Flower Induction in June-bearing Strawberry by Intermittent Low Temperature Storage

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In this paper, we describe a new artificial flower-inducing treatment, intermittent low temperature storage, which we suggest could be a low-cost and useful technology for the forcing of June-bearing strawberries. Tray-grown ‘Nyoho’ plants were placed in a refrigerator (13°C, in the dark) for 4 days and then transferred to an outdoor shelter with 50% shading, also for 4 days (4D/4D). Plants were transferred at noon and this 4D/4D cycle was repeated twice. Similarly, 3D/3D and 2D/2D cycles were applied 3 and 4 times, respectively. The outdoor conditions during the period of treatment from late August to mid-September were as follows: mean daily temperature, 22.5 to 29°C; and day length (sunrise to sunset) 12.2 to 13.1 h. The efficient use of a refrigerator could be achieved by alternately subjecting two groups of plants to the same duration of low temperature in the dark and outdoor conditions. Treated plants were grown on peat bags and flowering performance was compared with untreated controls and continuously cold-stored (12D) plants. In intermittently cold-stored plants, flowering was significantly earlier than that in untreated controls by 6 to 10 days. Compared to the continuous 12D plants, flowering was 15 and 4 days earlier in 4D/4D plants within the plots planted on September 13 and 17, respectively. The 2 to 4 days under outdoor conditions imposed in the low temperature storage apparently induced uniform flower initiation through the improvement of carbohydrate nutrition in strawberries. Although further studies are required to establish the most effective treatment procedures, intermittent low temperature storage could be a useful new flower-inducing technology for early forcing of June-bearing strawberries.

Key Words: flower initiation, Fragaria ×ananassa Duch. ‘Nyoho’, secondary inflorescence, tray plants.

Introduction

In Japan, strawberry (Fragaria ×ananassa Duch.) is one of the most popular and important fruits, particularly for Western-style cakes and sometimes for Japanese sweets, because of its attractive taste, flavor, color, and shape. The demand for strawberries usually exceeds the supply, and the market price often rises considerably during the Christmas season. Thus, an early yield of forced strawberries is quite important for both growers and consumers. When the flower-differentiated plants of early June-bearing cultivars are transplanted in mid-

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Several means have been applied practically in an effort to enhance flower initiation, such as waiting-bed plant production in high elevation nurseries, “Yama-age” (Fujishige, 2006; Ninomiya, 1954); partial root pruning in waiting beds, “Dankon-zurashi” (Fujimoto and Kimura, 1970); and nitrogen starvation for pot-grown plants (Arai et al., 1980). Following the breeding of two early and productive new cultivars, ‘Nyoho’ (Akagi et al., 1985) and ‘Toyonoka’ (Honda et al., 1985), two artificial flower-inducing treatments were established: (1) night cooling, i.e., approximately 16 h of low temperature treatment under conditions of darkness with an 8-h period of solar radiation and ambient temperature, and (2) continuous low temperature storage under darkness. In both these treatments, 10 to 15°C of low temperature has been recommended (Fushihara et al., 1989; Kumakura and Shishido, 1993; Ueki et al., 1993) and the treatments are continued until flower bud differentiation is visible. A stable flower-inducing effect can be expected using the former procedure, as the duration of treatment can be extended until flower bud differentiation; however, the treatment requires expensive facilities. The costs associated with the latter treatment are considerably lower than those of the former, as the procedure can be conducted using prefabricated cold rooms that have been used for precooling of picked strawberries. Sometimes, however, the inductive stimulus is insufficient even when the plants are treated for a long period (Ito and Saito, 1962; Furuya et al., 1988; Shishido et al., 1990).

Although tray plant production is becoming increasingly popular because of its ease and low-cost requirements, tray plants are inferior to conventional pot-grown plants in terms of earliness and uniformity of flowering (Yoshida and Morimoto, 2010). Thus, a low-cost means for inducing flower initiation is necessary for tray-grown strawberry plants. Flower buds usually differentiate around September 20 in tray-grown large plants of ‘Nyoho’, and flowering is uniform when they are planted in late September; however, a number of such plants can flower satisfactorily even when young or small plants are planted earlier (Yoshida and Motomura, 2011). It is assumed that ambient temperature and day length in late August or early September may provide inductive stimuli for sensitive strawberry plants, or at least have no inhibitive effect on flower initiation in early June-bearing cultivars. Matsumura (1994) also reported that ambient conditions in late August do not inhibit flower bud differentiation of insufficiently flower induced ‘Nyoho’ strawberry.

