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RESEARCH ARTICLE

Biennial sugar beets capable of flowering without vernalization treatment

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Abstract A biennial sugar beet (*Beta vulgaris*) generally takes two years to flower and complete its life cycle. In year one, the plant grows vegetatively and then enters a cold winter period. In year two, the plant grows reproductively and initiates flowering under long-day conditions. Among biennial beets that grow vegetatively in outdoor field conditions, two test strains were preliminarily found to flower early under 24-h daylength conditions without being exposed to cold temperatures. To confirm the this phenomenon's genetics, crossings between the test strains and normal biennials yielded hybrid derivatives of F_1, F_2, and BC_1F_1, and bolting rate was investigated both in an outdoor field under natural daylength conditions and in a greenhouse with an artificial 24-h daylength. The test strains and hybrid derivatives did not bolt in the outdoor field, similar to the biennial control strains. This enables assessment of important agronomic traits, such as yield, which cannot be evaluated using an annual control strain in which all plants are bolted. However, under 24-h daylength conditions, the test strains bolted without vernalization treatment, unlike the biennial control strains, but similar to annuals. Hybrid derivatives' bolting rates suggest that the flowering characteristics of the test strains are mainly controlled by a single dominant gene. The flowering characteristics and the hypothetical responsible gene were named 'BLOND' and '*Bd*', respectively. Because seed production in BLOND is estimated to take at least four months, similar to that of the annual

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beet, BLOND's bolt rate may be applicable for the speed breeding of sugar beets.

Keywords Bolting · BLOND · *Bd* · Day length · Vernalization

Introduction

More than a decade is needed to breed and develop cultivars of many crops. This ensures homogeneity by genetically fixing cultivars over multiple generations (Jamali et al. 2020) and speed breeding is particularly important in sugar beets.

Sugar beet (*Beta vulgaris*) is planted in temperate and subarctic regions and is the second most important sugar crop after sugarcane. It has an absolute vernalization requirement that includes a certain period of low temperature or winter to advance to a single generation (Bosemark 2006). Such biennial crops require additional years to advance by one generation compared to major annual crops, such as corn, wheat, soybean, and rice (Hickey et al. 2019).

Normal biennial beets can only grow vegetatively and accumulate sucrose in their roots during the first year of growth, resulting in high production. However, to obtain seeds, it is necessary to induce flowering from root through prolonged vernalization and subsequent long-day treatments. Because the onset of reproductive growth (i.e., bolting stem elongation and flowering) in the first year causes a decrease in sucrose yield and quality in roots, breeding of biennial beets that are less susceptible to bolting has been selected in the breeding process.

Sugar beets require two growing seasons to produce seeds by conventional methods (Fig. 1). Seeds

