DEVELOPMENTAL RESPONSES OF GARLIC TO TEMPERATURE AND PHOTOPERIOD

Respuestas en desarrollo del ajo a la temperatura y el fotoperíodo

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ABSTRACT

Bulb initiation and maturity of four Chilean clones of garlic (*Allium sativum* L.) were studied in controlled and field conditions in order to assess temperature and photoperiod requirements of selected clones. The effect of cold storage temperature prior to planting and photoperiod on bulb initiation was assessed in two clones of garlic. In one experiment bulbs were stored at 4, 7, 10, 13 and 16°C for 20, 40 or 60 days, and planted in pots in a heated greenhouse with a 14 h photoperiod. In a second experiment bulbs of the same clones were stored at 4 and 10°C, and room temperature (>13°C) for 30 days, and later grown in a heated greenhouse with 8, 10, 12, 14 or 16 h of photoperiod. In the field, days from emergence to bulb initiation and to maturity were evaluated in 13 clones planted on six sequential planting dates. Percentage of bulbing increased and the number of days from emergence to bulb initiation decreased, as storage temperature decreased and duration of storage increased. Plants of studied clones did not form bulbs with photoperiods of 8, 10 or 12 h with any storage temperature. All plants of both clones formed bulbs when photoperiod was over 14 h and storage temperature was 4 or 7ºC. A reduction on the period emergence-bulb initiation was observed as planting date was delayed from April to September.

Key words: cold requirement, storage temperature, ceiling photoperiod, *Allium sativum* L.

RESUMEN

Se estudió la bulbificación y maduración de clones de ajo (*Allium sativum* L.) chilenos bajo condiciones controladas y de campo a fin de determinar los requerimientos de temperatura y fotoperíodo de los clones seleccionados. Se estimó el efecto del almacenaje en frío previo a la plantación y del fotoperíodo en la bulbificación de dos clones de ajo. En un experimento se almacenaron bulbos a 4, 7, 10, 13 y 16°C por 20, 40 ó 60 días, y se plantaron en macetas en invernadero calefaccionado, con 14 h de fotoperíodo. En un segundo experimento se almacenaron bulbos de los mismos clones a 4 y 10°C y temperatura ambiente (>13°C) durante 30 días, y luego se hicieron crecer en invernadero calefaccionado con fotoperíodos de 8, 10, 12, 14 ó 16 h. En el campo, se evaluaron los días desde emergencia a inicio de bulbificación y a madurez en 13 clones plantados en seis fechas consecutivas. El porcentaje de bulbificación aumentó y el número de días desde emergencia a bulbificación disminuyó a medida que la temperatura de almacenaje fue menor y el tiempo de almacenaje aumentó. Las plantas de los clones estudiados no formaron bulbos con fotoperíodos de 8, 10 ó 12 h, con cualquier temperatura de almacenaje. Todas las plantas de ambos clones formaron bulbos cuando el fotoperíodo estuvo sobre 14 h y la temperatura de almacenaje fue 4 ó 7°C. Se observó una reducción en el período emergencia-inicio de bulbificación, a medida que la fecha de plantación se atrasó desde abril a septiembre.

Palabras clave: requerimiento de frío, temperatura de almacenaje, fotoperíodo crítico, *Allium sativum* L.

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INTRODUCTION

Garlic (*Allium sativum* L.) is an important crop in Mediterranean regions. In Chile, it is planted in a wide range of environments, however the main commercial production is found in the Mediterranean zone in the central part of the country. In Mediterranean areas the crop is exposed to a great seasonal fluctuation of temperature and photoperiod (day length), both having a strong influence on the growth and development of garlic (Takagi, 1990; Pooler and Simon, 1993).

Clove sprouting, leafing, bulb initiation and maturation are the main phenological stages in garlic. Clove sprouting and emergence are controlled mainly by temperature in the absence of dormancy (Takagi, 1990; Barrera *et al*., 1998). Also, linear relationships between the number of leaves and thermal time above 0ºC have been found in garlic growing in field conditions (Buwalda,1986; Espagnacq *et al*., 1987; Bertoni *et al*., 1992; Cabrera, 1993; Portela, 2000).

