‘つけな中間母本農2号’の抽だいに及ぼす苗令の影響

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Effects of Plant Stages on bolting of the Breeding Line 'Leafy Green Parental Line No.2' (*Brassica rapa* L. *pekinensis* group)

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Summary

Bolting characteristics of a late bolting breeding line 'Leafy Green Parental line No.2' (PL-No.2, previously known as Ano No.3 or FNC31・63) of *Brassica rapa* L. *pekinensis* group, syn. *B. campestris* L. *pekinensis* group, or Chinese cabbage, were examined with regard to its plant stages. Seedling sensitivity to low temperatures was very low, irrespective of plant stages; differentiation of flower buds occurred very late. Bolting and flowering were induced by a 16-hr photoperiod. However, a long-day with supplementary lighting was ineffective in initiating bolting and subsequent flowering. Seedlings grown under 16-hr photoperiod required nearly 70 days to anthesis. As the plants grew, the time from the onset of the long-day treatment to flowering became less than 50 days. The long-day sensitivity of 'PL-No.2' increased as the plants grew.

Key Words: bolting, *Brassica rapa* L., day length, photoperiod, plant stage.

Introduction

In spring cultivation of Chinese cabbage (*Brassica rapa* L. *pekinensis* group, syn. *B. campestris* L. *pekinensis* group), it is important to avoid early bolting. As Chinese cabbage is a seed vernalization plant, hotbed nursing is a practical way to prevent early bolting (Guttormsen and Moe, 1985a). After the first bolting resistant Chinese cabbage cultivar 'Nozaki-Harumaki' was bred in 1938 (Itsumi, 1977), breeding of bolting resistance has continued in Japan. Nowadays, many F_1 cultivars for spring cultivation have appeared and their bolting resistance is higher than that of 'Nozaki-Harumaki'. However, bolting is still a serious factor which affects the yield and quality of spring grown Chinese cabbage. Highly bolting resistant cultivars are in demand not only in Japan, but also in high latitude countries in Europe, where Chinese cabbage is becoming more popular (Elers and Wiebe, 1984a, 1984b; Mero and Honma, 1984a, 1984b; Guttormsen and Moe, 1985a, 1985b; Mero, 1985; Moe and Guttormsen, 1985; Pressman and Shaked, 1988).

Yui and Yoshikawa (1991) found late bolting individuals within 'Osaka-Shirona-Bansei' of the *B. rapa* L. *pekinensis* group. From these they bred 'Leafy Green Parental Line No.2' (PL-No.2, previously known as FNC31・63 or Ano No.3). It flowers under long-day conditions without any chilling treatment. Yui and Yoshikawa (1992) showed that the critical day length for 'PL-No.2' is around 14-hr and the chilling treatment has only a slight effect on flowering. Takahashi et al. (1994) classified turnip cultivars of the *B. rapa* L. *rapifera* group into 5 types, according to their responses to low temperatures and photoperiod. Using their classification,'PL-No.2' belongs to Type V(five) ; its reproductive growth is induced by long-day rather than low temperatures. However in their report, Type V turnip cultivars are said to be sensitive to low temperatures because they quantitatively promote reproductive development. It is important for 'PL-No.2', a bolting resistant selection, that its flowering is not seriously affected by low temperatures (Yui and Yoshikawa 1991, 1992). In their reports, however, the plant stages of seedlings were not given any consideration; there is no information on the relationship between plant stages and low temperatures or day-length sensitivity. The purpose of this report is to examine the detailed flowering characteristics of 'PL-No.2', as a bolting resistant parental stock, especially from the standpoint of its plant stages.

Materials and Methods

Experiment 1. Chilling treatments at different plant stages

Seeds of 'PL-No.2' and a common Chinese cabbage cultivar 'Muso' (Takii & Co.,Ltd.) were sown in 8 cm

