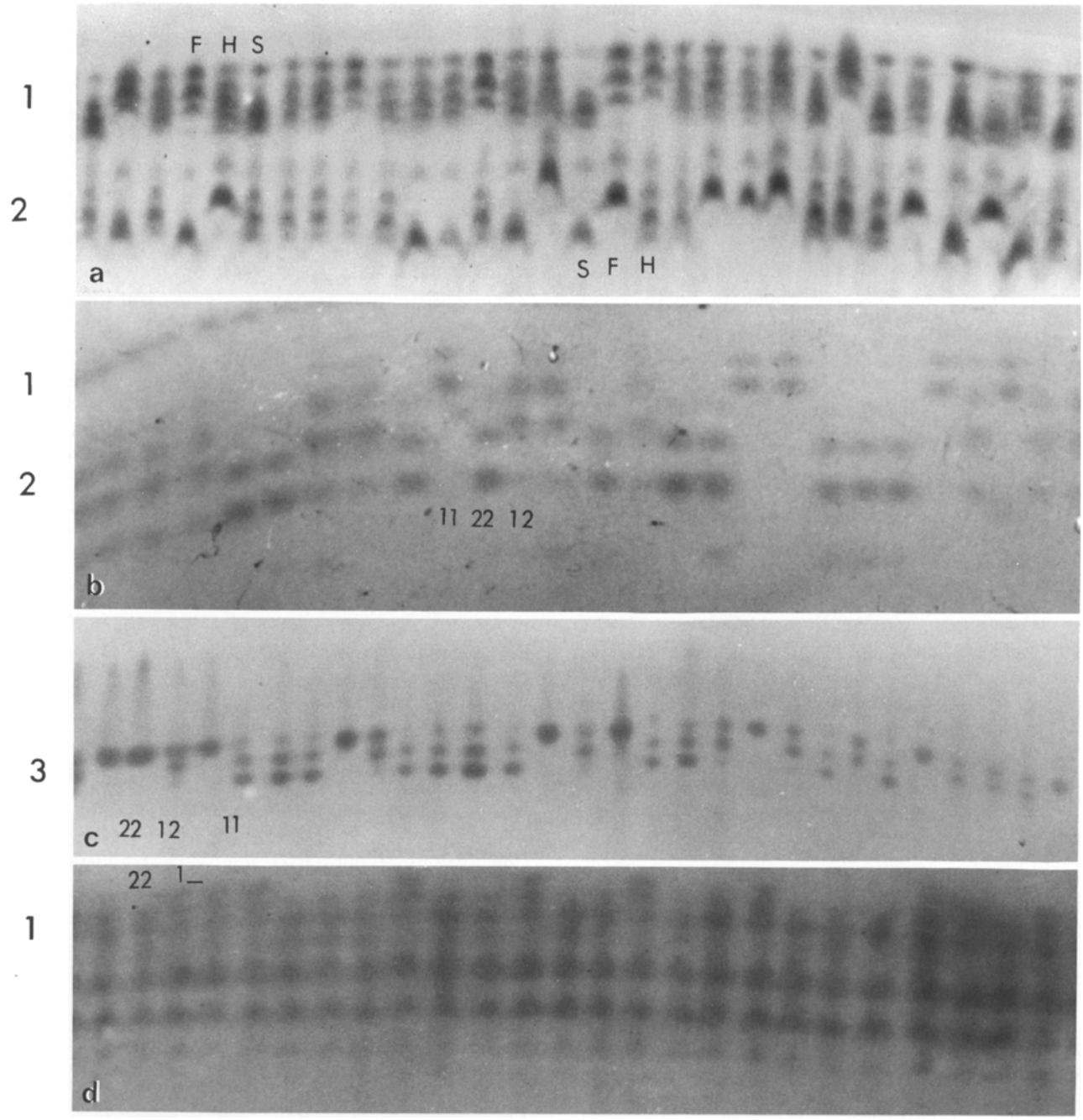


**Fig. 2a–d.** Zymograms for 'T.U.52-70R' × A143 $F_2$ progeny segregating for five isozyme loci. Anodal migration is *above*, origin is *below*. **a** Segregation for *Mdh-1* and *Mdh-2*. Letters show examples of slow (*S*), fast (*F*) and FS heterozygous (*H*) genotypes for each locus. **b** Segregation for *Tpi-2*. Numbers show examples of genotypes 11, 22 and heterzygous 12. **c** Segregation for *Pgi-3*. Numbers show examples of genotype *11* (3 banded with darker band close origin), *22* (single banded) and heterozygous 12 (three banded with lighter band close to the origin). **d** Segregation for *6pgd-1*. Numbers show examples of homozygous genotype 22 and phenotypes 11 or 12. Since phenotype 11 was dominant over 22, this progeny was tested for 3:1 segregation

The only exceptions were segregations for *Mdh-1, Pgi-2* and *Pgm-2* in one of the families (data not shown). Also, the pooled data for *Adh-1* deviated from the expected ratio. However, when each family was tested independently they fit the 1:2:1 ratio. *6pgd-1* was tested for the 3:1 ratio because of the inability to differentiate between the 12 heterozygous and the 11 homozygous phenotypes (Fig. 2d). Table 4 presents the recombination frequencies calculated for each pair of loci. Two way contingency tests revealed two linkage

**Table 4.** Linkage values observed for nine enzyme coding loci, habit and anthocyanin pigmentation

| | *Mdh-1* | *Mdh-2* | *Pgi-3* | *A* | *6pgd-1* | *Got-1* | *Adh-1* | *Tpi-2* | *Pgm-2* | *Sdh-1* | *Hb* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Mdh-1* | | ns[b] | ns | ns | ns | ns | 0.14 | ns | ns | 0.11 | ns |