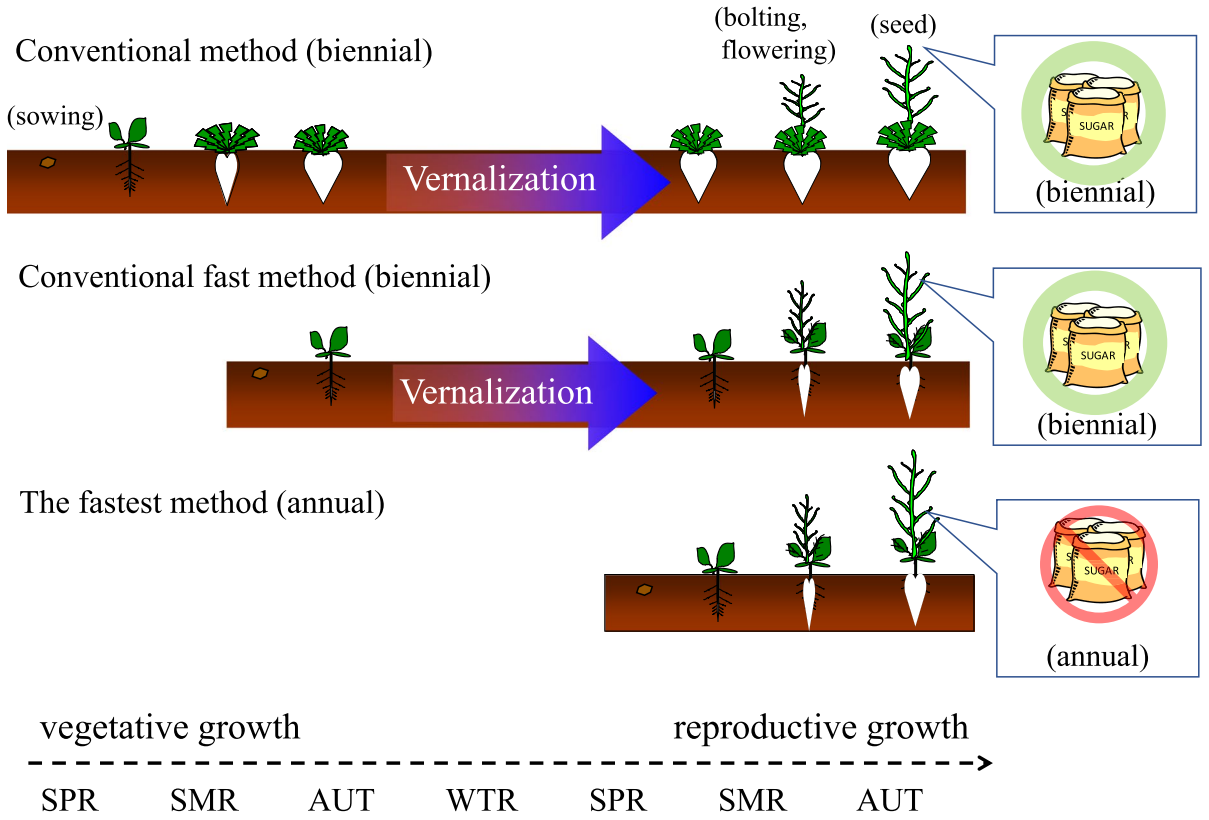

Fig. 1 Differences in seed production period and yield performance using seed production methods in sugar beet. Biennial sugar beet can ensure high sugar production in the field by continuing to grow vegetatively until harvest, but they require a vernalization period of several months to induce flowering for obtaining seeds. On the other hand, annual beet responds to the long day conditions of summer and starts flowering in a short period of time without the need for vernalization, but it is not suitable for sugar production because flowering cannot be suppressed in the field

are sown in the spring of the first year, and by the fall of that year, enlarged roots called stecklings are obtained through vegetative growth. The stecklings are then vernalized during winter and replanted the following spring to initiate reproductive growth and the seeds are harvested by fall (Kockelmann and Meyer 2006). Kuroda et al. (2015) reported another method for producing seeds in a relatively short period by taking advantage of the response to vernalization at the seedling stage (Fig. 1). Young seedlings were grown and vernalized during winter, replanted in spring, and harvested in fall. Nevertheless, long-term vernalization treatments are essential for both methods of seed production because plants must be exposed to cold temperatures (5 to 8 °C) for 8–14 weeks to achieve vernalization (Milford 2006).

The seed production period is significantly reduced if vernalization is not required. For example, there are annual strains of the same species as sugar beets that do not have vernalization requirements (Abegg 1936). When these annual strains are grown outdoors in the field from late April to early May, the typical growing season in Hokkaido, bolting and flowering are induced under natural daylength conditions in June (Shimamoto et al. 1990; Abe et al. 1997) and seeds can be harvested by fall. This method is likely to be the shortest method in effect, with seed production possible in approximately four months (Fig. 1). However, a major drawback of this method is that the extremely high frequency of bolting prevents root enlargement, making it impossible to evaluate important agronomic traits such as yield in the field. Currently, speed breeding is not compatible with high yields.