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diameter pots on May 30 and again on July 2, 1991. They were grown for 75 days (75-day seedlings) and 42 days (42-day seedlings) until Aug.13 under 23 ℃ with 12-hr of natural photoperiod, by using a sliding roof chamber (non-inductive conditions). All plants grew vegetatively in the chamber. They were put in a chilling chamber kept at 5 ℃ and constant light at 4,000 lx (100 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) for 79 days from Aug.13 to Oct.31. After the chilling treatment, the seedlings were transferred into the sliding roof chamber kept at 23 ℃ and a 16-hr photoperiod, which consisted of 9-hr of natural daylight and 7-hr of supplementary light with incandescent and fluorescent lamps. Light intensity of the supplementary lamps was about 100 lx (20 $\mu mol \cdot m^{-2} \cdot sec^{-1}$). Control seeds which received no chilling were sown in the sliding roof chamber on Dec.10. As the plants grew, they were transplanted into 12 cm, then into 18 cm diameter pots before the roots occupied the entire soil volume. Days to flowering and the number of leaves on the main stems were recorded. Four months after the chilling treatment was completed, leaf numbers of the seedlings which exhibited no flower buds were recorded. Ten plants were examined in each treatment.

Experiment 2. Long-day treatments at different plant stages with chilling treatment

1. Material and sowing

Seeds of 'PL-No.2' were sown in 5 cm diameter pots on Aug.12, 1991, and cultivated under natural day and temperature.

2. Chilling treatment

Seven days after sowing, the plants in the cotyledonary stage were put into a chilling chamber, where the temperature and light conditions were the same as in Exp. 1. They were chilled for 65 days from Aug.19 to Oct.23.

3. Day length treatment

When the chilling treatment was completed on Oct.23, plants were transferred to two phytotrons with 12-hr photoperiod (non-inductive short-day) and 16-hr photoperiod (inductive long-day); both phytotrons were kept at 25/20 ℃ (day/night) under a light intensity of 20,000 lx(180 $\mu mol \cdot m^{-2} \cdot sec^{-1}$). Twenty days after the chilling treatment was completed, '12-16 ①' plants were moved from the 12-hr short-day phytotron to the 16-hr long-day phytotron, and those of '16-12 ①' were moved from 16-hr to 12-hr regimen. Thirty-six days after the chilling treatment was completed, plants of '12-16 ②' were moved from the 12-hr short-day phytotron to the 16-hr long-day phytotron, and those of '16-12 ②' were moved from 16-hr to 12-hr. The unchilled control plants in the day length treatments were kept in 12-hr and 16-hr day length phytotrons for treatments '12-12' and '16-16', respectively.

4. Sampling for bud analysis

All plants were transplanted to 15 cm diameter pots after the chilling treatment. Twenty-nine days later, they were transplanted again to 18 cm diameter pots to accommodate the roots. Days to flowering and the number of leaves on the main stem were recorded while they were grown in the phytotrons for three months. When the experiment was completed, seedlings which had not yet flowered were dissected to examine whether they had differentiated flower buds. Twelve plants were examined in each treatment of this experiment.

Experiment 3. Long-day treatments at different plant stages without chilling treatment

1. Material and sowing

Seeds of 'PL-No.2' were sown in 5 cm diameter pots on Oct.11, 1991 and put in the same two phytotrons as Exp. 2 without chilling treatment. The sowing date was determined to synchronize the growth of plants in Exp. 2 and 3.

2. Day length treatment

Thirty-two days after sowing, plants of treatment '12-16 ①' were transferred from the 12-hr short-day phytotron to the 16-hr long-day phytotron, while those of a treatment '16-12①' were moved from 16-hr to 12-hr phytotrons. Forty-eight days after sowing, plants of treatment '12-16②' were moved from the 12-hr short-day phytotron to the 16-hr long-day phytotron, and those of treatment '16-12 ②' were moved from 16-hr to 12-hr. The control plants were retained in their original 12-hr or 16-hr phytotrons as treatments '12-12' and '16-16', respectively.

3. Plant and sampling as in Part 4, Exp.2.

All plants were transplanted to 15 cm diameter pots 12 days after sowing. Forty-one days after sowing, as the plants grew, they were transplanted again to 18 cm diameter pots before the roots fully spread within the pots. They were grown in the phytotrons for three months. Data were recorded as in Exp. 2.