For continuous low temperature storage in the dark, growers have to raise aged large pot plants and control nitrogen nutrition to obtain a sufficient treatment effect (Furuya et al., 1988). Kakumu et al. (1991) reported that the inductive effect of low temperature storage on flower initiation was improved by exposing the plants to 8 h of solar radiation several times. Nishizawa et al. (1997) reported the similar effect of an inserted light period and a significant decrease in carbohydrate content during continuous storage. When the natural temperature and day length are not inhibitive to flower initiation, we can hypothesize that repeated combination of a few days of inductive low temperature treatment in the dark and the same duration of non-inhibitive natural conditions may effectively induce initiation and subsequent differentiation of flower buds in June-bearing strawberries.

A certain amount of labor is required to transfer strawberry plants between a refrigerator and outdoors; however, twice as many plants can be treated compared to continuous storage by alternately subjecting two groups of plants to the same duration of low temperature and outdoor conditions. Here, we report new findings on intermittent low temperature storage, a new effective and low-cost flower-inducing treatment for June-bearing strawberries.

**Materials and Methods**

**Experiment 1. Effect of intermittent low temperature storage on flowering**

On July 19, 2008, runner plants of ‘Nyoho’ strawberry having more than 3 expanded leaves were excised from mother plants grown on the table top substrate. Old leaves were removed from the plants, with the exception of the youngest fully expanded leaf, and they were planted in trays having thirty-five 130-mL cells, spaced 7 × 7 cm apart (Suku-suku tray; Marusan Sangyo, Tochigi), filled with a mixture of peat moss, perlite, and granulated rock wool [2 : 1 : 1 (v/v)]. Trays were placed under 50% shading and given 2 min of intermittent mist irrigation at 30-min and 3-h intervals during the day and night, respectively, for 1 week. These plants were supplied with ca. 30 mL (1 L/tray, 35 plants) of 25% Ohtsuka A nutrient solution (N, 4.3 mM; P, 0.6 mM; K, 2.0 mM; Ca, 1.0 mM; Mg, 0.5 mM; S, 0.5 mM; plus microelements; Ohtsuka Chem., Osaka) every 2 days, from July 28 until August 23. During the period from August 23 to 25, uniformly growing plants were selected, old leaves were removed remaining 2 expanded leaves, and the medium surrounding the crown was removed to strip the basal part of the crown as described previously (Yoshida and Motomura, 2011).

Trays were placed in prefabricated refrigerators at approximately noon and brought out 2, 3, or 4 days later. The same number of trays was alternately treated in the same way, and 2, 3, and 4 days of low temperature (LT)/ ambient cycles (2D/2D, 3D/3D, and 4D/4D) were repeated 4, 3, and 2 times, respectively. Thus, intermittently cold-stored plants, which were alternately transferred to outdoor conditions, were subjected to one to three ca. 13-h (natural day length) and two 6- to 7-h periods of sunlight with two to four times of 10 h or longer natural dark periods at ambient temperature. A scheme of the intermittent low temperature treatments is shown in Figure 1.
We used a storage temperature of 13°C as several authors have reported that a temperature of 10 to 15°C is optimal for storage under conditions of continuous darkness (Fushihara et al., 1989; Kumakura and Shishido, 1993; Ueki et al., 1993). During ambient temperature treatment, plants were placed under 50% shading in a rain shelter with untreated control plants. Continuous low temperature storage for 12 days (12D) was also applied to the other tray plants. Seventy plants in 2 trays and 35 plants in 1 tray were subjected to the intermittent and continuous low temperature storage treatments, respectively. Sixty-four, 48, and 32 uniformly growing plants were selected from those plants subjected to intermittent storage, untreated control, and continuous storage treatments, respectively, and were planted on peat bags filled with 16 L of pH-modified peat moss. These were planted on September 13, 17, and 21 (Fig. 1) and managed practically as described previously (Yoshida et al., 2002).

