Floral Development and Bolting of Spinach as Affected by Photoperiod and Integrated Photosynthetic Photon Flux During Transplant Production

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Abstract. We recently showed that spinach (*Spinacia oleracea* L.) transplants produced under a short photoperiod and low air temperature were characterized by a delay of bolting and short flower-stalk length at harvest (Chun et al., 2000a). The present study was conducted to determine whether these changes are caused by the short photoperiod itself or by the lower integrated photosynthetic photon flux (*IPPF*). Shoot and root dry weights of transplants increased significantly with increasing *IPPF*, but were not affected by a change in the photoperiod. However, the floral development indices of transplants were significantly greater under a 16- than under a 10- or 13-hours/day photoperiod, but were not affected by a change in *IPPF*. The percentage of bolted plants 3 days after transplanting (DAT) increased significantly with increasing photoperiod (from 0% at 10 hours/day to more than 85% at 16 hours/day). Flower-stalk length increased with increasing photoperiod (e.g., at 14 DAT, from 15 mm at the shorter photoperiods to 80 mm at 16 hours/day), but was not affected by a change in *IPPF*. These results show that the delay of bolting that occurs when the photoperiod is reduced during transplant production is due to the delay of floral development and not to retarded vegetative growth as a result of reduced *IPPF*.

A long photoperiod causes spinach plants to bolt and flower (Garner and Allard, 1920; Hartmann et al., 1988; Kim et al., 2000), and bolted plants lose their market value (Kagawa, 1997). We recently proposed a method for producing spinach transplants in a closed system (Chun et al., 2000a). In this method, the photoperiod is shortened and air temperature is lowered, resulting in short flower stalks at harvest, even when the transplants are subsequently cultured under long-day and high air temperature conditions.

Increasing the photoperiod but maintaining a constant photosynthetic photon flux (*PPF*) resulted in increased vegetative growth of transplants, and promoted floral development and elongation of flower stalks (Chun et al., 2000a; Kim et al. 2000). However, it was not clear from these studies whether the inhibition of bolting and flower-stalk elongation that occurred with a shorter photoperiod was

due to delayed floral development or retarded vegetative growth as a result of the decreased integrated photosynthetic photon flux (*IPPF*). The *IPPF* (mol·m^{-2}) is the product of photoperiod (h/d), *PPF* (mol·m^{-2}·s^{-1}), and production period (d). Vegetative growth (e.g., the increase in dry weight) depends on the *IPPF* (Kitaya et al., 1998), and vegetative growth affects the floral development of quantitative long/short-day plants (Thomas and Vince-Prue, 1997). However, few studies have examined the effects of *IPPF* on floral development of qualitative long/short-day plants, including spinach.

To clarify the effects of the photoperiod during transplant production on bolting of spinach, we investigated floral development in transplants grown under different photoperiods but the same *IPPF*, and under the same photoperiod but different *IPPFs*. In addition, bolting in these plants during subsequent culture was also investigated.

Materials and Methods

Transplant production. In the present study, *S. oleracea* L., cv. Dimple (Sakata Seed Co., Yokohama, Japan), was used. This cultivar is a semi-savoy type and its critical daylength for bolting initiation is between 13 and 15 h/d (Chun et al, 2000b). Five seeds were sown in each cell (20 mm in diameter) of transplant production trays (48 cells per tray, 300 × 200 × 40 (H) mm; Taiyo Kogyo Co., Tokyo) that had been filled with 3 g (dry weight) of granules of rock-wool (Nichias Co., Tokyo). The

trays with seeds were wrapped with opaque plastic sheets and placed in a walk-in growth chamber for 3 d for germination. During this period, the air temperature was maintained at 20 ± 1 °C and the relative humidity at 70% ± 5%.

The trays with germinated seeds were moved to three growth chambers (Koitotron 3HN-35MLA; Koito Industries, Yokohama, Japan) and cultured for 11 d. Each chamber [100 × 100 × 150 (H) cm] had a microwave-powered lamp as described by Kozai et al. (1995). The lamp (Solar 1000™ Lighting System SAB; Fusion Lighting, Rockville, Md.) was used as the sole source of light. The lamp was essentially a point lighting source, so *PPF* varied with distance from the lamp. To achieve a uniform *PPF* at the surface of the tray, a screen frame was placed 50 cm from the lamp and circular shading material made of white plastic net (mesh size 0.8 mm, GN-2300; Nihon Wide Cloth Co., Osaka, Japan) was placed near the center of the screen. The photo-/dark periods in the three chambers were set at 10/14, 13/11, and 16/8 h/d, respectively. In each chamber, the *PPF* at the surface of the trays was set at two different values (Table 1) by placing additional semicircular shading materials on one half of the screen. The number of pieces of shading material was chosen so that, although there were six different treatments, there were only three different *IPPFs* (≈80, 125, and 200 mol·m^{-2}; Table 1).

