Inheritance of Vernalization Requirement in Carrot

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ABSTRACT. Carrot (*Daucus carota* L.) is a cool-season vegetable normally classified as a biennial or winter annual species, requiring vernalization to induce flowering. Nevertheless, some cultivars adapted to warmer climates require less vernalization and can be classified as early flowering or annual. The genetic control of this phenotypic difference has not been previously studied in carrot. The authors evaluated F_1, F_2, and BC_1 progenies of an intercross between the early-flowering carrot ('Criolla INTA') and a late-flowering biennial carrot. F_1 progenies were completely annual. Observed segregation ratios in the F_2 and BC_1 families were not significantly different from expected segregation ratios under the hypothesis of a single dominant gene conditioning the early-flowering habit. The authors conclude that annual habit is dominant over biennial and is a monogenic character.

In almost all crop species we know as biennials, early flowering or annual plants are usually found. These crops include carrot, beet [*Beta vulgaris* L. var. *crassa* (Alef) J.Helm], sugar beet (*Beta vulgaris* L. var. *saccharifera* Alef.), cabbage (*Brassica oleracea* L. var. *capitata* L.), turnip (*Brassica rapa* L.), and clover (*Trifolium repens* L.). The occurrence of annuals causes considerable economic loss to the grower. Carrot is normally classified as a biennial species that requires vernalization to induce flowering. During the first year it produces a basal rosette of leaves and stores carbohydrates in its hypertrophic root (Whitaker et al., 1970). The stage of growth when carrot seedlings are not responsive to low-temperature vernalization is known as juvenility. This condition usually ends when carrot plants have initiated 8 to 12 leaves, and storage roots are larger than 4 to 8 mm in diameter (Atherton et al., 1990; Galmarini and Della Gaspera, 1996; Galmarini et al., 1992; Lona and Crnko, 1967). After a vernalization period, with temperatures between 0 and 10 °C, and long days, floral stem elongation and flowering are induced (Atherton and Basher, 1984; Dickson and Peterson, 1958; Sakr and Thompson, 1942). Carrot roots quickly become very lignified after vernalization, even before the floral stalk elongates, so that the initiation of flowering results in a complete loss of commercial value (Rubatzky et al., 1999).

The level of response to cold treatments is cultivar dependent. Late-flowering cultivars require ≈11 to 12 weeks at 5 °C to be permanently vernalized (Atherton et al., 1990; Hiller and Kelly, 1979; Rubatzky et al., 1999). Early-flowering cultivars, as shown by Dias Tagliacozzo and Valio (1994) and suggested by Galmarini and Della Gaspera (1996), require shorter vernalization periods (1–4 weeks) than late-flowering cultivars. 'Criolla INTA' is a cultivar that can be classified as annual or early flowering because it has short vernalization requirements; Galmarini and Della Gaspera (1996) reported that this cultivar has the same juvenile period as the cultivar Flakkee (biennial), suggesting that its annual behavior results from a lower cold requirement after juvenility rather than a shorter juvenile period.

At La Consulta, Mendoza, Argentina (lat. 33°42′S, long. 69°04′W), the flowering habit defines the sowing time, depending on whether the growers want to produce seed or root crops

(Oliva, 1987). For seed production, using the seed-to-seed method, late-flowering cultivars must be sown early in the summer (January) to flower late the following spring (November), whereas the early-flowering cultivars can be sown in the summer, autumn and early winter (from January–July), and they are able to flower the same year of sowing (Astorquizaga et al., 2004; Galmarini and Della Gaspera, 1996).

The genetics of vernalization response and flowering habit have been studied in several biennial species, including the *Brassica* species, *B. napus* L., *B. rapa,* and *B. oleracea* (Bagget and Kean, 1989; Ferreira et al., 1995). In *B. oleracea* the annual habit is clearly dominant and seems to be controlled by several factors, and the inheritance pattern for flowering behavior depends on the particular parents used (Bagget and Kean, 1989; Walkof, 1963). There are several studies of the physiological bases of vernalization response in carrot (e.g., Atherton et al., 1990), but there are no studies about the genetics of this trait.

