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 F1, F2, and BC1 progenies of an intercross between the early-flowering carrot (‘Criolla INTA’) and a late-flowering biennial carrot. F1 progenies were completely annual. Observed segregation ratios in the F2 and BC1 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 Crmko, 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; Hille 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 (Astorquiza 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 F1 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 F1 plants were noted. Two F2 families derived from self-pollinated individual F1 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 F1 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...
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. F2 families, backcross families, F1, 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 F2 and their respective parents. On the first
sowing date, the following populations sizes were evaluated:
280 plants from F2a, 203 plants from F2b, 61 plants from BC1a,
66 plants from BC1b, 201 plants from BC1c, and 51 plants from
BC1d. On the second sowing date, 61 plants from F2a and 51
plants from F2b were evaluated. Individual plants and popula-
tions 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 $\chi^2$ calculations.

Results

For all three sowing dates during the first year, all plants of
the early-flowering parents and the F1 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 F1 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 F1 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

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

| Progeny pedigree* | Generation\(\uparrow\) | Families (no.) | Family identification |
|-------------------|------------------|----------------|-----------------------|
| L1 × E1 × E1      | BC1P2            | 2              | BC1a, BC1b            |
| L2 × E2           | F2               | 2              | F2a, F2b              |
| (L2 × E2) × E2    | BC1P2            | 2              | BC1c, BC1d            |

*L1 and L2, late-flowering parents; E1 and E2, early flowering parents.
\(\uparrow\)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.

| Year       | May   | June  | July  | August | September | October |
|------------|-------|-------|-------|--------|-----------|--------|
| 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. 1. Phenological stages of carrot flowering plants. (A) First floral stem internode elongated. (B) Floral primordial. (C) Plant with flowering primary umbel.

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.
Table 3). Segregation ratios in the F₂ and BC₁ 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 F₂ families there was higher variability for the distribution of flowered and nonflowered plants; only 63.9% of F₂a plants flowered whereas 80.4% of F₂b plants flowered.

The highest proportion of plants of the F₁ 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 BC₁ generation, the date when the highest proportion of plants bolted was the same (BC₁a and BC₁d) or even earlier (BC₁b and BC₁c) than their corresponding annual parents (Fig. 5). For both F₂ families, a shorter cycle was observed when the sowing date was delayed (Fig. 6).

Discussion

Segregation patterns of the F₂ and BC progenies confirmed a strong dominance for annual habit. Dominance of the early-flowering habit was clearly demonstrated by the F₁ families in both years, and by the segregation patterns of the BC and F₂ 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 F₂ families and BC₁ P₂ 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.

| Generation | Progeny pedigree | Early flowering | Late flowering | Expected ratio | \( \chi^2 \) | \( P \) |
|------------|------------------|----------------|---------------|----------------|---------|-------|
| F₂         | L₂ × E₂          | 206 (73%)      | 76 (27%)      | 3:1 (211.5:70.5) | 0.572   | 0.50  |
| F₂         | L₂ × E₂          | 155 (76.4%)    | 48 (23.5%)    | 3:1 (152.2:50.7) | 0.198   | 0.75  |
| BC₁ P₂     | (L₂ × E₂) × E₂   | 194 (96.5%)    | 6 (3.5%)      | 1:0 (201:0)     | 0.24    | 0.75  |
| BC₁ P₂     | (L₂ × E₂) × E₂   | 50 (98.1%)     | 1 (1.9%)      | 1:0 (51:0)      | 0.019   | 0.90  |
| BC₁ P₂     | (L₁ × E₁) × E₁   | 60 (98.3%)     | 1 (1.7%)      | 1:0 (61:0)      | 0.0163  | 0.90  |
| BC₁ P₂     | (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 were grown in the field in La Consulta, Mendoza, Argentina. The sowing date was 4 May 2005. P₂, early-flowering parent.

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 so 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 F₂b family (left) and its biennial mother L₂ (right) on 10 Nov. 2005.
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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