Bulb initiation is promoted by previous exposure of cloves to low temperature (cold-storage) and also by long photoperiod during crop development (Racca *et al*., 1981; Takagi, 1990; Rahim and Fordham, 2001). An important clone variation in cold-storage temperature and photoperiod requirements for bulb initiation has been found in garlic (Takagi, 1990; Burba, 1992). Also, an interaction between cold-storage temperatures and photoperiod has been reported by several authors (Racca *et al*., 1981; Ledesma *et al*., 1983; Takagi, 1990). For instance, cold temperatures can shorten the critical photoperiod (Takagi, 1990). At higher temperatures, bulb formation can be induced under long photoperiods, or continuous light, since photoperiod essentially replaces the cold-temperature stimulus that is normally necessary for bulb initiation (Racca *et al*., 1981; Ledesma *et al*., 1983).

Despite the fact that garlic is an obligate apomictic species (with no sexual reproduction), an important variation in phenological and productive traits was found among 66 garlic clones collected in Chile (Matus *et al*., 1999). In this paper we analysed the effects of temperature and photoperiod on development of Chilean clones growing in glasshouse and field conditions. The aim was to determine temperature and photoperiod requirements of selected clones.

MATERIALS AND METHODS

A series of experiments were carried out in glasshouse and field conditions in order to evaluate the effect of temperature and photoperiod on bulb initiation and maturity.

Glasshouse experiments

Two experiments were carried out in a heated glasshouse, located in Chillán (36º03' S; 72º06' W), during 1996. In the first experiment the effect of five pre-planting storage temperatures (4, 7, 10, 13 and 16°C), and three storage periods (20, 40 and 60 days), on bulbing of two clones of garlic, Akukeli (non-bolting type) and Rosado-INIA (bolting type), were evaluated. The experimental design was completely randomized in a factorial arrangement. Each treatment had six replicate pots with one plant per pot. Mother bulbs were stored after harvest in shelves at room temperature (18-25°C) from January 8 until March 31, April 20, or May 10 depending of the following storage periods under different temperatures. Then bulbs were transferred to controlled-temperature chambers and after temperature treatments, peripheral cloves were selected and disinfected by immersion in 1.5% v/v sodium hypochlorite solution for 5 min. Cloves were planted in pots (2 L capacity) which contained a mixture of 50% soil and 50% sand v/v on May 30. The substrate was fertilized with 56 g P_2O_5 and 28 g N per m^3 of soil-sand mixture. Plants were grown in a heated glasshouse (minimum temperature of 18°C) and received natural light from sunrise till 17:00 h, and then incandescent lamp provided supplementing light to get a constant photoperiod of 14 h. Plants were irrigated 2-4 times per week and pots were hand weeded when necessary.

In the second experiment the effect of cold-storage temperature prior to planting and photoperiod during growth were evaluated in clones Rosado-INIA and Akukeli. The experimental design was a completely randomized in a factorial arrangement that included three storage temperatures, five photoperiods and two clones. Each treatment had five replicate plants. Mother bulbs were stored in shelves at room temperature ranging from 18 to 25°C during four months. On May 1 the bulbs were stored for 30 days in chambers at 4°C and 10°C, and at storeroom temperature, which was always above 13°C. After the storage-temperature treatments, peripheral cloves were selected, disinfected, and planted in pots in a similar way as the first experiment, on May 31. In the glasshouse, plants received 8 h of natural light between 9:00 and 17:00 h, and then were artificially illuminated with 0, 2, 4, 6, or 8 h of light, in closed chambers, provided by 100 W Plantilux light bulbs. Mean minimum and maximum temperatures during the experiments were 17 and 25ºC, respectively. Plants were irrigated 2-3 times per week and weeding was done by hand.

Phenological observations were carried out on individual plants every three days. Parameters evaluated included number of days from planting to emergence, number of days from emergence to bulb initiation, and percentage of plants that produced bulbs. Bulb initiation was defined as the stage when the ratio between the diameter of the pseudostem and the equatorial diameter of the bulb being formed decreased to 0.5 (Mann, 1952). The criteria to determine maturity for harvesting were bulbing ratios of 0.2 - 0.3 (Ledesma *et al.*, 1997), and between 38 to 50% of leaves in senescence. Additionally, the number of cloves per bulb was evaluated at harvest.