The current work was designed to study the genetic control of vernalization response in carrot by evaluating progeny of crosses between early- and late-flowering cultivars.

Materials and Methods

Four individuals plants of 'Criolla INTA' (early-flowering parents: E1, E2, E3, and E4) were intercrossed with plants of two male-sterile biennial lines (late-flowering parents: L1 and L2), from the Instituto Nacional de Tecnología Agropecuaria (INTA) breeding program to generate four F_1 families (L1 × E1, L2 × E2, L2 × E3, and L2 × E4) that were evaluated during the first year. 'Criolla INTA' is a cultivar obtained by mass selection of annual local carrot populations of Argentina. It has cylindrical roots, a blunt end, an orange cortex and core, and its average root length is 12 to 15 cm. 'Criolla INTA' often restores fertility to cytoplasmic male-sterile hybrids, and several male-fertile F_1 plants were noted. Two F_2 families derived from self-pollinated individual F_1 plants from L2 × E2, two backcross families derived from (L1 × E1) × E1 and two from (L2 × E2) × E2, were evaluated the second year (Table 1).

FIRST YEAR. The parents and F_1 were direct seeded in the field at La Consulta, Mendoza, Argentina (lat. 33°42′S, long. 69°04′W), on three different sowing dates (3 Mar., 20 Apr., and 6 June 2004) to ensure they received a range of exposures to low

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Table 1. Segregating families used in this study of the inheritance of vernalization requirement in carrot.

Progeny pedigree[z]	Generation[y]	Families (no.)	Family identification
(L1 × E1) × E1	BC$_1$P2	2	BC$_1$a, BC$_1$b
L2 × E2	F$_2$	2	F$_2$a, F$_2$b
(L2 × E2) × E2	BC$_1$P2	2	BC$_1$c, BC$_1$d

[z]L1 and L2, late-flowering parents; E1 and E2, early flowering parents.
[y]P2, early-flowering parent.

Table 2. Monthly average minimum, maximum, and mean temperatures May through October in 2004 and 2005 at La Consulta, Mendoza, Argentina.

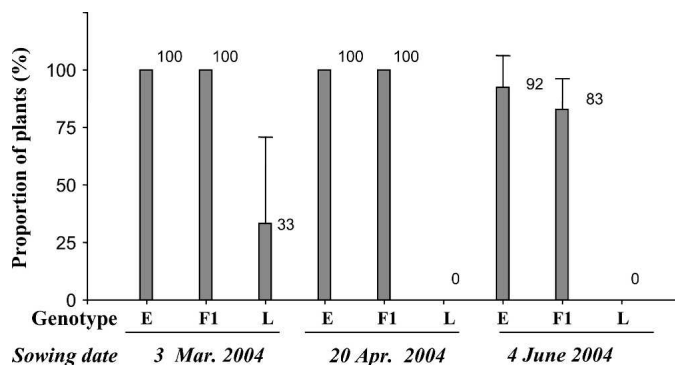|	May	June	July	August	September	October
Year				Temperature (°C)		
2004						
Minimum	1.8	−0.6	−1.3	0.9	3.7	6.5
Maximum	13.4	15.8	13.4	16.1	20.1	23.9
Mean	7.6	7.6	6.0	8.5	11.9	15.2
2005						
Minimum	0.4	0.3	−1.0	0.9	2.4	5.3
Maximum	17.7	13.5	14.7	14.6	17.1	23.3
Mean	9.1	6.9	6.9	7.8	9.8	14.3

Fig. 2. Proportion of flowering carrot plants at three sowing dates for early-flowering parents (E), late-flowering parents (L), and their crosses (F1) during the first year. For each category the mean and SD are presented.

temperatures (Table 2). For each sowing date, a randomized block design with three repetitions was used. Each plot had 20 plants on average, seeded in rows 0.8 m apart, with plants spaced 0.25 m apart within rows.