Experiment 2. Effect of intermittent low temperature storage on carbohydrate nutrition

Runner plants of ‘Nyoho’ were planted in trays on July 22, 2010, grown as in Expt. 1, and subjected to intermittent (3 cycles of 3D/3D) or continuous 15 days of LT treatments from August 22 to September 6. At the beginning and end of the treatments, the 2 expanded leaves left on the plants as described previously and roots of treated and untreated plants were taken and dried at 80°C for 48 h. Three replications of tissue samples consisting of 2 pooled plants were weighed and ground with a mortar. Concentrations of total soluble sugars and starch in leaf blades and roots were colorimetrically determined as described by Takeuchi et al. (2001) and Sugiyama and Ooshiro (2001), respectively. Sixteen plants were planted on peat bags for each treatment on September 10 and managed as in Expt. 1.

Air temperature in the rain shelter was measured with T thermocouple 1.2 m above ground, almost equal to the top of the plant canopy. Measured values were recorded at 10-min intervals and daily mean, maximum and minimum values were calculated. Flowering of primary and secondary (on the upper-most branch crown) inflorescences was recorded and the number of leaves on the branch crown (NL, leaves emerged between inflorescences) was also counted. Statistical data analysis was conducted using Excel spreadsheets.

Results

Experiment 1. Effect of intermittent low temperature storage on flowering

As shown in Figure 2, the daily means of ambient temperature ranged from 24.8 to 28.6°C, with the exception of September 15, on which the minimum temperature was 22.5°C. The day length of 13.1 h on August 27 decreased to 12.2 h on September 21 (sunrise to sunset at Okayama Meteorological Observatory).
The mean flowering dates of primary and secondary inflorescences are shown in Table 1. Apart from the plants treated with 12D of continuous LT from August 31 and subsequently planted on September 13, all the LT-treated plants flowered earlier than the untreated control plants planted on the same date. Intermittent low-temperature storage significantly enhanced flowering in tray-grown plants of ‘Nyoho’ strawberry.

Among the plants that were treated with 2 to 4 days of LT/ambient cycles and planted on the same date, no significant difference was observed in the mean flowering date of the primary inflorescences; however, 2D/2D treatment seemed less effective than 3D/3D and 4D/4D treatments among the plots planted on September 13 (Fig. 3). In the plots planted on September 17, flowering of 4D/4D plants was slightly earlier (1.8 days on average) than that of 3D/3D plants (Table 1). There was also no significant difference in the flowering of primary inflorescences between alternately treated plants within the plots planted on September 13 (2D/2D, 4D/4D) and 17 (3D/3D, 4D/4D).

Regarding the planting date, late planting resulted in uniform but delayed flowering in untreated controls (Table 1 and Fig. 4). Among the plants treated with 3D/3D cycles, flowering was earliest in the plants treated from August 27 and planted on September 13 (AUG27-SEP13, Fig. 4); however, no significant difference was observed in the remainder. Sharp increases in the flowering rate of treated plants indicate that not only the earliness but also the uniformity of flowering was improved by the treatments.

As shown in Figure 5, less than 40% of 12D-AUG31 plants planted on September 13 responded to treatment and flowered before October 31. The remaining 60%
flowered after November 20, which was later than the
untreated controls (Fig. 5A). In the plants planted on
September 17, flowering was enhanced in 70% of 12D-
SEP4 plants and no negative effects were observed in
the remainder (Fig. 5B). More than 90% of 4D/4D plants
for which treatments were begun on the same days
flowered before November 5, which was considerably
earlier than the controls. These observations revealed
that the interruption of continuous LT by 4 days of
ambient temperature (25–28°C on average) and day
length (12.5–12.8 h) in early September significantly
stimulated flower initiation in ‘Nyoho’ strawberries.
Similar results were also obtained in 2009 (Ozaki and
Yoshida, 2010) and also in 2010 (Expt. 2, data not shown).

