Effect of Photoperiod, Temperature and True-leaf Stage in Bolting Rate of Chicory (*Cichorium intybus* L. var. *sativus*)

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ABSTRACT

Root chicory (*Cichorium intybus* L. var. *sativus*) is potential alternative medicinal and sugar crop which accumulates a high amount of linear polyfructan, inulin in its roots. A problem in root production is that over-wintered stock plants often flower. Once the plant becomes reproductive, stem elongation and root growth slows and floral buds arise from every node, rendering the plants useless for propagation. The objectives of this research was to examine the effectiveness of manipulating environmental factors containing photoperiod, temperature and number of leaf states.

The experiment was performed in growth chamber to create two photoperiods (8 h, and 16 h) with three temperature regimes (5℃/3℃, 10℃/8℃ and 15℃/13℃ day/night temperature) for a total of six treatments on three type of true-leaf stage of plant. Data of bolting rate, shoot and root length, shoot and fresh weight was invetigated in each treatments. This is the first report on changes in bolting rate and shoots and roots production during a whole growing season and differences in the effect of cold and photoperiod treatment depending on the true-leaf stage of plant.

Key words **:** Bolting, Leaf stage, Photoperiods, Root chicory, Temperatures

INTRODUCTION

Chicory varietie, *Cichorium intybus* L. var. *Sativus* is alternative sugar crops which accumulate linear fructose pripolymers (fructans) and inulin in their roots. The fructans, and the fructose resulting from fructan hydrolysis, can be used in the food industry (e.g., as dietary fibers and low calorie sweeteners), and in addition also have several non-food medical and industrial applications (Fuchs, 1993). Chicory is a perennial plant native to Europe and is bred and cultivated for its roots that are still used as a coffee substitute. Also, the medicinal properties of chicory can be found in its leaves and large taproot, which act as a tonic, diuretic and laxative. Like burdock and dandelion (related Compositae plants), chicory root stimulates the biliary tract, and thereby improves digestion. Traditionally, chicory root has been given for liver

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health maintenance, skin eruptions, rheumatism, gallstones, constipation, malaria, and fever. In Oriental medicine, chicory is believed to relieve headache, inflammation, sore throat, and skin allergies. Chicory leaves can be used externally as a poultice and applied to swellings, inflammations and sore eyes. The young leaves can be edible and are most often used in salads much like the garden endive. Since the sowing and harvesting technique of sugarbeet and potato can be used in root chicory production, no investment into new agronomic technique is necessary, but controlling of flower induction is very important for efficient production of root (yield and quality) in Chicory.

Chicory is a cold requiring (thermoinduction), obligatory long day plant for flower induction (Hartman, 1956; Rappaport and Wittwer, 1956; Doorenbos and Riemens, 1959; Harrington *et al.*, 1959). Thermoinduction of flowering is an epigenetic process, restricted to a single sexual generation (Burn *et al.*, 1993). Each generation requires vernalization, i.e. the response to cold is not transmitted to the progeny, suggesting that the program is reset during gamete formation (Lang, 1965). However, chicory is very sensitive to spring vernalization and early sown fields are thus more or less prone to bolting on the first year, depending on the environmental conditions. A period of low temperature is an essential part of the life cycle of many cold required species. In chicory flower development is induced at higher temperatures (20-25 ℃), but subsequent elongation of flower stalk and proper flower is dependent on an extended period of low temperature (<10℃) (Paulet, 1985). This dependence of flowering on low temperature is a well-known phenomenon in this cold requiring plant species such as calnola (Brassica napus cv. Crystal; Zanewich and Rood, 1995), winter wheat (*Triticum aestivum* L.; Reda *et al.*, 1978), *Thalaspi arvense* L (Hazebroek *et al.*, 1993) radish (*Rapanus sativus* L.; Michniewicz *et al.*, 1981; Suge, 1970) and also chicory (*Cichorium intybus* L; Joseph *et al.*, 1983)

Flowering plants may be categorized as long day (LD), short day (SD) or day neutral (DN) according to their response to photoperiod. SD plants flower when the period of darkness exceeds a critical length, whereas LD plants flower when the dark period does not exceed a critical length. DN plants flower in response to a stimulus other than photoperiod, such as temperature or total light energy, independent of the amount of day light (Dole and Wilkins, 1999). Temperature plays an important role in the growth and development of crops. Higher temperatures tend to encourage rapid growth, while cooler temperatures may be used to slow growth. Manipulation of temperature is often used in the flori- and rhizo-culture industry to control growth and flowering response of many plant species. Photoperiod and temperature, two factors which individually play roles in bud initiation on some species, can interact with each other to affect a plant's flowering ability.

A problem in root production is that over-wintered stock plants often flower. Once the plant becomes reproductive, stem elongation and root growth slows and floral buds arise from every node, rendering the plants useless for propagation. The objectives of this research were to examine the effectiveness of manipulating environmental factors containing photoperiod, temperature and number of leaf states.