During the transplant production period, the air temperature and relative humidity (RH) in each chamber were maintained at 20 ± 1 °C and 70% ± 5% RH. The CO_2 concentration during the photo- and dark periods was maintained at 700 and 350 μmol·mol^{-1}, respectively. Trays were irrigated once a day with diluted commercial nutrient solution (High Tempo; Sumitomo Chemical Co., Tokyo) containing (mmol·L^{-1}): 12.0 NO$_3$–N, 0.8 NH$_4$–N, 30.6 P, 7.3 K, 10.2 Ca, and 4.2 Mg. The pH and electrical conductivity (EC) of the nutrient solution were 6.0 and 140 mS·m^{-1}, respectively. Shoot and root dry weights of 16 transplants from each replication were measured 14 d after sowing (DAS).

At 14 DAS, meristems of transplants were viewed and photographed using a digital microscope (VH-6300; Keyence Co., Osaka, Japan). The floral development of an apical meristem can be divided into four stages, according to Eguchi and Ichikawa (1940) and Kim et al. (2000). When a transplant had a flat shoot apex with only subtending leaf primordia, it was characterized as being in the vegetative stage (no floral differentiation) and was given a score of 0. When the apex formed a hemispherically shaped dome, the transplant was classified as being in the flower cluster initiation stage and was given a score of 1. If the diameter of the dome was significantly larger than that at the flower cluster initiation stage, it was classified as being in the flower cluster differentiation stage and was given a score of 2. And when many flower clusters and bracts had formed, the apex was classified as being in the flower cluster formation stage and was given a score of 3. Five transplants from

Table 1. Photoperiod, *PPF*, and *IPPF* of each treatment during an 11-d transplant production.

Chamber no.	Photoperiod (h/d)	PPF^z ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	$IPPF^y$ ($mol \cdot m^{-2}$)	Treatment code
1	10	200 ± 5	81.2	P10, I80
		320 ± 12	126.7	P10, I125
2	13	155 ± 10	80.0	P13, I80
		390 ± 40	200.8	P13, I200
3	16	200 ± 6	120.4	P16, I125
		320 ± 26	202.7	P16, I200

^{z}Photosynthetic photon flux on the empty shelf.

$^y IPPF = PPF \times$ photoperiod $\times$ 11 (number of days for transplant production).

each treatment were destructively sampled to determine the stage of floral development.

Culture after transplanting. At 14 DAS, the plants were transplanted to four modified nutrient film technique (NFT) (Cooper, 1973), hydroponic systems installed in a walk-in growth chamber and were grown for 14 more days. Cool-white fluorescent lamps (FPL55EX-N; Matsushita Electronics Co., Osaka, Japan) were used as an artificial light source and the average *PPF* on the planting panels (26 cm below the lamps) was $320 \pm 20 \ \mu mol \cdot m^{-2} \cdot s^{-1}$. The photo- and dark periods in the NFT systems were 16 and 8 h/d, respectively. The plants were placed on planting panels (Taiyo Kogyo Co., Tokyo) at a density of 63 cells/m². In the NFT system, the diluted commercial nutrient solution (High Tempo; Sumitomo Chemical Co., Tokyo) was continuously circulated between the nutrient tank and the culture beds. The EC of the solution was $280 \ mS \cdot m^{-1}$. The average air temperatures during the photo- and dark periods were 33 and 31 °C, respectively, and the average solution temperature was 22 °C.

For 20 plants from each replication, the percentage of bolted plants was observed throughout the culturing period [0 to 14 d after transplanting (DAT)] and flower-stalk length was measured 5, 10, and 14 DAT. At harvest (14 DAT), shoot and root dry weights and leaf number of 14 plants sampled from each replication were measured.

A completely randomized design was used with two replications. A tray for transplant production represented a replication and an analysis of variance (ANOVA) test and Duncan's multiple range test at $P \leq 0.05$ were used to compare means in each treatment.