Once per week each plot was evaluated. Individual plants were scored as being vegetative until the first floral internode elongated. This was soon followed by the appearance of floral primordia. For each plot, the following six dates were noted: when 50% of the plants had their first floral stem internode elongated (Fig. 1A); when 100% of the plants had their first floral stem internode elongated, when 50% of plants had floral primordia in the primary umbel, and when 100% of plants had floral primordia in the primary umbel (Fig. 1B); and when 50% of the plants displayed flowering in primary umbel and when 100% of the plants displayed flowering in primary umbel (Fig. 1C). With these data, the proportion of flowering plants, weeks from sowing to first elongated internode, weeks to primordia

appearance, and weeks to flowering were calculated. Analysis of variance and mean separation were carried out for each sowing date.

SECOND YEAR. F$_2$ families, backcross families, F$_1$, and parents were sown on 4 May 2005 in the field at La Consulta, Mendoza, Argentina. A second sowing was carried out on 14 June 2005 including F$_2$ and their respective parents. On the first sowing date, the following populations sizes were evaluated: 280 plants from F$_2$a, 203 plants from F$_2$b, 61 plants from BC$_1$a, 66 plants from BC$_1$b, 201 plants from BC$_1$c, and 51 plants from BC$_1$d. On the second sowing date, 61 plants from F$_2$a and 51 plants from F$_2$b were evaluated. Individual plants and populations were scored as done the first year. Percentage of flowered plants for each population, and weeks from sowing date to first floral stem internode elongated for individual plants were calculated. Goodness-of-fit was evaluated by comparing observed and expected data with single-df χ^2 calculations.

Results

For all three sowing dates during the first year, all plants of the early-flowering parents and the F$_1$ flowered, whereas only one of the late-flowering parents (L2) flowered in the first sowing date. No late-flowering plants flowered in the later sowing dates (Figs. 2 and 3).

The flowering cycle of the F$_1$ progenies was the same as the early-flowering parents. Although the early-flowering parents and F1 showed a shorter cycle when the sowing date was delayed, the late-flowering parents did not flower at all (Fig. 3).

During the second year, all the individual plants evaluated from the early-flowering parents (E1 and E2) flowered on the first sowing date (4 May 2005), whereas none of the L1 plants (late-flowering parent) and only 12% of L2 plants flowered. From the F$_1$ populations, 100% of L1 × E1 plants and 88% of L2 × E2 plants flowered. The distribution of flowered and nonflowered plants in segregating generations supported the hypothesis that a single dominant gene controlled the early-flowering habit (Fig. 4 and

Fig. 1. Phenological stages of carrot flowering plants. (**A**) First floral stem internode elongated. (**B**) Floral primordial. (**C**) Plant with flowering primary umbel.
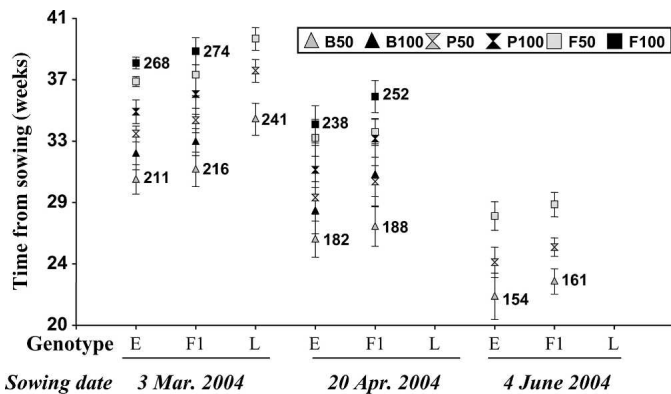
Fig. 3. Evolution of carrot flowering at different sowing dates during the first year. Weeks from sowing to 50% bolted plants (B50), 100% bolted plants (B100), 50% of plants with floral primordia (P50), 100% of plants with floral primordia (P100), 50% of flowering plants (F50), and 100% of flowering plants (F100) are presented for early-flowering parents (E), late-flowering parents (L), and their crosses (F1) at each sowing date. For each category the mean and SD are presented. Values after B50 and F100 categories are average values for days after sowing for these two categories. Flowering did not reach completion for L with the 3 Mar. sowing date or for E and F1 for the 4 June sowing date. No flowering was observed for L on either the 20 Apr. or 4 June sowing dates, so no values are presented.

Fig. 4. Photograph of F_2b family (left) and its biennial mother L2 (right) on 10 Nov. 2005.