With respect to secondary inflorescences that
differentiate on the uppermost branch crowns, flowering
was also enhanced by intermittent low temperature
storage (Table 1). The difference in flowering date
between the control and treated plots was smaller than
that for the primary inflorescence (Fig. 5). As described
by Shishido et al. (1990), plants in each 12D plot can
be divided into 2 groups, enhanced-flowering and non-
enhanced-flowering plants. Flowering interval (FI:
difference between flowering date of the primary and
secondary inflorescences) was longer in enhanced plants
than in non-enhanced plants. FI was also longer in plants
planted on September 13 than in those planted at a later
date. Similarly, flowering of primary inflorescences was
earlier in enhanced 12D and 4D/4D plants than in control
plants, whereas, in contrast, that of secondary
inflorescences was later in 12D plants than in 4D/4D
and control plants. Although there was no significant
difference between 4D/4D and 12D plants with respect
to the number of leaves on the branch crown (NL), FI
was significantly longer in 12D plants than in 4D/4D
plants when compared within plants having the same
NL (Table 2). These observations may indicate that
growth and development of the branch crown were
retarded in 12D plants compared with 4D/4D plants,
particularly in those plants in which flowering was
successfully enhanced.

**Experiment 2. Effect of intermittent low temperature
storage on carbohydrate nutrition**

Changes in dry weight and non-structural carbohy-
drate concentration during 15 days of treatments are

![Figure 5. Changes in the proportion of flowering ‘Nyoho’ strawberry
plants planted on September 13 (A) and 17 (B) as affected by
12 days of continuous low temperature storage and intermittent
low temperature storage with 2 cycles of 4 days storage at 13°C
and 4 days ambient condition. The number of plants subjected
to intermittent storage, continuous storage, and control
treatments was 64, 32, and 48, respectively. See Figure 1 for
details of treatments.](image)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of leaves between inflorescences</th>
<th>Flowering interval of primary and secondary inflorescences (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enhanced†</td>
<td>Non-enhanced†</td>
</tr>
<tr>
<td>Planted on 13 September</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4D/4D-AUG31</td>
<td>5.0 (52)†</td>
<td>4.3 (12)</td>
</tr>
<tr>
<td>12D-AUG31</td>
<td>4.4 (11)</td>
<td>4.4 (20)</td>
</tr>
<tr>
<td>Planted on 17 September</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4D/4D-SEP4</td>
<td>4.5 (45)</td>
<td>4.4 (19)</td>
</tr>
<tr>
<td>12D-SEP4</td>
<td>4.8 (20)</td>
<td>4.5 (12)</td>
</tr>
<tr>
<td>Significance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4D/4D vs 12D</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>SEP13 vs SEP17</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Interaction</td>
<td>**</td>
<td>NS</td>
</tr>
</tbody>
</table>

† Leaves differentiated on the uppermost branch crown before secondary inflorescence.
‡ Plants flowered in October were classified as enhanced.
§ Values in parentheses are No. of flower enhanced, non-enhanced, and total plants.
* Values in parentheses are No. of plants having each No. of leaves between inflorescences and total enhanced plants.
NS, **, ***: non-significant, significant at 1% or 0.1% by 2-way ANOVA.
shown in Table 3. Whereas both leaf and root dry weight significantly decreased in continuously treated 15D plants and increased in untreated control plants, a significant increase in root and no significant change in leaf dry weight were observed in intermittently stored 3D/3D plants. Concentration of starch was much lower than that of soluble sugars in strawberry roots and leaves, and most of the large and significant changes in total non-structural carbohydrate (TNSC) were caused by the changes in soluble sugars. TNSC in leaves and roots also decreased in 15D plants, but no significant difference was observed in 3D/3D and control plants except for leaves of control plants. Carbohydrate nutrition was significantly improved by the interruption of continuous LT by sunlight in the outdoor conditions.