| N[a] | | 211 | 218 | 198 | 216 | 215 | 175 | 129 | 215 | 218 | 185 |
| *Mdh-2* | | | ns | ns | ns | 0.28 | ns | ns | ns | ns | 0.36 |
| N | | | 230 | 218 | 233 | 231 | 191 | 237 | 232 | 129 | 202 |
| *Pgi-3* | | | | ns | ns | ns | ns | ns | ns | ns | ns |
| N | | | | 217 | 247 | 246 | 205 | 252 | 246 | 251 | 224 |
| *A* | | | | | ns | ns | ns | ns | ns | ns | 0.38 |
| N | | | | | 222 | 178 | 104 | 225 | 220 | 224 | 191 |
| *6pgd-1* | | | | | | ns | ns | ns | ns | ns | ns |
| N | | | | | | 242 | 213 | 248 | 242 | 248 | 213 |
| *Got-1* | | | | | | | ns | ns | ns | ns | ns |
| N | | | | | | | 202 | 247 | 247 | 246 | 213 |
| *Adh-1* | | | | | | | | ns | ns | 0.07 | ns |
| N | | | | | | | | 206 | 201 | 205 | 174 |
| *Tpi-2* | | | | | | | | | ns | ns | ns |
| N | | | | | | | | | 247 | 252 | 218 |
| *Pgm-2* | | | | | | | | | | ns | ns |
| N | | | | | | | | | | 246 | 213 |
| *Sdh-1* | | | | | | | | | | | ns |
| N | | | | | | | | | | | 217 |

[a] Number of individuals
[b] Independent

groups. One formed by *Adh-1*, *Sdh-1* and *Mdh-1*, where the recombination frequency between *Adh-1* and *Mdh-1* was 14%. *Sdh-1*, located in the middle, was closer to *Adh-1* with a recombination frequency of 7% (Fig. 3). The second linkage group consisted of *Got-1*, *Mdh-2*, the annual habit gene *Hb* and the anthocyanin gene A (Fig. 3). The closest linkage in this group was observed between *Got-1* and *Mdh-2* with a recombination value of 28%.