Results

At the end of the transplant-production period (14 DAS), shoot and root dry weights of the transplants had increased significantly with increasing *IPPF* (Fig. 1), but prolonging the photoperiod did not increase dry weight within *IPPF* treatments. The floral development indices of transplants grown under a 16-h photoperiod were significantly greater than those under the 10- or the 13-h photoperiod, while no difference was found among the 10- and the 13-h photoperiod treatments (Fig. 1). Furthermore, transplants grown under the same photoperiod showed the same floral development index regardless of the *IPPF*.

Of the plants grown under a 16-h photoperiod, >85% had bolted 3 DAT, while 15% to

40% of the plants in the 13-h photoperiod treatments and none of the plants in the 10-h photoperiod treatments had bolted at this time (Fig. 2A). The percentage of bolted plants in the 16-h photoperiod treatments reached 100% 7 DAT. However, the percentage of bolted plants 14 DAT did not differ between the 13- and the 16-h photoperiod treatments. Under 10-h photoperiods, the percentage of plants that bolted after 11 DAT was greater at the higher *IPPF*. The flower-stalk lengths of plants 5 DAT in the 16-h photoperiod treatments were ≈10 mm, while those in the other treatments were 0 mm (Fig. 2B); at harvest, they were ≈80 mm under the 16-h photoperiods, but only ≈15 mm in the other treatments. The *IPPF* did not affect flower-stalk length within photoperiods. There were no significant differences in shoot and root dry weights among treatments at harvest (data not shown), but leaf number was greater in the 16-h photoperiod treatments (Fig. 3).

Discussion

Growth and floral development at transplanting. Several studies have shown a rela-

tion between *PPF* or *IPPF* and plant growth. Ikeda et al. (1988) and Knight and Mitchell (1988) reported that increasing *PPF* promoted vegetative growth of lettuce (*Lactuca sativa* L.). Since only one photoperiod was used in these studies, it can be concluded that vegetative growth is also dependent on *PPF*. Kitaya et al. (1998) demonstrated that the dry weight of lettuce increased with increasing daily *IPPF*. In that study, daily *IPPF* values ranging from 5.8 to 25.9 $mol \cdot m^{-2} \cdot d^{-1}$ were achieved by using different combinations of photoperiods (from 16 to 24 h/d) and *PPFs* (from 100 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Chun et al. (2000b) reported that increasing the photoperiod from 11 to 15 h/d with *PPF* of 310 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during transplant production promoted bolting and flower-stalk elongation of 'Dimple' spinach plants that were subsequently cultured under long-day conditions.

As shown in Fig. 1, vegetative growth of spinach transplants (on a dry weight basis) was dependent on the *IPPF* rather than on the photoperiod itself. These transplants, which were grown under different photoperiods but which exhibited the same vegetative growth, were used for examining the effects of the photoperiod and *IPPF* during transplant production on the bolting characteristics. By using such plants, the effects of *IPPF* on vegetative growth could be avoided.

Spinach has been classified as an absolute or a qualitative long-day plant (Boswell, 1935; Garner and Allard, 1920; Kagawa, 1997; Metzger and Zeevaart, 1980; Salisbury and Ross, 1978; Vince-Prue, 1975). However, Eguchi and Ichikawa (1940) found that spinach differentiated reproductive structures between 15 and 30 DAS under natural conditions throughout the year, and Kim et al. (2000) reported that the differentiation occurred ear-
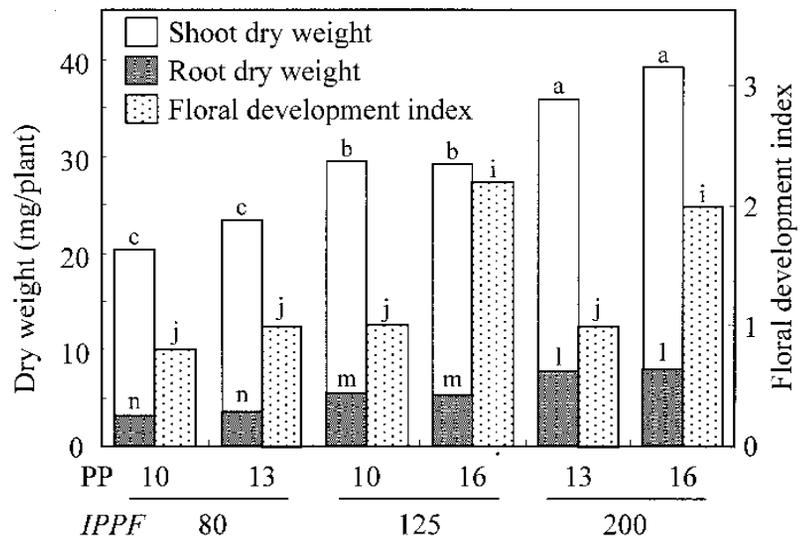