**Discussion**

It is well known that photoperiodicity in June-bearing strawberries is a temperature-dependent response to the dark period of a day (Darrow, 1966; Ito and Saito, 1962). Plants treated with intermittent low temperature storage were exposed to 2, 3, and 4 days of ambient temperature and day length. Thus, 4D/4D plants were subjected to three ca. 13-h periods and two 6- to 7-h periods of natural light, excluding dawn and dusk. Flower initiation in ‘Nyoho’ strawberries was significantly stimulated by such 2 to 4 days of critical conditions in early September—day lengths in excess of 12.5 h and temperatures above a daily mean of 25°C (Fig. 2). Ito and Saito (1962) reported that the flower buds of strawberries often failed to differentiate under continuous LT in the dark and that strawberries required a longer duration of inductive stimulus when day length was shorter than 8 h. Further, the concentration of soluble sugars in strawberry plants decreases during continuous LT in the dark (Nishizawa et al., 1997). Furuya et al. (1988) reported that continuous LT may cause a shortage of photosynthate in strawberry plants and result in supraoptimal nitrate accumulation leading to unsuccessful flower induction. Kakumu et al. (1991) and Uematsu (1989) reported that the inductive effect of continuous LT in the dark was increased by inserting an 8-h period of sunlight with ambient temperature and by applying a foliar spray of sucrose solution, respectively. These results suggest that poor carbohydrate nutrition may inhibit flower induction of strawberries.

In strawberry plants in which exposure to sunlight interrupted continuous LT, activated photosynthesis significantly improved plant growth and carbohydrate nutrition (Table 3). Improved carbohydrate nutrition may be one of the most important physiological factors affecting early and uniform flowering. Such an improvement in combination with the accumulated stimulus of LT overcame the partly negative or neutral effect of ambient high temperature (daily maximum of 26.2 to 32.5°C during treatment) and critical day length (12.5–12.8 h) for flower induction.

Along with poor carbohydrate nutrition, etiolated growth of emerging leaves and chlorophyll degradation in expanded leaves are observed in plants treated with continuous LT; however, no etiolated symptom was observed in intermittently treated plants and they began to grow much more vigorously after planting than continuously treated plants (Data not shown). The improved growth and carbohydrate nutrition after planting may affect the development of the primary inflorescence and also for the initiation and development.

**Table 3.** Effect of intermittent or continuous low temperature storage on growth of and non-structural carbohydrate concentration in leaves and roots of strawberry ‘Nyoho’.

<table>
<thead>
<tr>
<th>Date</th>
<th>Treatment</th>
<th>Dry weight (mg/plant)</th>
<th>Non-structural carbohydrate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Leaves</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sugars</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sugars</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sugars</td>
</tr>
<tr>
<td>AUG 22</td>
<td>—</td>
<td>718 b</td>
<td>4.96 b</td>
</tr>
<tr>
<td>SEP 6</td>
<td>Control</td>
<td>994 a</td>
<td>6.41 a</td>
</tr>
<tr>
<td></td>
<td>3D/3D</td>
<td>726 b</td>
<td>4.98 b</td>
</tr>
<tr>
<td></td>
<td>15D</td>
<td>496 c</td>
<td>2.07 c</td>
</tr>
<tr>
<td>AUG 22</td>
<td>—</td>
<td>387 c</td>
<td>11.72 a</td>
</tr>
<tr>
<td>SEP 6</td>
<td>Control</td>
<td>721 a</td>
<td>9.81 a</td>
</tr>
<tr>
<td></td>
<td>3D/3D</td>
<td>514 b</td>
<td>10.31 a</td>
</tr>
<tr>
<td></td>
<td>15D</td>
<td>324 d</td>
<td>5.92 b</td>
</tr>
</tbody>
</table>

1 Beginning and ending date of treatments.
2 Total soluble sugars colorimetrically measured by phenol-sulfuric acid method.
3 Mean dry weight of outer 2 leaves including petiole and carbohydrate concentration in leaf blade.
of the secondary inflorescence on the branch crown.

In conclusion, the ambient temperature and day length in early September significantly stimulated flower initiation when they were intermittently inserted into a period of LT at 13°C under conditions of darkness. There was no significant difference in flowering date among the plants treated with 2D/2D to 4D/4D cycles, but a 4D/4D cycle may be more efficient practically than a 3D/3D or 2D/2D cycle, as the labor cost required to transfer the plants is lowest with this treatment. Further practical and physiological research is still required to establish effective treatment procedures; however, on the basis of the results of this study, we suggest that intermittent low temperature storage could be a new practical technology for inducing flower initiation in June-bearing strawberries.

### Literature Cited