Table 3). Segregation ratios in the F_2 and BC_1 families were not significantly different from that expected under the hypothesis of a single dominant gene conditioning the early-flowering habit (Table 3).

On the second sowing date (14 June 2005), 100% of E2 plants flowered whereas 6% of L2 plants flowered. For the F_2 families there was higher variability for the distribution of flowered and nonflowered plants; only 63.9% of F_2a plants flowered whereas 80.4% of F_2b plants flowered.

The highest proportion of plants of the F_1 generation (L1 × E1 and L2 × E2) on the first sowing date bolted 1 week later than their annual parents (E1 and E2), whereas for the BC_1 generation, the date when the highest proportion of plants bolted was the same (BC_1a and BC_1d) or even earlier (BC_1b and BC_1c) than their corresponding annual parents (Fig. 5). For both F_2 families, a shorter cycle was observed when the sowing date was delayed (Fig. 6).

Discussion

Segregation patterns of the F_2 and BC progenies confirmed a strong dominance for annual habit. Dominance of the early-flowering habit was clearly demonstrated by the F_1 families in both years, and by the segregation patterns of the BC and F_2 families in the second year. This does not agree with Wijnheijmer et al. (1989), who suggested that biennial behavior was dominant, although these authors were working with both wild (*D. carota* ssp. *carota*) and cultivated carrots, and they did not study crosses between them. It does, however, agree with preliminary observations of Dickson (1958), who supported the hypothesis that annual flowering is dominant over biennial flowering, although his evidence was inconclusive.

Segregation of F_2 families and BC_1P2 could be explained by the action of a single gene. Differences between the two late-flowering parents could be the result of allelic differences or action of other genes.

Variation in the vernalization requirement has been observed since domesticated carrots arrive in Europe from Arab countries in the 13th or 14th century (Banga, 1957). Early-flowering carrot cultivars are grown in warm temperatures and subtropical production areas of South America and Asia today (Simon et al., in press). Because flowering is an important adaptive characteristic for carrot root and seed production, understanding the genetics of this trait is valuable for breeding programs. For example, introgression of genes between annual and biennial forms will broaden the genetic base of each group,

Table 3. Observed and expected segregation ratios for flowering rate in carrot populations under the hypothesis that early flowering is dominant and monogenic.

		Observed segregation ratio				
Generation	Progeny pedigree	Early flowering	Late flowering	Expected ratio	χ^2	P
F_2	L2 × E2	206 (73%)	76 (27%)	3:1 (211.5:70.5)	0.572	0.50
F_2	L2 × E2	155 (76.4%)	48 (23.5%)	3:1 (152.2:50.7)	0.198	0.75
BC_1P2	(L2 × E2) × E2	194 (96.5%)	6 (3.5%)	1:0 (201:0)	0.24	0.75
BC_1P2	(L2 × E2) × E2	50 (98.1%)	1 (1.9%)	1:0 (51:0)	0.019	0.90
BC_1P2	(L1 × E1) × E1	60 (98.3%)	1 (1.7%)	1:0 (61:0)	0.0163	0.90
BC_1P2	(L1 × E1) × E1	62 (93.9%)	4 (6.1%)	1:0 (66:0)	0.24	0.75