MATERIALS AND METHODS

Cichorium intybus L. (colleted origin of In-Jae) were sowed in 5.7 cm pots using a soilless potting mix consisting of peat moss, perlite and vermiculite. Plants were grown on for 5 days using clear water irrigation. A string of six 100 watt incandescent light bulbs was used to extend the photoperiod to 16h. 5 days after transplant (DAT), 60 uniform plants were selected and transported to pots (26.5° × 35.5) and plants were placed in growth chambers. The experimental plants

were seperated in three stand age classes (state of three, six, nine leaves) by development stage. The chambers had 16 fluorescent tubes and 6 incandescent bulbs for a total photosynthetic photon flux density of 411μmol · $m^{-2}s^{-1}$. Temperatures may vary from the set point ± 0.5 ℃ Treatments consisted of three photoperiods, 8 and 16 h, with three temperature regimes, 5℃/3℃, 10℃/8℃ and 15℃/13℃ day/night temperature, with 10 replications of each. Day and night temperature corresponded with the photoperiod: for example, under 8 h warm treatment, plants received 8 h at 15℃ and 16 h at 13℃. Data collected included bolting rate, shoot and root length, shoot and fresh weight being measured upon termination of the experiment, 9 weeks after initiation of treatment. Data were analyzed using t-tests (LSD), GLM and correlations in SAS Version 8 (SAS Institute, Inc., Cary, N.C.).

RESULTS AND DISCUSSIONS

Seed genrmination of chicory

Sprouting rate of chicory appeared from 75% to 96%, and could germinated more than 70% within 2 or 3 days at 10-30℃ by non-dependent light (Table 1). Time to sprouting was longer but the sprouting rate not appeared difference greatly at 10℃ when compared with at 20℃. Dhellemmes (1987) reported that chicory has a relatively high minimum temperature for germination of 10℃. Later, Gianquinto and Pimpini (1989) reported that chicory emerged most rapidly at temperatures between 20℃ and 26℃. In addition, root chicory exhibited a slow leaf growth at low temperature (Meijer and Mathijssen, 1992).

The vegetative growth of a chicory plant can be divided into two phases. In the first part, leaf biomass starts to develop from 45 days after sowing. The number of leaves reaches a maximum around 120 days after sowing. Afterwards the leaf number decreases because of senescence of the oldest leaves. In the second part, especially from 2 to 5 months after sowing, an increase in fresh root weight occurs. The maximum root yield in biomass is achieved around 160 days after sowing .

Bolting rate of chicory

Bolting rate was little correlated with photoperiod treatment, however, there was a positive correlation between temperature and bolting rate (Fig. 1.). Plants under each treatment were flowered, with the higher visible buds on the daylength of 16 h treatment at 10℃ compared with other treatment. The bolting rate was highest in three true-leaf stage on the daylength of 16 h treatment at 10℃ by seventy-two percent. The bolting rate was decreased according to the development of true-leaf stage in bud with 70.2% at 3 true-leaf stage, 53.3% at the 6 true-leaf stage and 50.0% at 9 true-leaf stage. Several crops can only perceive photoperiods after they have reached maturity (Dole and Wilkins, 1999). Time to flower was also delayed at 15℃ when the photoperiod was shortened by 8 hours. In the chrysanthemum species, as reported previously (Cathey, 1957), we found that cooler temperatures extended the

Table 1. Germination characteristics of seeds in *Cichorium intybus* L.

Treatment	Temperature(℃)	% of germination after seeding			
		Total	1day	2days	3days
Dark	10	73.7	0	51.7	20.0
	20	77.4	0	70.7	5.0
	30	80.0	0	75.3	4.0
Light	30	79.7	0	75.5	4.0

critical photoperiod, while warmer temperatures shortened the photoperiod required for optimum flower development.

Critical photoperiod can also be affected by varying the temperature. *Xanthium pennsylvanicum* has a critical photoperiod of 8.5 hours at 21℃ versus a critical photoperiod of 11 hours at 4℃ (Long, 1939). The studies with *Primula obconica* Hance (Karlsson and Werner, 2002) also found interactions with time to flower, photoperiod and temperature. Flowering was delayed by 11 days when the long day photoperiod was kept consistent (16 hours) but the temperature was decreased from 20℃ to 16℃. Similarly, the time to flower was delayed by 11 days when the photoperiod was reduced to short days, or 8 hours, and the temperature held consistent at 16℃.

The erratic bolting was consistent throughout all the treatments. This indicates that a another factor as temperature than photoperiod triggers the reproductive mechanism in *cichorium intybus* L. Armitage (1989) found photoperiod induced flowering in *Trachelium caeruleum* Linn., a plant produced did not gain 100% bud initiation under the critical photoperiod. Armitage postulated another factor contributed to floral initiation, and noted that 100% flower initiation had been achieved with injections of 1000 mg · L^{-1} CO_2 (Geertsen and Bredmose, 1987).