Long daylength and low temperature are important external environmental factors that promote the flowering of sugar beets (Chroboczek 1933; Owen 1940; Stout 1946). In other long-day plants, such as wheat, barley, chickpeas, and oilseed rape, the advancement of generations can be promoted by extending the day length (Ghosh et al. 2018; Watson et al. 2018). To resolve the dilemma of useful agronomic traits in the field and short-term seed production in sugar beets, in situations where annuals and biennials are currently incompatible, a discovery of strains that initiate flowering only under certain specific daylength conditions while continuing to grow normally in the field would be useful for speed breeding. A preliminary investigation of sugar beet breeding strains from these perspectives revealed two interesting strains in which the plant behaves biennially and the roots enlarge without the occurrence of bolting when grown outdoors; however, in these strains, flowering is induced without vernalization treatment when grown in a greenhouse with 24-h daylength (Kuroda unpublished data).

The *B* locus found in the annual strain was involved in bolting and is controlled by a single dominant gene. The dominant *B* allele leads to an annual habit, whereas plants with the recessive *b* allele require vernalization and exhibit biennial habits (Abegg 1936; Boudry et al. 1994; El-Mezawy et al. 2002). The causative gene of the *B* locus is *BvBTC1* and a partial loss-of-function for annual habit occurs in biennial beets (Pin et al. 2012). Based on the polymorphisms in the *BvBTC1* gene, there are two classes of haplotypes; the first class consists of three haplotypes (‘a’ to ‘c’), including a biennial parent for molecular mapping, whereas the second consists of eight haplotypes (‘d’ to ‘k’) including an annual parent for molecular mapping. In the process of breeding and selection, a specific biennial haplotype (haplotype ‘a’) tolerant to bolting has been selected (Pin et al. 2012). However, some Japanese biennial strains, including the two strains found in the preliminary investigation described above, have been recognized with annual haplotypes such as ‘g’ and similar to ‘f’ named ‘o’ (Kuroda et al. 2019). Focusing on those strains is also important to understand the genetic mechanisms involved in the control of bolting, apart from the *B* locus.

In this study, differences in flowering characteristics were investigated for two years using two test strains under two growth conditions, an outdoor field and a greenhouse, to examine the possibility of combining speed breeding and high yield in sugar beets. In addition, artificial crosses were performed between strains with different flowering characteristics to produce several progenies, and the heritability of these characteristics was investigated.

Materials and methods

Plant materials

Flowering characteristics were investigated using two test strains, ‘NK-420 mm-O’ and ‘NK-422 mm-O’,

derived from biennial sugar beet populations of different origins in 1991 and 1976, respectively. One of the two strains, 'NK-420 mm-O' (also known as N2n-35-70-2-4), had flowering characteristics similar to the annual beet in the greenhouse, which could be explained by a single dominant gene model (Kuranouchi et al. 1990). However, there are no detailed data on flowering characteristics under outdoor field conditions. As for the other strain ('NK-422 mm-O'), there is only a single record of an annual-like flowering trait, and no additional information is available. The haplotypes at *BvBTC1* in the test strains are both 'g' (Kuroda et al. 2019).

Typical annual and biennial beets were used as controls for the test strains. For the annual control, the strain 'TA-33BB-O' was used. This strain usually bolts under long-day conditions in a greenhouse and exhibits the same phenomenon when grown under outdoor field conditions (Shimamoto et al. 1990; Abe et al. 1997). For the biennial control, 'NK-280 mm-O', 'NK-310 mm-O', 'NK-377 mm-O' and/or 'Monohikari' were used.

In addition, these materials were crossed to evaluate the heritability and segregation of the flowering characteristics of the test strains. For crossing using the test strains, F_1 and F_2 were obtained by crossing the test and biennial strains ('NK-377 mm-CMS' or 'NK-310 mm-O'), and BC_1F_1s were obtained by crossing the F_1 and biennial strain ('NK-310 mm-O'). For crossing using the annual strain (used as a reference), F_1 was obtained by crossing the annual ('TA-33BB-O') and biennial ('NK-377 mm-CMS') strains, and BC_1F_1 was obtained by crossing the F_1 and biennial ('NK-310 mm-O') strain. Note that the nuclear genome composition of 'NK-310 mm-O' and 'NK-377 mm-O' are almost the same, thus, crossing the F_1 ('NK-377 mm-CMS' × 'NK-420 mm-O') with 'NK-310 mm-O' was considered as backcrossing. The haplotypes in the biennial lines of the breeding parents are not uniform but contain less 'a' (12%) and more 'g' (22%) and 'o' (66%) (e.g. 'NK-310 mm-O', Kuroda et al. 2019).