From data obtained in the first experiment the relative rate of bulb induction (RBI) was calculated. RBI was defined as the ratio between days from emergence to bulb initiation (E-BI) of the most inductive cold temperature, and E-BI at other temperatures. Also, cumulative cold days (CCD, ºC days) was calculated as:

$$CCD = (Tc - T)\, N$$

where Tc is the ceiling temperature below which there is bulb induction, T is the storage temperature and N is number of days.

Field experiment

Time to sprout emergence, bulb initiation and maturity of 13 clones of garlic were determined on six sequential planting dates (April 15, May 14, June 15, July 15, August 13, and September 15, 1993) at Chillán (36º03' S, 72°00' W). Fifteen cloves per clone and planting date were planted in 2.5 m long rows. For a particular planting date, the 13 clones were planted in a single row, and the position of a clone in the row was randomized. The distance between rows (planting dates) was 60 cm. Before planting cloves were disinfected with a 1% sodium hypochlorite solution. Plots received the equivalent of 100 kg ha^{-1} P_2O_5 as normal superphosphate (prior to planting), and 135 kg ha^{-1} N as urea (1/3 on planting, 1/3 at 30 days and 1/3 at 60 days after planting). Plants were irrigated as necessary and weeds were controlled by hand.

Phenological observations were carried out three times a week and the duration of the different phenological stages was related to temperature and photoperiod. Temperatures were obtained from a meteorological station located 200 m from the experiment. For the period planting-sprout emergence the daily mean temperature at 0.02 m depth was used, and from sprout emergence-bulb initiation, and bulb initiation-maturity the screen air temperature at 1.5 m. Daily variation of day length or photoperiod was calculated using an equation that relates sun declination and latitude with photoperiod (Smithsonian Institution, 1963).

RESULTS AND DISCUSSION

Effect of pre-planting storage temperature and storage duration

The storage temperature regime had little effect on days to emergence of clone Akukeli and had no effect on clone Rosado-INIA (data not shown). Both storage temperature and duration had a great effect on bulb initiation percentage (Table 1). Clones formed bulbs when the storage temperature was 4 or 7ºC at any storage duration. Clone Akukeli did not initiate bulbing when stored at 16°C, regardless of the duration of storage. Clone Rosado-INIA, however, initiated bulbing after being stored for 60 days at 16°C (Table 1). The number of days from emergence to bulb initiation increased as the storage temperature increased above 7°C (Table 1). Additionally, it was observed that the number of days between emergence and bulbing decreased as the number of storage days increased (Table 1).

The relative rate of bulb induction (RBI) was greater at 4ºC and declined as the storage temperature increased (Figure 1A). From the polynomial equations of Figure 1A it was determined that the ceiling temperature for bulb induction (Tc) was 16 and 18ºC for the clones Akukeli and Rosado-INIA, respectively. It was found a curvilinear relationship between cumulative cold days and RBI (Figure 1B), where maximum bulb induction was obtained at 540 and 660ºC days, in clones Akukeli and Rosado-INIA, respectively.

Although bulb initiation occurred in the non-bolting type clone Akukeli, it was considered anomalous due to the lower number of cloves per bulb formed (1-2.2) compared to the bolting type clone Rosado-INIA (6.2-9.5) (data not shown). In natural field conditions, clone Akukeli has a higher number of cloves (14 to 19) per bulb, compared to clone Rosado-INIA which normally has 10 to 14 cloves per bulb. This effect has been described by Takagi (1990) in several garlic clones of temperate climates. It seems that long photoperiods and high temperatures immediately after planting promote rapid storage-leaf differentiation, as a consequence cloves may form axillary branching before resulting in single-cloved bulbs (Brewster, 1994).

The inductive effect of cold storage depends not just on temperature but on its duration, and clones differed in cold requirements for bulb induction. Exposure to temperatures below 16-18ºC (depending on clone), with an optimum of 4°C was necessary to induce bulbing. Differences between clones in cold requirement has also been reported by Braz *et al.* (1997). They found that one clone (cv. Chines) was not affected by the duration (0, 10, 20, 30 and 40 days) of the pre-planting cold treatment at 4ºC, whereas other two clones (cvs. Contestado and Quiteria) did not form cloves with less than 20 days of pre-planting cold treatment.