Results

Experiment 1. Chilling treatments at different plant stages

The unchilled control 'Muso' plants did not differentiate any flower buds (Table 1). All the chilled seedlings of 'Muso' differentiated flower buds and flowered within 23 days. The 42-day and 75-day old seedlings produced 54.3 and 71.5 leaves, respectively. The difference corresponds to the difference of vegetative growth before the chilling treatment. Only one out of 10 'PL-No.2' seedlings flowered among the 42-day and 75-day old seedlings. However, the flowers on the two plants were abnormal, as their shoots did not elongate well. The control, 42-day and 75-day old 'PL-No.2' seedlings developed 37.3, 64.8 and 76.6 leaves, respectively, but no flowers.

Table 1. Effect of chilling treatments at different plant stages of breeding line 'PL-No.2' and Chinese cabbage 'Muso' on bolting (Exp.1)

Cultivar or line	Plant stage at chilling treatment	Plants with flower buds(%)	No. of leaves at flowering	Days to flowering^z	Differentiated leaf number of plants in vegetative stage
	control	0	–^{y}	–	37.3
PL-No.2	42 days	10	(46)^x	(81)^x	(64.8)^w
	75 days	10	(55)^x	(102)^x	(76.6)^w
	control	0	–	–	86.4
Muso	42 days	100	54.3	23	–
	75 days	100	71.5	16	–

^{z} Days from Oct.31, when the chilling teratment was completed.
^{y} '–' means no plants there.
^{x} Figures with parentheses are the data of one plant which flowered.
^{w} Figures with parentheses are the data of nine plants which were in a vegetative stage at the end of Exp.1.

Table 2. Effect of long-day treatments at different plant stages of breeding line 'PL-No.2' on bolting. (With chilling treatment, Exp. 2)

	Day length treatment^z	Flower bud Differentiation (%)	Flowering (%)	Days to flowering^y	No. of leaves at flower bud differentiation	No. of leaves^x
Control	12-12	8	0	–	37.0	39.9
	16-16	100	100	66(66)	29.0	–
12-16	12-16①	100	100	77(57)	33.2	–
	12-16②	100	100	74(38)	33.4	–
16-12	16-12①	42	0	–	39.8	39.6
	16-12②	58	17	78	33.9	37.2

^{z} See 'Materials and Methods'.
^{y} Days to flowering from the end of chilling treatment (figures in parentheses indicate the days to flowering from the beginning of the 16-hr long-day treatment).
^{x} Number of leaves of plants in a vegetative stage at the end of Exp. 2.

Experiment 2. Long-day treatments at different plant stages with chilling treatment

In the control 16-16 treatment, all plants flowered while producing 29.0 leaves, whereas no 12-12 plants flowered (Table 2). In 12-16 ① and 12-16 ② treatments, the plants produced 33.2 and 33.4 leaves, respectively, on the main stem and flowered. The analysis of variance for 16-16, 12-16 ① and 12-16 ②, where all plants flowered, revealed that the leaf numbers differed significantly ($P<0.01$). The time of flower bud differentiation in 12-16 ① and 12-16 ② was significantly later than that in 16-16. In 16-12 ① and 16-12 ② treatments, 42% and 58% of the plants, respectively, differentiated flower buds, but none in 16-12 ① and only 17% of the 16-12 ② seedlings reached anthesis; their bolting scapes did not elongate normally.

Experiment 3. Long-day treatments at different plant stages without chilling treatment

All control 16-16 seedlings flowered while producing 31.9 leaves, whereas no 12-12 plants flowered (Table 3). In 12-16 ① and 12-16 ② treatments, all plants flowered while forming 35.3 and 34.6 leaves, respectively, on the main stem. The analysis of variance for 16-16, 12-16 ① and 12-16 ②, where all plants flowered, revealed that the leaf numbers differed significantly ($P<0.01$). Flower bud differentiation in 12-16 ① and 12-16 ② occurred significantly later than that in 16-16. In 16-12 ① and 16-12 ② treatments, 17% and 33% of the seedlings differentiated flower buds, respectively, while none in 16-12 ① and only 8% in 16-12 ② reached anthesis. As in Exp.2, the bolting scape of one plant in 16-12 ② did not elongate normally.