Prefixes such as 'NK-420 mm' or 'TA-33BB' denote the nuclear genotype of strains, and suffixes such as '- O (O-type)' or 'CMS (cytoplasmic male sterility)' denote cytoplasmic type. All the seeds of the beet strains described above were bred at the Hokkaido Agricultural Research Center (HARC).

Field test

Seeds were sown in paper pots (Nippon Beet Sugar Manufacturing Co. Ltd., Japan) on the 9th day of April in both 2014, and 2015, and the seedlings were grown for approximately 30 days. The seedlings were initially grown in a greenhouse and the temperature was gradually reduced from 20 to 10 °C for hardening. Two-to three-leaf seedlings were transplanted to experimental fields at HARC (Memuro, Hokkaido, Japan, 42.9°N, 143.1°E), at a planting density of 22.5 cm between the plants and 60 cm between the rows, on May 9, 2014, and May 13, 2015. Except for natural sunlight, no additional light was applied during the growing period. Bolting individuals were counted in late August (August 21, 2014, and August 26, 2015) to evaluate flowering characteristics. Bolting rate was calculated by dividing the number of bolting plants by the total number of plants examined. In 2014, the root weight (g) was measured for the parental strains and F1s at harvest (October 7). Tukey's multiple range test was performed to determine the differences in the root weights of these materials.

Greenhouse test

The greenhouse test was conducted during two seasons–summer and winter–at temperatures not below 15 °C during the test periods. For the summer seasons of 2014 and 2015, the seedlings were grown at the same time as in the field test described above. Two-to-three-leaf seedlings were transplanted into pots (diameter 20 cm × height 20 cm) at a density of nine individuals per pot in May (specifically, on May 8, 2014, and May 15, 2015) and then grown in a greenhouse at HARC. The temperature was set to 20 °C and the light conditions were 24-h daylength, with daytime sunlight (~ 100,000 lx) and nighttime illumination (300 lx), for all growing periods. An incandescent bulb (200 W) placed approximately one meter above the plants was used for nighttime illumination. The lighting hours ranged from 18:00 to 5:00. Bolting individuals were counted in June (June 11, 2014, and June 30, 2015), and bolting rates were calculated. For the winter seasons of 2013 and 2014, seeds were sown in a paper pot in early November (November 5, 2013, and 2014), and seedlings were transplanted in December (December 11, 2013, and 2014). Except for the longer illumination time, which ranged from 16:00 to 7:00, the winter

test was performed in the same manner as the summer test. Bolting individuals were counted in June (June 6, 2013, and June 12, 2014), and bolting rates were calculated. Assuming that the flowering characteristics of the test strain were determined by a single dominant gene based on a preliminary study (Kuranouchi 1990), the chi-square test was used to evaluate the segregation distortion from Mendelian inheritance for each generation. Tukey's multiple-range test was performed to determine the differences in the number of days to bolting by strain. Broad-sense heritability (H_B) for each test strain was estimated as the ratio of total genotypic variance to phenotypic variance, based on the method of Kelly and Bliss (1975), using bolting data obtained from 2013 to 2015 as follows:

$$H_B = \{ VF_2 - (VP_1 + VP_2 + VF_1) /3 \} / VF_2$$

where VP_1, VP_2, VF_1, and VF_2 indicate the variances in the parent1, parent2, F_1, and F_2 generations, respectively.

Incubator test

The experiments were conducted in an incubator (Sanyo Co. Lid, MLR-351) to clarify whether the induction of flowering was dependent on day length. Seeds of 'TA-33BBmm-O, 'NK-310 mm-O', 'NK-420 mm-O', and 'NK-422 mm-O' were sown in paper pots on December 21, 2015, and seedlings grown for 30 days. Three seedlings of each strain were planted in Jiffy pots (Denmark), each 10 cm in diameter, on January 21, 2016. The seedlings were grown under incandescent illumination (IL) conditions of 24-h, 18-h, or 14-h daylength at a temperature of 20 °C. An incandescent lamp (100 W) placed approximately 50 cm above the pots was used as the light source (700 lx). In addition, as a control of an incubator test, the greenhouse evaluation was also performed under two light conditions of daytime sunlight only (SL) and 24-h daylength with daytime sunlight and nighttime illumination (SL + IL, 16:00 to 7:00, the same conditions as in the greenhouse test described above) in a greenhouse set to a temperature of 20 °C. Bolting individuals were counted on March 4, 2016, and the bolting rates and stem lengths were calculated. The Mann–Whitney U test was used to test the difference between the strains tested and the biennial control 'NK-310 mm-O'.