Production of shoot in chicory

Photoperiod had no statistically significant effect on shoot height, but longer photoperiods (16h) produced plants with higher shoot fresh weight in 3 and 6 true-leaf stage (Fig. 2, 3). Means for shoot height were not statistically different between the each temperature treatment. However, there was a significant difference in the mean shoot height of the longer photoperiods (16h) treatment on 3 true-leaf stage. Shoot height was affected by photoperiods than temperature in each true-leaf stage of chicory (Fig. 2).

Both photoperiod and temperature had a no significant effect on shoot fresh weight in 9 true-leaf stage at relatively higher temperature, but the shoot fresh weight depends on photoperiod and temperature in lower ture-leaf stage. In 3 true-leaf stage, the shoot fresh weight appeared higher at 5℃ than 10℃, in contrary higher shoot fresh weight appeared at 10℃ than 5℃ in 6 true-leaf stage (Fig. 3). Plants produced under treatments of cooler temperatures showed a deeper green leaf than those under treatments of warm temperatures, regardless of photoperiod.

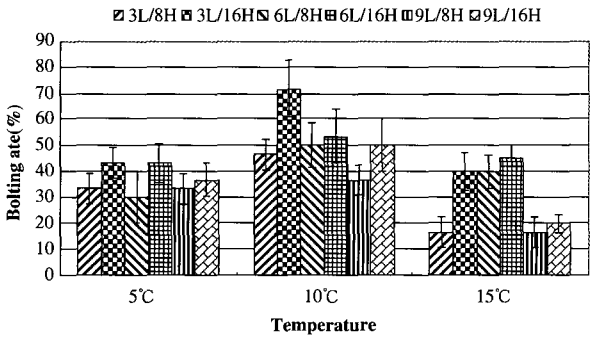

Fig. 1. Effect of photoperiod, temperature and true-leaf stages on the bolting rate in *Cichorium intybus* L. (3L; 3 true leaf stage; 6L: 6 true leaf stage; 9L: 9 true leaf stage; 8H: 8 hour of daylength; 16H: 16 hour of daylength).

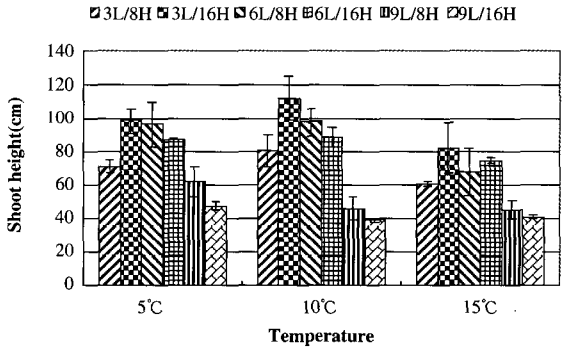

Fig. 2. Effect of photoperiods, temperature and true-leaf stages on the shoot length in *Cichorium intybus* L.(3L; 3 true leaf stage; 6L: 6 true leaf stage; 9L: 9 true leaf stage; 8H: 8 hour of daylength; 16H: 16 hour of daylength).

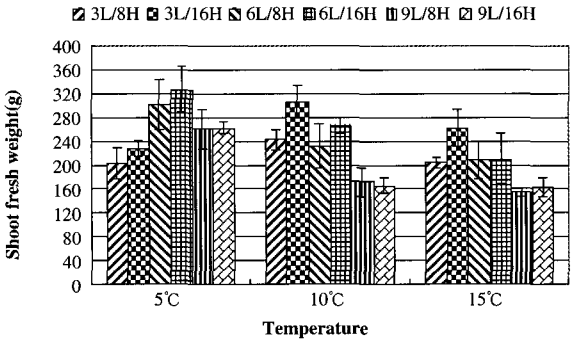

Fig. 3. Effect of photoperiods, temperature and true-leaf stages on the shoot fresh weight in *Cichorium intybus* L. (3L; 3 true leaf stage; 6L: 6 true leaf stage; 9L: 9 true leaf stage; 8H: 8 hour of daylength; 16H: 16 hour of daylength).

