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A model plant for vernalization studies

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Abstract

Vernalization requirements of *Raphanus sativus* L. 'Early 40 Days', 'Chinese Radish Jumbo Scarlet', 'Everest', and 'Minowase Early Long White' were studied to determine their potential as model plants for vernalization studies. Leaf number below the inflorescence and days to anthesis decreased as vernalization time increased from 0 to 15 days at 6.5°C for all cultivars. 'Early 40 Days', 'Everest', and 'Minowase Early Long White' flowered but 'Chinese Radish Jumbo Scarlet' did not flower under long-day-conditions (inductive) at 18°C without vernalization, i.e. 'Chinese Radish Jumbo Scarlet' has an obligate vernalization requirement. 'Chinese Radish Jumbo Scarlet' was completely vernalized in 5–10 days. Use of 'Chinese Radish Jumbo Scarlet' as a model plant for vernalization studies is discussed. © 1997 Elsevier Science B.V.

Keywords: Japanese radish; *Raphanus sativus* L.; Photoperiod; Flower induction; Stem elongation

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1. Introduction

Vernalization is the low temperature induction of flowering in responsive plants (Chouard, 1960). Optimum vernalization temperatures range from 6–10°C for most plants (Lang, 1959; Chouard, 1960). The time required for complete vernalization varies with species. For example, *Lunaria biennis* L. requires 9 weeks (Wellensiek, 1958) and *Apium graveolens* L. (celery) and *Secale cereale* L. (winter rye) require 6 weeks (Ramin and Atherton, 1991; Friend, 1965).

The length of the vernalization treatment required for complete vernalization is related to whether a species has an obligate or facultative vernalization requirement. Plants that require a short vernalization period (20–30 days), such as *Brassica campestris pekinensis* L., are often facultative in their vernalization requirement (Suge, 1984); photoperiod is usually the primary flower induction stimulus but vernalization can

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substitute for or hastens flowering (Lang, 1959). In contrast, plants requiring a long vernalization period for complete flower induction are often obligate in their vernalization requirement, i.e. vernalization alone induces flowering.

The goal of the research presented here was to identify and characterize a model plant to study the interaction between vernalization and other environmental factors. An ideal model plant for vernalization studies would: 1) be cold obligate, 2) be rapidly vernalized, 3) be receptive to vernalization early in development, 4) be daylength insensitive during vernalization, and 5) be available from seed or readily available from cuttings.

Preliminary research focused on studies using *L. biennis*, *A. graveolens*, and *Raphanus sativus* L. (Japanese radish). *L. biennis* and *A. graveolens* are daylength insensitive during vernalization, but have a long juvenile period (7–9 weeks) and a 9-week vernalization requirement (Wellensiek, 1958; Ramin and Atherton, 1991). Therefore, these plants have a long experimental cycle (approximately 16–18 weeks). The *Arabidopsis thaliana* L. *fca* mutant has also been used in vernalization studies but has a facultative vernalization requirement (Bagnall, 1993; Martinez-Zapater and Somerville, 1990). Our efforts, therefore, focused on *R. sativus* because it can be vernalized as a germinating seed (Nakamura, 1985; Wiebe and Alpers, 1983) and it can reportedly be vernalized in 20 days. Extensive breeding has produced a diverse range of cultivars that differ in morphology and eco-physiological responses to flowering (Nakamura, 1985). Here, we characterized variation in vernalization requirement and photoperiod effect during vernalization on flowering of four *R. sativus* cultivars in an effort to identify a cultivar for future vernalization studies.

2. Materials and methods

Seeds of *R. sativus* 'Early 40 Days', 'Chinese Radish Jumbo Scarlet', 'Everest', and 'Minowase Early Long White' were obtained from American Takii (Salinas, CA). Cultivars were harvested within 60 days of each other and reportedly had a short vernalization requirement (T. Ueda, American Takii, personal communication). Seed was imbibed for 24 h in the dark at 25 ± 1°C in 5.5 cm petri dishes on moist Whatman no. 1 filter paper (Yoo and Uemoto, 1976). The emerging radicle was approximately 1–2 mm long after imbibition when vernalization treatments were initiated.