Results

Field test

In the outdoor field, bolting rates were 0% for the biennial control strains and 100% for the annual control strain over the two years. For strains obtained by crossing the annual reference and biennial reference strains, bolting rates of F_1 were 100% for both years, and those of BC_1F_1 were 44% (26 / 59 individuals) in 2015. In the chi-square test, assuming a single dominant control gene, the segregation ratios of bolting and non-bolting were not significantly different from the separation ratio of 1:1 in the BC_1F_1 generation ($p > 0.05$). The two test strains, which were 'NK-420 mm-O' and 'NK-422 mm-O', showed the same bolting tendency as the biennial reference strains and continued vegetative growth without any bolting (Table 1). For strains obtained by crossing the test strains and biennial reference strains, bolting rates of F_1, F_2, and BC_1F_1 were 0% over the two years. The results suggest that the bolting tendency of the test strains was similar to that of the biennial strains, and unlike the annual strain in outdoor field conditions, and that the bolting tendency of the annual strain was controlled by a single dominant gene. The root weights of the two test strains (735.1 g and 773.4 g) were heavier than those of the annual line (54.2 g) (Table 1, $p < 0.05$). Similarly, the F_1 root weights (1084.4 g and 1274.2 g) of the test and biennial strains were also heavier than the F_1 root weights (395.5 g) of the biennial and annual strains (Table 1, $p < 0.05$).

Greenhouse test

In the summer greenhouse, bolting rates were 0% for the biennial reference strains and 100% for the annual reference strain over the two years. The two test strains exhibited the same bolting tendency as the annual reference strain and the bolt rate was 100% (Table 2). For strains obtained by crossing the annual reference and biennial reference strains, bolting rate of F_1 was 100% in 2014. For strains obtained by crossing the test strains and biennial reference strains, the bolting rates of two F_1s, F_2s, and BC_1F_1s ranged from 84% (38 / 45 individuals) to 100%, 83% (115 / 139 individuals) to 98% (146

Table 1 Bolting tendency and genetics of the two test strains under natural sunlight conditions in the summer outdoor field

Materials	Bolting tendency				Weight/root (g)	
	2014		2015		2014	
	N_T	N_B	N_T	N_B	average	sd
NK-420 mm-O	0	11	0	42	773.4	496.8[bc 5]
NK-422 mm-O	0	30	–	–	735.1	620.8[bc]
F_1 (NK-377 × NK-420)	0	30	–	–	1084.4	518.0 [cd]
F_1 (NK-377 × NK-422)	0	30	–	–	1274.2	927.7[d]
F_2 (NK-310 × NK-420)	–	–	0	120	–	–
F_2 (NK-310 × NK-422)	–	–	0	120	–	–
BC_1F_1 (NK-377 × NK-420 × NK-310)	–	–	0	120	–	–
BC_1F_1 (NK-377 × NK-422 × NK-310)	–	–	0	85	–	–
TA-33BB-O	30	30	15	15	54.2	19.4[a]
Biennial strains [1]	0	30	0	38	–	–
F_1 (NK-377 × TA-33BB)	12	12	–	–	395.5	259.7[ab]
BC_1F_1 (NK-377 × TA-33BB × NK-310)	–	–	26	59 ns [4]	–	–

[1]Monohikari (2014), NK-377 mm-O (2015)

[2]NT: total number of individuals tested

[3]*significant difference at 5% level in the chi-squared test compared to the expected segregation ratio of 3: 1 (F_2) or 1: 1 (BC_1F_1)

[4] ns: no significant difference at 5% level in the chi-squared test compared to the expected segregation ratio of 3: 1 (F_2) or 1: 1 (BC_1F_1)

[5]Differential alphabet indicates that there is a significant difference at 5% level in the Tukey–Kramer multiple comparison test

/ 149 individuals), and 48% (71 / 147 individuals) to 55% (82 / 149 individuals), respectively, in 2015. In the chi-square test, assuming a single dominant gene control, the segregation ratios of bolting and non-bolting were found to be significantly different from the separation ratio of 3:1 in the F_2 generation ($p < 0.01$), but did not differ from the separation ratio of 1:1 in the BC_1F_1 generation ($p > 0.05$).