Effect of photoperiod and pre planting storage temperature

Bulbing is stimulated by long photoperiods and warm temperatures, provided plants have been previously exposed to low temperatures, either as stored bulbs or after planting in the field. Photoperiods shorter or equal to 12 h inhibited bulb initiation in both clones, regardless of storage temperature previous to planting (Table 2). When plants were grown at 14 h bulb initiation

Table 1. Percentage of plants that reached bulb initiation in two garlic clones as influenced by storage temperature and storage duration. In parentheses are days from emergence to bulb initiation.

Cuadro 1. Porcentaje de plantas que alcanzaron el inicio de la bulbificación en dos clones de ajo, en respuesta a la temperatura de almacenaje y su duración. Entre paréntesis están los días desde emergencia a inicio de bulbificación.

Days of storage	Clone Akukeli					Clone Rosado - INIA				
	Storage temperature (º C)									
	4	7	10	13	16	4	7	10	13	16
20	100	100	n.b.[1]	n.b.	n.b.	100	100	17	17	n.b.
	(63)	(60)	-	-	-	(64)	(61)	(129)	(100)	-
40	100	100	50	50	n.b.	100	100	100	100	n.b.
	(45)	(50)	(76)	(76)	-	(54)	(51)	(73)	(73)	-
60	100	100	100	100	n.b.	100	100	100	100	100
	(48)	(43)	(56)	(56)	-	(42)	(42)	(61)	(66)	(78)

[1] n.b.: no bulbing.
- : without information.

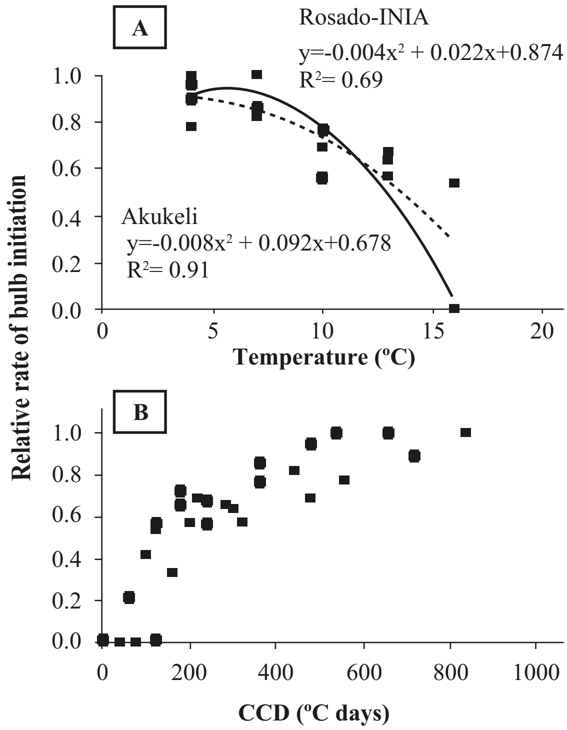

Figure 1. Relation between relative rate of bulb initiation and storage temperature (A), and cumulative cold days (CCD) (B). Squares are clone Rosado-INIA and circles are clone Akukeli. For the calculation of CCD, Tc was 16ºC and 18ºC, in clones Akukeli and Rosado-INIA, respectively.

Figura 1. Relación entre tasa relativa de inicio de bulbificación y temperatura de almacenaje (A), y días frío acumulados (CCD) (B). Cuadrados corresponden al clon Rosado INIA y los círculos al clon Akukeli. Para el cálculo de CCD, Tc fue 16°C y 18°C en los clones Akukeli y Rosado INIA, respectivamente.

Table 2. Percentage of plants reaching bulb initiation in two garlic clones as influenced by temperature treatment previous to planting and by photoperiod during the growth period. In parentheses are days from emergence to bulb initiation.

Cuadro 2. Porcentaje de plantas que lograron el inicio de bulbificación en dos clones de ajo, en respuesta a tratamientos de temperatura previo a la plantación y al fotoperíodo durante el período de crecimiento. Entre paréntesis están los días desde emergencia a inicio de bulbificación.