Table 3. Effect of long-day treatments at different plant stages of breeding line 'PL-No.2' on bolting. (Without chilling treatment, Exp. 3)

	Day length treatment[z]	Flower bud Differentiation (%)	Flowering (%)	Days to flowering[y]	No.of leaves at flower bud differentiation	No. of leaves[x]
Control	12-12	0	0	-	-	36.4
	16-16	100	100	77(77)	31.9	-
12-16	12-16①	100	100	83(63)	35.3	-
	12-16②	100	100	85(49)	34.6	-
16-12	16-12①	17	0	-	30.5	36.7
	16-12②	33	8	73	33.8	35.3

[z] See 'Materials and Methods'.
[y] Days to flowering from sowing (figures in parentheses indicate the days to flowering from the beginning of 16-hr long-day treatment).
[x] Number of leaves of plants in a vegetative stage at the end of Exp. 3.

Discussion

Exposure to low temperature is essential for reproductive growth for most of Cruciferae plants (Shinohara, 1959). In *B. oleracea* L., which includes cabbage and broccoli, there is a juvenile or basic growth phase (Ashizawa, 1977; Kagawa, 1965; Thomas, 1980). When the seedlings are in the juvenile phase, they grow vegetatively and are not seriously affected by environmental factors, which induce reproductive growth if the seedlings are in reproductive phase. In *B. rapa*, however, low temperature induces seedlings into the reproductive growth; the timing of the exposure to chilling has little effect (Kagawa, 1966; Elers and Wiebe, 1984a). 'PL-No.2' of *B. rapa* is a unique line as it is somewhat unresponsive to low temperatures during its cotyledonary stage; it is induced to flower by long-day conditions (Yui and Yoshikawa, 1991, 1992).

We can conclude from the results of Exp.1 (Table 1) that low temperature sensitivity of 'PL-No.2' is very low irrespective of its plant stages. Results of Yui and Yoshikawa (1991) and Exp.1 also show that long-day treatment with high light intensity (20,000 lx) induces flowering of 'PL-No.2'. However, long-day treatment with supplementary lamps is ineffective in inducing flowering. This suggests that light intensity is an important factor in inducing 'PL-No.2' into the reproductive growth.

Data from Exp.2 and 3 (Table 2, 3) show that, irrespective of the chilling treatment, the number of days to flowering from the onset of the 16-hr long-day treatment shortened as the plants grew. Furthermore, 20-days to 48-days of long-day treatment during the early stages in four 16-12 treatments were not sufficient to induce flowering. This indicates that in the early stages of development,'PL-No.2' has a low sensitivity to long-days. Ajisaka et al. (2001) reported the segregation of bolting in the F_2 population between a late bolting line derived from 'PL-No.2' and a common Chinese cabbage. The authors reported that 180-day old seedlings of late bolting plants began to flower after only 23 days of the 16-hr long-day treatment. It demonstrates that long-day sensitivity of 'PL-No.2' increases as the plant grows. Friend (1985) noted that irradiance, not day length, is an important factor to induce flowering of *Brassica*. In Exp.2 and 3, most of the plants in 12-12 and 16-12 did not differentiate flower buds although they had more than 35 leaves (Table 2, 3). In 16-16, all plants, although they had less leaves than those in 12-12 and 16-12, differentiated flowers. This indicates that irradiance or growth quantity caused by photosynthesis is not the only factor to induce flowering.

Our results confirm 'PL-No.2' sown in early summer does not bolt even under the long-day conditions in northern Japan (Sato Yutaka, Uchiumi Toshiko, personal communications). If the late bolting characteristic is introduced into heading Chinese cabbage, it will be useful not only for spring cultivation, but also for summer cultivation in highlands and at higher latitudes, including European countries.

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‘つけな中間母本農２号’の抽だいに及ぼす苗令の影響

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摘　要

ハクサイと同種の *Brassica rapa* L. *pekinensis* group に属する晩抽性育種素材‘つけな中間母本農２号（旧系統名安濃３号またはFNC31・63）’を用いて，抽だいに及ぼす苗令の影響を検討した．植物体の苗令に関わらず，この系統の花芽分化は非常に遅く，低温に対する感受性も非常に低かった．また，この系統の抽だい開花は16時間の長日処理によって誘起されたが，補光による長日は効果がなかった．発芽直後から植物体を16時間の長日条件下に置いた場合，開花するまでに70日程度を要した．これに対して，苗令が進むと長日条件下に置かれてから開花するまでの日数は50日以下になった．この結果から，‘つけな中間母本農２号’の長日感受性は，植物体の生長に伴って高くなると考えられた．