In the winter greenhouse, similar to the results of summer greenhouse test, bolting rates were 0% for the biennial reference strains and 100% for the annual reference strain in the two years. The two test strains exhibited the same bolting tendency as the annual reference strain. The bolting rates was between 95% (21/22 individuals) to 100% (Table 2). For strains obtained by crossing the annual reference and biennial reference strains, the bolting rate of BC_1F_1 was 68% (61/90 individuals) in 2014. For strains obtained by crossing the test strains and biennial reference strains, bolting rates of two F_2s and BC_1F_1s ranged from 63% (169/270 individuals) to 74% (193/262 individuals) and from 47% (42/90 individuals) to 53% (48/90 individuals), respectively. In the chi-square test, assuming a dominant one-gene control, the segregation ratios of bolting and non-bolting were significantly different from the separation ratio of 3:1 in the F_2 generation in one of the two cases ($p < 0.01$) but did not differ from the separation ratio of 1:1 in the BC_1F_1 generation ($p > 0.05$).

There was no significant difference between the number of days until bolting for the annual and test strains. However, 'NK-422 mm-O' had slightly more days until flowering than 'NK-420 mm-O' and 'TA-33BB-O'. The F_1s of the biennial and test strains also tended to be slightly slower to bolt than the annual and test strains (Table 3). These results suggest that bolting in the test strains occurs without exposure to low temperatures, similar to the annual strain, and is likely controlled by a single or a small number of dominant genes with strong effects. The broad sense heritability (H_B) of 'NK-422 mm-O' was relatively high (0.66) and that of 'NK-420 mm-O' was extremely high (1.00). Both test strains showed that genetic factors can explain most of the early flowering characteristics under 24-h daylength conditions.

Table 2 Bolting tendency and genetics of the two test strains under 24-h light conditions in a greenhouse

Materials	Summer greenhouse				Winter greenhouse				H_B [6]
	2014		2015		2013		2014		
	N_T	N_B	N_T	N_B	N_T	N_B	N_T	N_B	
NK-420 mm-O	8	8	12	12	6	6	27	27	1.00
NK-422 mm-O	36	36	11	11	22	21	27	27	0.66
F_1 (NK-377 × NK-420)	45	45	–	–	–	–	–	–	–
F_1 (NK-377 × NK-422)	45	38	–	–	–	–	–	–	–
F_2 (NK-310 × NK-420)	–	–	139	115* [4]	–	–	270	169*	–
F_2 (NK-310 × NK-422)	–	–	149	146*	–	–	262	193 ns	–
BC_1F_1 (NK-377 × NK-420 × NK-310)	–	–	149	82 ns 5	–	–	90	48 ns	–
BC_1F_1 (NK-377 × NK-422 × NK-310)	–	–	147	71 ns	–	–	90	42 ns	–
TA-33BB-O	36	36	24	24	18	18	27	27	–
Biennial strains [1]	18	0	18	0	18	0	27	0	–
F_1 (NK-377 × TA-33BB)	16	16	–	–	–	–	–	–	–
BC_1F_1 (NK-377 × TA-33BB × NK-310)	–	–	–	–	–	–	90	61*	–

[1]Summer: Monohikari (2014), NK-377 mm-O (2015)

Winter: NK-280 mm-O (2013), NK-377 mm-O (2014)

[2]NT: total number of individuals tested

[3]NB: number of bolting individuals

[4]*: significant difference at 5% level in the chi-squared test compared to the expected segregation ratio of 3: 1 (F_2) or 1: 1 (BC_1F_1)