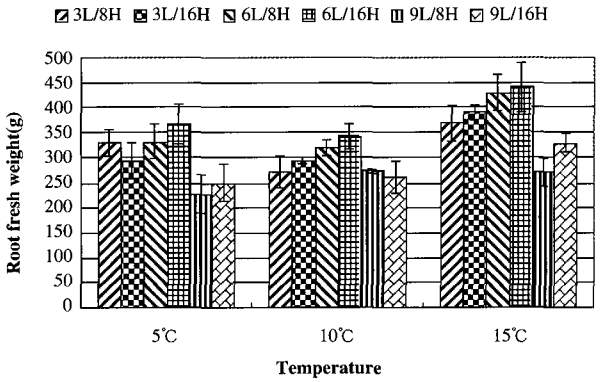

Fig. 5. Effect of photoperiods, temperature and true-leaf stages on the root fresh weight in *Cichorium intybus* L. (3L; 3 true leaf stage; 6L: 6 true leaf stage; 9L: 9 true leaf stage; 8H: 8 hour of daylength; 16H: 16 hour of daylength).

Production of root in chicory

There was no correlation between the root length and bolting rate, but there was a negaitive correlation between root fresh weight and bolting rate (Fig. 4, 5). Means for root length were not statistically different between the all experiments. Photoperiod had a no significant effect on root fresh weight in all true-leaf stage and the root fresh weight showed lowest at 10℃ that bolting rate was the highest among each temperatures. The root fresh weight was highest in 6 true-leaf stage at 15℃ by mean of 425g.

Growing shoots and bolting of plants under cooler temperatures (10℃) resulted in lower root fresh weight than the relatively warmer treatment (15℃). The growing of root on 9 true leaf stage was significantly smaller than those produced in 3 and 6 true leaf stage, regardless of photorperiods and temperatures.

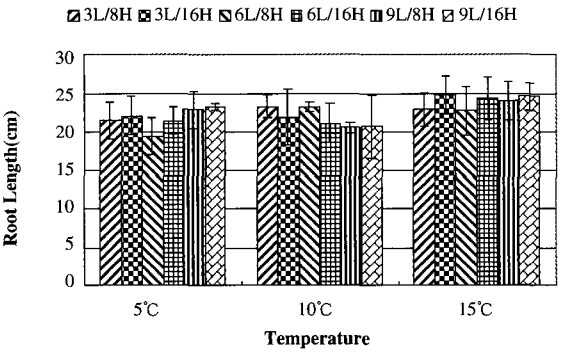

Fig. 4. Effect of photoperiods, temperature and true-leaf stages on the root length in *Cichorium intybus* L. (3L; 3 true leaf stage; 6L: 6 true leaf stage; 9L: 9 true leaf stage; 8H: 8 hour of daylength; 16H: 16 hour of daylength).

The difference in bolting rate and shoots and roots production response to the cold treatment depending on the number of true-leaf stage has not been studied in other plant species. However, it seems to be an important phenomenon in chicory. The observation that the bolting rate and shoots and roots production response to the treatments of cold and photoperiods varies depending on true-leaf stage of plant hints to the presence of a development or other regulated program. Induction of bud initiation was contained continuous two phase in chicory, first plant obtained sensibility of long daylight, following bud induction by long daylight condition (Gianquinto, 1997). With this result separately, we founded that the sensitivity of chicory with respect to bolting and flowering changes during the growing season. In order to be induced to flower, chicory must undergo a low temperature treatment (especially 10℃) which can be applied to germinating seeds or to the entire plant at the end of the first

growing season. Also, the bud formation and bolting was not desireable in root chicory, because production of root was decreased by flowering greatly. The bolting and flowering were more induced strongly when seeds was sowed at early spring because bolting helped by long daylight and exposure of low temperature. And, low temperature was quantitative physiology factor in plant and long periods of low temperature was more effective for flowering. Undesirable bolting and flowering by low temperature could be inhibited by treatments of higher temperature (15-18℃) relatively, in order to stabilize effect of low temperature treatments (deveranlization). As figure 1 and 5, bolting was an adverse effect on production of roots. This result suggested that bolting rate inhibited by treatments of higher temperature was prevented loss of nutritive substance in the soil for root gowing.

Flowering is controlled by not only change of environmental condition and developmental regulation but also cultivation of bolting resistance cultivar containing gene transformed plants in chicory. Although mechanisms for the epigenetic control of gene expression were not well understood, there was increasing evidence that flowering pathways and that GA (gibberellins) metabolism was required for the promotion of flowering by vernalization and flowering in noninductive photoperiods. Removal of the flower bud and leaves, both major auxin sources, before the rapid elongation of the floral stalk reduces floral stalk elongation considerably, whereas application of indole-3-acetic acid (IAA) reverses this effect (Okubo and Uemoto, 1985; Saniewski, 1989). The auxin induced floral stalk elongation was inhibited by application of paclobutrazol, a GA biosynthesis inhibitor (Saniewski, 1989), suggesting that GA biosynthesis is also necessary for auxin-induced floral stalk elongation. Therefore, study of interaction between floral stalk elongation and GA metabolism is a prerequisite for flowering control in chicory. We remain hopeful that investigations of possible analogues with cold requiring process such as vernalization, and the interaction of auxin and GA in stalk elongation process in chicory.

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