Seedlings were vernalized (6.5 ± 1°C) under either 8 (SD) or 16 h (LD). Optimum vernalization temperature for *R. sativus* is 5–7°C (Nakamura, 1985; Sagwansupyakorn et al., 1986). SD was delivered from 0100 to 0900 h, and LD was delivered from 0100 to 1700 h. Light pollution in SD treatments was eliminated by placing seedlings in a plastic bag from 0100–0900 h each day. Treatments (petri dishes) were randomized within the chamber. Seedlings were vernalized for 5, 10, 15, or 20 days. The PPFD was 55–60 μmol m^{-2} s^{-1} (400 watt metal halide lamps; General Electric, Energy Technics, York, PA). The R:FR ratio (650–670: 720–740 nm) was 2.0 (LiCor LI-1800 spectroradiometer, Lincoln, NE).

Following vernalization, seedlings were planted in 10 cm diameter standard plastic pots (254 cm^3) in a soilless medium (50% sphagnum, 25% perlite, and 25% vermiculite) (Baccto Soil, Michigan Peat, Houston, TX). Planted seedlings were then placed in a glasshouse maintained at constant 18 ± 2°C with a 16-h (LD) photoperiod to promote flower development (Nieuwhof, 1987; Nakamura, 1985; Wiebe, 1985). Post-vernalization photoperiod was composed of natural light (October 1–November 30, 1992, St. Paul, MN) supplemented with 400-W metal halide lamps (General Electric, 55 μmol m^{-2} s^{-1}) from 1800 to 2200 h. Plants were watered daily and fertilized four times a week with a solution containing $CaNO_3$ and KNO_3 (7.2 mM N and 2.6 mM K). Plants were fertilized with $MgSO_4$ (0.35 mM Mg) supplemented with essential micronutrients once a month (S.T.E.M., W.R. Grace Co., Fogelsville, PA).

Data were collected on days to anthesis (DTA) and leaf number below the inflorescence at anthesis. DTA was the number of days from the end of vernalization treatment to anthesis. The experimental design was a 3-way factorial. Main effects were cultivar (four levels), photoperiod (two levels), and vernalization time (four levels). The experiment was replicated twice over time with nine and six plants per treatment per replicate, respectively. Seedlings not exposed to low temperature (18°C under LD) served as controls. Data were analyzed using an analysis of variance procedure (SYSTAT, Evanston, IL). Mean separation was conducted using Tukey's test for mean separation (H.S.D.). Statistical significance was determined throughout the paper using an alpha level of 0.05.

3. Results

Increasing vernalization time decreased DTA across cultivars (Table 1). Increasing vernalization time from 5 to 15 days under SD decreased DTA on 'CRJS' more than with 'Minowase', 'Early 40 Days', and 'Everest' (Table 1). Increasing the vernalization time beyond 15 days under LD did not decrease DTA in 'CRJS', 'Minowase', or 'Everest' plants. Increasing the vernalization time beyond 15 days under SD decreased DTA on 'Everest' plants (Table 1).

The effect of photoperiod on DTA varied with vernalization time (Table 1). For instance, DTA was shorter under LD than SD in 'Early 40 Days' and 'CRJS' when vernalized for 10 days. In contrast, DTA was reduced under SD in 'Minowase', 'CRJS', and 'Everest' when plants were vernalized for 20 days (Table 1).

Cultivars varied in their vernalization requirement. 'Minowase', 'Early 40 Days', and 'Everest' did not require vernalization for flowering (Table 2); they flowered after 47 days when grown under LD at 18 ± 2°C without a cold treatment. In contrast, vernalization was essential for flowering of 'CRJS' (Table 2).

Node number in 'CRJS' and 'Minowase' was higher under SD than LD, when vernalized for 10 days (Table 1). When 'Minowase' was vernalized for 5 and 20 days, node number was smaller under SD than LD. 'CRJS', 'Everest', and 'Early 40 Days' node number per plant was not affected at 5 or 20 days of vernalization under LD or SD.

Node number decreased as vernalization time increased across all cultivars (Table 1). 'CRJS' node number decreased from 17.4 to 11.9 nodes per plant as vernalization time increased from 5 to 15 days under LD (Table 1). Further vernalization did not decrease node number in 'CRJS'. The decrease in node number associated with vernalization treatment was higher on 'CJRS' than the other cultivars.