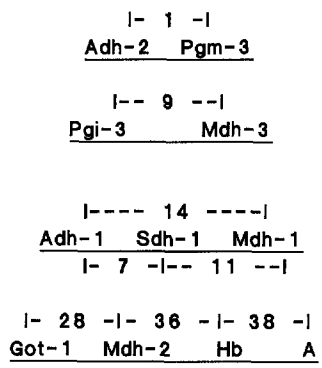


**Fig. 3.** Celery linkage groups and recombination frequencies. First two groups from the top adapted from Arus and Orton (1984)

## Discussion

Our results demonstrate that annual habit in celery is determined by a single gene, *Hb* with incompletely dominant expression. Most of the segregations fit mendelian ratios, with the exception of two progenies. Most likely, these are spurious segregations due to environmental effects or experimental error. In several other crops such as Brussel sprouts (Friend 1985; Wellensiek 1960) and lettuce (Ryder 1985) annual habit has also been reported as a partially dominant trait determined by a single gene. In cabbage, however, biennial habit was reported as dominant over annual habit (Yarnell 1956). Previous celery reports (Bouwkamp and Honma 1970; Emsweller 1934) indicate that premature seeding in biennial strains is dominant. It is possible that the gene *Vr* (vernalization response) reported by Bouwkamp and Honma (1970) and the gene *Hb* reported in this study are the same. The gene *Vr* however, was detected in easy bolting biennial material which required a 2 week vernalization to bolt, while *Hb* was found in annual material which did not require cold treatment but responded to it. Allelic tests are necessary to determine whether *Vr* and *Hb* are the same.

The annual strain responded to cold treatment by accelerated bolting. Short vernalization exposures of only 7 days were sufficient to cause this phenomenon. The expression of the gene *Hb* varies according to the biennial parent used in the hybridization. Hybrids with the fast bolting parent A112 will flower earlier than those with the slow bolting parent R. This differential response might be due either to the presence of different *Hb* alleles in the biennial parents, or to modifier genes acting at other loci. The second pos-

sibility is more likely due to the complexity of the bolting and flowering process in celery (Hanisova and Krekule 1975; Pressman and Sachs 1985).

Regarding the linkages observed in our study, although the loci *Hb* and *Mdh-2* and *A* were linked on the same chromosome, the observed recombination frequencies for these genes is too large to use *Mdh-2* or *A* as a useful seedling marker for annual habit. The fact that these linkages were observed in both progenies when tested independently and in the pooled data indicates that they are genuine. Other linkages in celery have been reported by Bouwkamp and Honma (1970), who detected 18.75% recombination between petiole color and pinnae number. Arus and Orton (1984) reported the inheritance and linkage relationships of eight genes in celery. Of these we have studied the anthocyanin gene *A*, and the enzyme coding loci *Adh-1*, *Pgm-2*, *Pgi-3* and *Sdh-1*. Arus and Orton (1984) found *Pgm-2* and *Sdh-1* on the same chromosome with a recombination frequency of 39% based on data obtained from one progeny. Our data, based on two progenies, did not confirm this linkage. The inheritance of petiole anthocyanin was first reported by Towsend et al. (1946). He also reported that green celery color was dominant over the yellow self-blanching type and conditioned by a single gene. This gene has been used as a marker by Honma and Lacy (1980) to identify celery-parsley hybrids. Other genes reported in celery are *Dt* for shallowly toothed leaf (Bouwkamp and Honma 1970) and *ms-1* for male sterility (Quiros et al. 1986). The present study adds five enzyme coding loci and the habit gene *Hb*, useful for the construction of celery linkage map. Figure 3 summarizes the linkages known so far in celery, combining our data with those from previous reports.

Due to the biennial nature of commercial celery, breeding work in this crop is a slow process. The gene *Hb* could be used to reduce generation time in backcrossing, as suggested by Ryder (1985) in lettuce. The development of additional genetic markers and the construction of a linkage map in celery opens the possibility of tagging genes of horticultural value.

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