Photoperiod (h)	Clone Akukeli			Clone Rosado - INIA		
	Storage temperature (º C)					
	4°C	10°C	> 13°C	4°C	10°C	> 13°C
8	n.b.[1]	n.b.	n.b.	n.b.	n.b.	n.b.
10	n.b.	n.b.	n.b.	n.b.	n.b.	n.b.
12	n.b.	n.b.	n.b.	n.b.	n.b.	n.b.
14	100 (67)	n.b.	n.b.	100 (58)	n.b.	n.b.
16	100 (54)	40 (96)	n.b.	100 (64)	80 (95)	60 (90)

[1] n.b.: no bulbing.

only occurred when bulbs received cold temperature (4ºC) during storage. Clone Akukeli did not initiate bulbing at any photoperiod when stored at room temperature, which was never lower than 13°C. However, in clone Rosado-INIA 60% of the plants produced bulbs when the storage was at room temperature and the photoperiod was 16 h (Table 2), indicating that this clone had lower requirements for cold temperature to induce bulbing.

According to Kamenetsky *et al.* (2004), long photoperiod is both obligatory and quantitative for the vegetative and reproductive processes in garlic, respectively. They found that the induction of dormancy in lateral cloves required over two weeks exposure to long photoperiod, whereas a regime of cold conditions and short photoperiod resulted in sprouting and growth of the axillary buds, and lack of dormancy.

Effect of planting date on development

As planting date was delayed from April to September the number of days from emergence to bulb initiation decreased for all clones (Table 3). A considerable variation between clones was observed on time to bulb initiation for all planting dates, particularly when photothermal conditions were least inductive (Table 3). Clone Rosado-INIA was on average the most precocious, while clone Rosé de Lautrec was the latest one.

Depending on planting date, mean temperature and mean photoperiod for the period from emergence to bulb initiation, ranged from 9.8 to 14.1°C, and from 11.3 to 13.7 h, respectively (Table 3). However, most of the clones planted on very different dates reached the state of bulb initiation around mid November, when photoperiod was about 14 h (Table 3). Clones Rosado Chileno and Rosado-INIA appear to have

Table 3. Phenological plasticity of 13 Chilean clones of garlic: number of days from emergence to bulb initiation (E-BI), under the most- and least-inductive photothermal (mean temperature for E-BI period, T, and mean photoperiod for E-BI period, P) conditions. The range of photoperiods (P) at the time of bulb initiation for the different planting dates is also shown.

Cuadro 3. Plasticidad fenológica de 13 clones de ajo chilenos: número de días desde emergencia a inicio de bulbificación (E-BI), bajo las condiciones fototermales (temperatura media para el período E-BI, T, y fotoperíodo medio para el período E-BI, P) más y menos inductivas. Se muestra también el rango de fotoperíodos (P) al momento de inicio de la bulbificación para las diferentes fechas de plantación.

Clone	Most-inductive conditions Sowing date 15/09/93			Least-inductive conditions Sowing date 15/04/93			Range of P (h)
	E-BI (days)	T (°C)	P (h)	E-BI (days)	T (°C)	P (h)	
Rosé de Lautrec	67	14.0	13.7	199	10.4	11.6	14.0-14.3
Morado Ruso	69	14.0	13.7	193	10.3	11.5	14.0-14.3
California	45	14.0	13.7	184	10.3	11.5	14.1-14.3
Uruguay LB-13	57	13.6	13.7	190	10.3	11.6	13.9-14.0
Akukeli	65	14.1	13.7	187	10.3	11.6	14.0-14.3
Cañete	66	14.1	13.6	184	10.4	11.9	14.1-14.3
Fukuchi White	67	14.0	13.7	184	10.1	11.4	13.7-14.2
Car	45	14.0	13.7	184	10.3	11.5	14.0-14.1
Quepe	52	13.6	13.7	179	10.2	11.5	13.8-14.2
Camiña Vallenar	51	13.5	13.6	178	10.3	11.7	14.0-14.1
Rosado Nortino	52	13.5	13.6	176	10.2	11.5	13.8-14.0
Rosado Chileno	52	13.5	13.6	162	9.8	11.3	13.2-13.8
Rosado-INIA	55	13.6	13.7	156	10.0	11.5	13.5-14.1

slightly lower requirements of photoperiod for bulb initiation. No clear relationship was found between days from bulb initiation to maturity and planting date (data not shown), fluctuating from 27 days for clone Car planted on April 15 and May 14 to 68 days for clone Rosado Chileno planted on September 15 .

The results indicated that Chilean clones of garlic have an obligate response to photoperiod for bulb initiation, with a ‘ceiling photoperiod’ around 14 h in most clones. When photoperiod is lower than this ‘ceiling photoperiod’ bulbing does not occur, whereas when it is greater, days to bulbing decrease.

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