[5] ns: no significant difference at 5% level in the chi-squared test compared to the expected segregation ratio of 3: 1 (F_2) or 1: 1 (BC_1F_1)

[6]H_B: Broad-sense heritability

Table 3 Days to bolting under 24-h light conditions in a greenhouse

Materials	Summer greenhouse		Winter greenhouse	
	2014	2015	2014	2015
NK-420 mm-O	36.0^{a} [1]	35.0^{a}	48.6^{a}	42.3^{a}
NK-422 mm-O	36.5^{a}	37.0^{b}	55.8^{b}	48.0^{b}
F_1 (NK-377 × NK-420)	37.0^{a}	–	–	–
F_1 (NK-377 × NK-422)	40.8^{b}	–	–	–
TA-33BB-O	36.0^{a}	35.1^{a}	51.9^{ab}	45.6^{ab}
F_1 (NK-377 × TA-33BB)	36.6^{a}	–	–	–

[1]Different alphabets indicate significant differences at 5% level in the Tukey kramer's multiple test

Incubator test

The bolting rate of the biennial control strain was 0% under all conditions (Table 4a). The rate of the annual control strain was 100% (in the 18-h IL, 24-h IL, and SL + IL), except under the conditions of a relatively short daylength in the incubator (14-h IL) or in the greenhouse (SL). Stem length tended to increase as day length increased in both the incubator and the greenhouse, with a maximum length of approximately 190 mm (Table 4b). The bolting tendencies of the two test strains differed depending on the test conditions. The same trends as those of the annual strain were observed in the rates of 'NK-420 mm-O', bolting rate was 100% except under the condition of a relatively short daylength (Table 4a). The bolting stem length exhibited the same trend as the annual strain. The stem length was longer under 24-h IL (400 mm) and SL + IL (383.3 mm) than under 18-h IL (13.3 mm) (Table 4b). On the other hand, the bolting rate of 'NK-422 mm-O' was 100% only in the greenhouse with nighttime illumination (SL + IL), whereas these were 0% under SL and all incubator conditions (14-h IL, 18-h IL, and 24-h IL) (Table 4a).

Table 4 a Bolting rate (%) under different light conditions. b Bolting stem length (cm) under different light conditions

Materials	Incandescent light (IL [1])			Winter sun light (SL [2])	
	14 h	18 h	24 h	24 h (SL + IL)	9–11 h (SL)
a					
NK-420 mm-O	0	3* [3]	3*	3*	0
(sd)	0	0	0	0	0
NK-422 mm-O	0	0	0	3	0
(sd)	0	0	0	0	0
TA-33BBmm-O	0	3*	3*	3*	0
(sd)	0	0	0	0	0
NK-310 mm-O	0	0	0	0	0
(sd)	0	0	0	0	0
Materials	Incandescent light (IL [1])			Winter sun light (SL [2])	
	14 h	18 h	24 h	24 h (SL + IL)	9–11 h (SL)
b					
NK-420 mm-O	0.0	13.3* [3]	400.0*	383.3*	0.0
(sd)	(0.0)	(6.1)	(85.4)	(41.6)	(0.0)
NK-422 mm-O	0.0	0.0	0.0	256.7*	0.0
(sd)	(0.0)	(0.0)	(25.0)	(49.3)	(0.0)
TA-33BBmm-O	0.0	93.3*	116.7*	190.0*	0.0
(sd)	(0.0)	(41.6)	(32.1)	(81.9)	(0.0)
NK-310 mm-O	0.0	0.0	0.0	0.0	0.0
(sd)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)

[1]Incubator: an incandescent lamp (700 lx)

[2]Glasshouse: daytime sunlight (~ 100,000 lx) and nighttime illumination by an incandescent lamp (300 lx, 18:00 to 5:00)

[3]Significance difference at 5% level from the biennial 'NK-310 mm-O' by using the Mann–Whitney U test