Table 1

Effect of photoperiod, vernalization time, and cultivar on days to anthesis (DTA) and leaf number (nodes) of *R. sativus* L. cvrs. 'Chinese Radish Jumbo Scarlet', 'Early 40 Days', 'Minowase Early Long White', and 'Everest'. Plants were grown under an 8 (short day) or 16 h (Long Day) photoperiod during vernalization

Vernalization time (*d*)	Photoperiod			
	Short day		Long day	
	DTA	Nodes	DTA	Nodes
Chinese Radish Jumbo Scarlet				
5	50 (a) a[a]	17 (a) a	49 (a) a	18 (a) a
10	39 (b) a	13 (b) a	45 (a) b	16 (a) b
15	34 (c) a	12 (bc) a	38 (b) b	13 (b) a
20	35 (c) a	12 (c) a	33 (c) b	12 (b) a
Early 40 Days				
5	32 (a) a	12 (a) a	32 (a) a	12 (a) a
10	26 (b) a	10 (b) a	29 (b) b	11 (b) a
15	29 (c) a	10 (b) a	26 (c) b	6 (c) a
20	26 (b) a	9 (c) a	30 (abc) a	9 (c) a
Minowase Early Long White				
55	39 (a) a	18 (a) a	37 (a) a	16 (a) b
10	30 (b) a	14 (b) a	31 (b) a	14 (b) b
15	29 (bc) a	12 (bc) a	28 (c) a	13 (c) a
20	32 (b) a	13 (b) a	27 (c) b	11 (d) b
Everest				
5	31 (a) a	14 (a) a	32 (a) b	14 (a) a
10	26 (b) a	11 (b) a	26 (b) a	11 (b) a
15	27 (c) a	11 (b) a	25 (c) b	11 (b) a
20	29 (c) a	10 (c) a	24 (d) b	10 (c) a

[a] Letters in parenthesis are comparisons of each cultivar across vernalization time. Letters not in parenthesis are comparisons of each cultivar across photoperiod.

Table 2

Comparison of vernalized and non-vernalized *R. sativus* L. 'Chinese Radish Jumbo Scarlet' (CRJS), 'Minowase Early Long White' (MINO), 'Early 40 Days' (E40) and 'Everest' (EVE) days from the end of vernalization to anthesis (days to anthesis) and leaf number at anthesis

Flower inducing condition	Cultivar			
	CRJS	E40	EVE	MINO
Days to anthesis				
Non-vernalized (18°C)	–[a]	39 (a)	42 (a)	47 (a)
Vernalized (6.5°C)	34	29 (b)	27 (b)	29 (b)
Leaf number				
Non-vernalized (18°C)	–	12 (a)	14 (a)	18 (a)
Vernalized (6.5°C)	12	10 (b)	11 (b)	12 (a)

[a] '–' indicates that plants did not flower, i.e. data could not be collected.

Letters in parenthesis denote mean comparisons across vernalization treatments.

4. Discussion

Our objective was to determine the degree of variation in flower induction by vernalization and photoperiod among the four *R. sativus* cultivars to identify a model cultivar appropriate for environment × vernalization studies. 'CRJS' was identified as a potential cultivar for use as a model plant (Table 2). Even though vernalization decreased DTA on 'Early 40 Days', 'Everest', and 'Minowase', these cultivars were inappropriate for use as model plants because they exhibited a facultative vernalization response since flowering occurred under LD without vernalization (Table 2).

In early experiments, 'CRJS' flower induction required a 5–10-day vernalization treatment. Subsequent experimentation consistently resulted in 100% 'CRJS' flowering after a 5-day vernalization treatment (Englen-Eigles, 1996). In 'CRJS', leaf number data (Table 1) do not support the conclusion that a 5-day vernalization treatment resulted in complete flower induction since a 10-day versus 5-day vernalization treatment still reduced node number (Table 1). Since variation in plant vernalization requirement was evident, selection for a still earlier vernalization response may be possible (Table 2).

Nakamura (1985) divided Japanese radish cultivars into two groups based on vernalization response: 1) slow bolting (flowering) cultivars that require low temperatures of prolonged duration, and 2) cultivars that are easily induced under a wide range of temperatures of shorter duration than the first group. 'CRJS' did not fit in the first group defined by Nakamura (1985). The cultivar did not require prolonged vernalization treatments as in group 1, yet 'CRJS' was the cultivar with the greatest DTA. 'CRJS' probably belongs in the second group.

5. Conclusion

'CRJS' is an alternative model plant to *A. thaliana*, *L. biennis* or *A. graveolens* for whole plant vernalization studies. *R. sativus* 'CRJS' may be a more appropriate plant for vernalization studies since it has an obligate vernalization requirement, requires a short vernalization treatment for flower induction, has a short DTA period, is readily available, and can be vernalized early in development. Studies utilizing *R. sativus* 'CRJS' for studying vernalization × light quality have already yielded significant results that increased our understanding of factors that affect Easter lily vernalization (Erwin and Englen-Eigles, 1996). Also, because 'CRJS' is rapidly vernalized, it may be useful for studies on vernalization regulation of gene expression.

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