Segregating generations were derived from the crosses L2 × E2 and L1 × E1 (L1 and L2, late-flowering parents; E1 and E2, early-flowering parents). Plants where grown in the field in La Consulta, Mendoza, Argentina. The sowing date was 4 May 2005. P2, early-flowering parent.
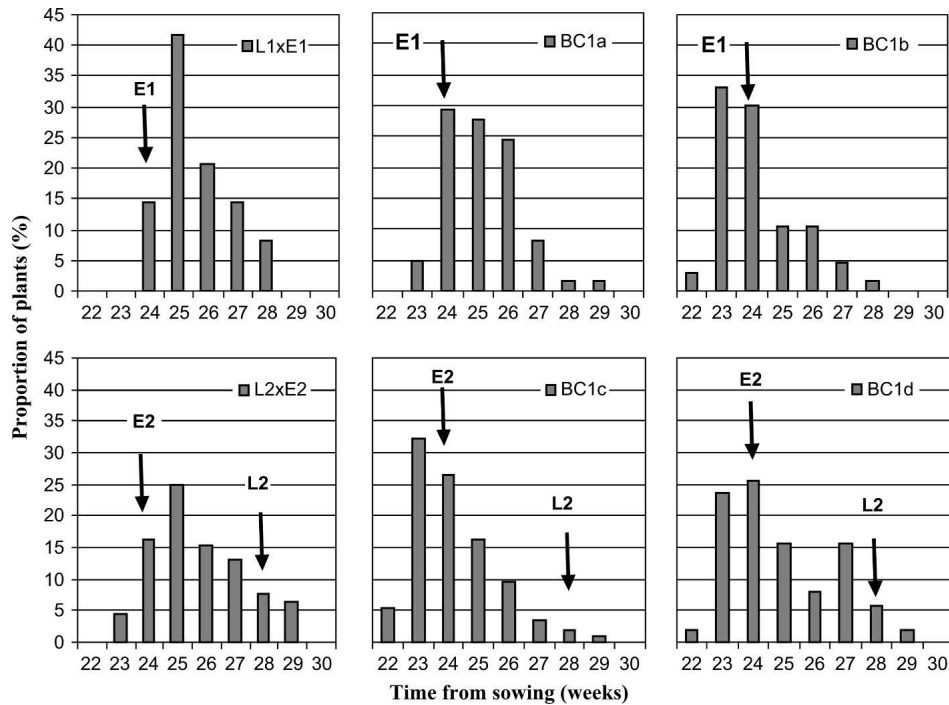
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Fig. 5. Proportion of carrot plants bolting for F₁ [L1 × E1, L2 × E2 (L1 and L2, late-flowering parents; E1 and E2, early-flowering parents)] and backcrosses to annual parent families (BC₁a, BC₁b, BC₁c, and BC₁d). The number of weeks from sowing date (4 May 2005) to evaluation date are presented on the x-axis. Weeks when the highest proportion of plants bolted for each parent (E1, E2, and L2) are represented by arrows. Because it did not flower, the L1 parent is not shown. Only 12% of L2 plants flowered.
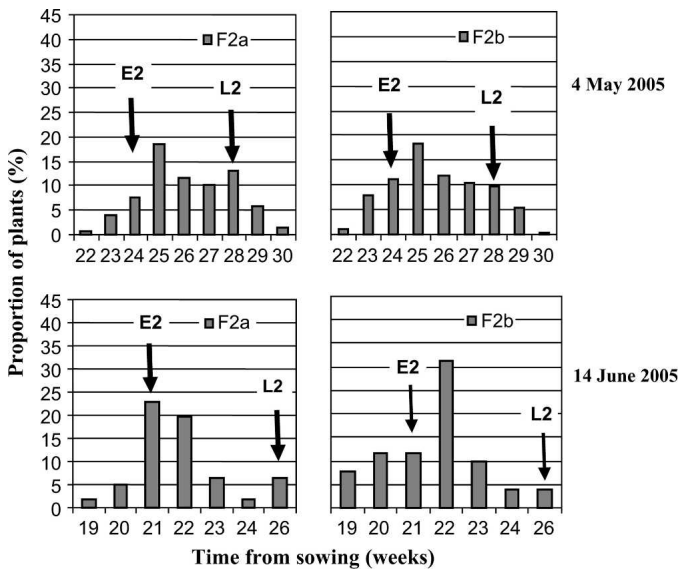

Fig. 6. Proportion of carrot plants bolting in each weekly evaluation for F₂ families in the first (4 May 2005) and second (14 June 2005) sowing dates. Weeks from sowing to evaluation date are presented on the x-axis. Dates when the highest concentration of plants bolted for each progenitor (E2 and L2) are represented by arrows. Only 12% and 6% of total L2 plants flowered during the first and second sowing dates respectively. E2, early-flowering parent; L2, late-flowering parent.

allowing exploitation of a heterotic effects that might occur. Our observations indicate that evaluation and, consequently, selection of flowering habit depends strongly on planting time and growing conditions.

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