Fig. 1. Shoot and root dry weights and floral development indices of 'Dimple' spinach transplants 14 d after sowing as affected by photoperiod (PP) and integrated *PPF* (*IPPF*) ($mol \cdot m^{-2}$) over an 11-d transplant production period. The floral development of a terminal apical meristem was divided into four stages according to Kim et al. (2000) and scored from 0 to 3, where 0 = no floral differentiation, 1 = flower cluster initiation stage, 2 = flower cluster differentiation stage, and 3 = flower cluster formation stage. Mean separation among fresh (a, b, c) and dry weights (l, m, n), floral development index (i, j) values by Duncan's multiple range test, $P \leq 0.05$.
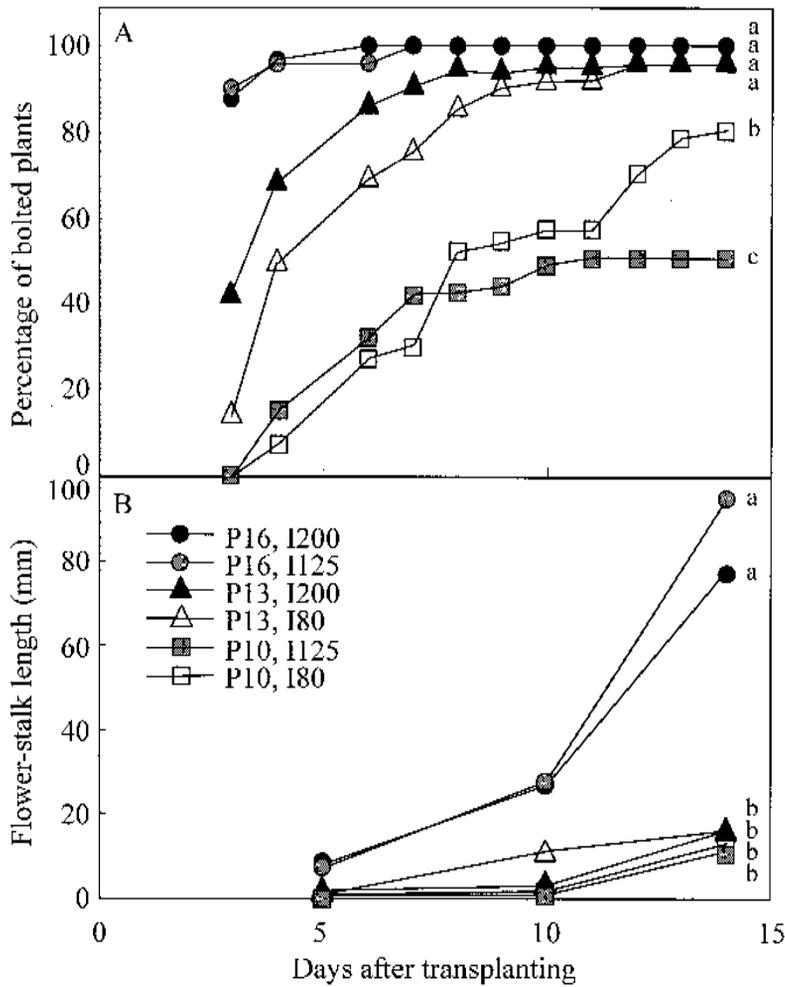
Fig. 2. (**A**) Time course of bolting percentage and (**B**) flower-stalk elongation of 'Dimple' spinach subsequently cultured under a 16-h photoperiod, as affected by photoperiod (PP) and integrated *PPF* (*IPPF*) over an 11-d transplant production period. Mean separation at 14 DAT by Duncan's multiple range test, $P \leq 0.05$. Treatment codes: P = photoperiod (h); I = *IPPF* (mol·m^{-2}).
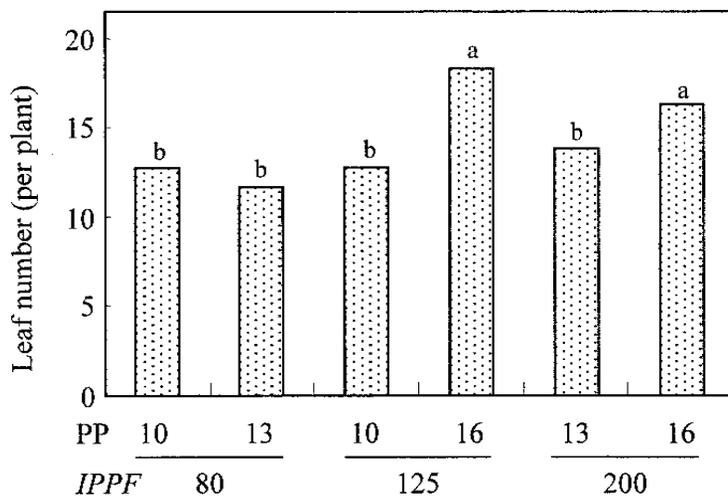

Fig. 3. Leaf number of 'Dimple' spinach subsequently cultured for 14 d under a 16-h photoperiod as affected by photoperiod (PP) and *IPPF* over an 11-d transplant production period. Mean separation by Duncan's multiple range test, $P \leq 0.05$.

lier than 17 DAS under artificial lighting. In agreement with Eguchi and Ichikawa (1940) and Kim et al. (2000), we found that the transformation from the vegetative to the reproductive state occurred in all photoperiods (Fig. 1). However, our results on the timing of development of reproductive structures could not be directly compared with those of Eguchi and Ichikawa (1940) and Kim et al. (2000) because of differences in lighting sources and other environmental factors. We found that floral development progressed further under 16-h photoperiods than in the other treatments, and that the floral development indices were not affected by *IPPF* when photoperiod was constant (Fig. 1). These findings suggest that the development of differentiated reproductive structures was not dependent on vegetative growth, but on the photoperiod, although floral initiation itself occurred regardless of the photoperiod and *IPPF*.

Growth and bolting at harvest. The photoperiod during transplant production had a significant effect on the percentage of bolted plants (Fig. 2A) and on flower-stalk length (Fig. 2B) of 'Dimple' spinach subsequently grown under a 16-h photoperiod. Chun et al. (2000b) reported that the critical day length for bolting of 'Dimple' spinach was longer than 13 h and shorter than 15 h. The percentage of bolted plants 3 DAT was significantly higher under 16-h photoperiods than in the other treatments. This raises the possibility that the bolting of the plants in the 16-h photoperiod treatments was initiated during the 11-d transplant-production period. However, for the plants in the 10- and the 13-h photoperiod treatments, bolting might have been initiated after these plants were transplanted and grown under a 16-h photoperiod. Even though no difference was found in the flower development indices between the 10- and the 13-h photoperiods (Fig. 1), the timing of development of reproductive structures in the 13-h photoperiods (earlier than 3 DAT) differed from that in the 10-h photoperiod (between 3 and 4 DAT). Kim et al. (2000) reported that flower-bud development of 'Dimple' spinach was accelerated by increasing the photoperiod from 8 to 16 h. However, more precise anatomical observations of the reproductive structures and more precise measurement of the percentage of bolted plants at transplanting (0 DAT) are necessary to clarify this matter.

The photoperiod during transplant production had distinct effects on the flower-stalk length (Fig. 2B) and on leaf number (Fig. 3B) at harvest, but no effect on dry weight. (Fig. 3A). However, the *IPPF* had no clear effect on bolting percentage, flower-stalk length, or leaf number. Based on this effect of photoperiod on bolting of spinach, Chun et al. (2000a) proposed that spinach transplants with delayed bolting could be produced by growing them under a short photoperiod using artificial lighting.

These present findings indicate that a longer photoperiod during transplant production enhances floral development and increases percentage bolting and flower-stalk length, but that the *IPPF* during transplant production

does not affect flowering characteristics. In this study, we were unable to determine why the percentage of bolted plants increased more rapidly after 11 DAT when *IPPF* was raised from 125 to 180 mol·m^{-2} on a 10-h photoperiod (Fig. 2A).

The present results indicate that the delay of bolting that occurs with shortening the photoperiod during transplant production is due to delayed floral development and not to retardation of vegetative growth as a result of the decreased *IPPF*.

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