Discussion

Unique flowering characteristics of the test strains were named BLOND

This study demonstrated that the flowering characteristics of the two test strains were clearly different from those of typical annual and biennial beets and that these varied in annual and biennial beets depending on the growth conditions. Bolting, a trait that occurs in the early stages of flowering, did not occur in the two test strains under outdoor field conditions, similar to that in the biennial beet; however, it promptly occurred without vernalization treatment under 24-h daylength greenhouse conditions, similar to that in the annual beet (Fig. 2). The specific flowering characteristics observed in the test strains suggest that their responses to vernalization and day length are different from those of annual and biennial beets. Based on these results, we hypothesized that the two strains retained vegetative growth and required vernalization to initiate flowering under outdoor field conditions (i.e., natural day length) but lost the vernalization requirement and initiated flowering under daylength conditions longer than natural daylength (e.g., 24-h daylength), and proposed to name their flowering characteristics 'BLOND' (Bolting by longer than natural day length, Table 5).

Previous studies have reported beet strains with flowering characteristics similar to those of BLOND. For example, Owen et al. (1940) showed that strain 'Clone70' did not initiate bolting in a Salt Lake City field without vernalization treatments, but initiated bolting under a 17-h daylength condition in a greenhouse (winter natural day length plus nighttime supplemental light). Smit (1983) reported that the strain 'G4' did not initiate bolting under natural daylength conditions in a greenhouse (14-h daylength) without

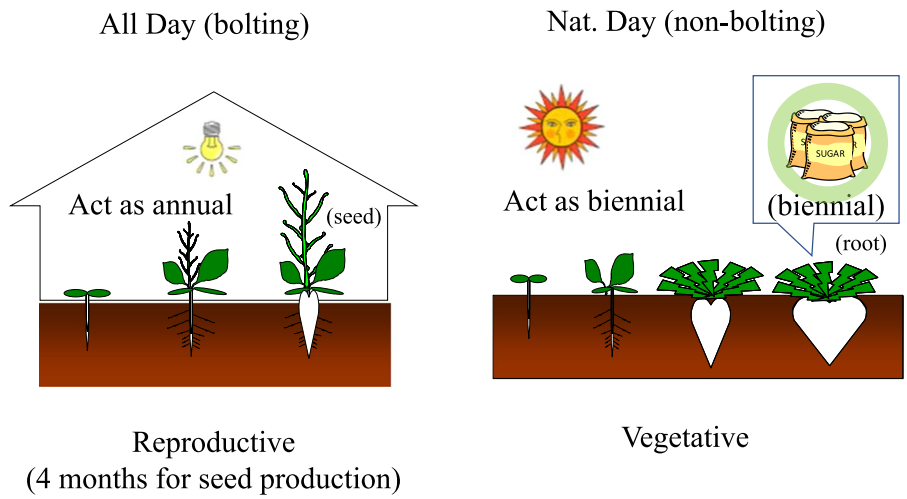

Fig. 2 Flowering characteristics of sugar beet strains suitable for fast breeding. These strains combine the advantages of flowering characteristics of biennial and annual beets. They continue to grow vegetatively like biennial beets under natural daylength conditions, but they can flower quickly under long days without exposure to cold temperatures like annual beets

Table 5 Bolting tendency without vernalization treatment

Type	Gene	Field (Summer)	24 h light
BLOND[1]	*Bd*	Non-bolting[2]	Bolting[3]
Biennial	*b*	Non-bolting	Non-bolting
Annual	*B*	Bolting	Bolting

[1]Bolting by longer than natural day length

[2]Suppress bolting during cultivation for sugar production

[3]Promote flowering during cultivation for seed production

vernalization treatments, but initiated bolting under a 24-h daylength condition in a greenhouse (natural daylength plus nighttime supplemental light). The flowering characteristics of these strains were similar to those of BLOND but not as sharply responsive to the emergence of bolting as the BLOND strains used in this study, which, along with the annual strains, attained almost 100% bolting rate at approximately 40–50 days after sowing, whereas 'Clone70' attained almost 50% bolting rate approximately 50 days after sowing, and 'G4' attained almost 70% bolting rate approximately 80 days after sowing.

Light conditions are the main factors affecting bolting

Two environmental factors are considered essential for inducing the reproductive growth of sugar beets: vernalization and daylength (Ream et al. 2012; Melzer et al. 2014). In this study, none of the outdoor or greenhouse conditions included exposure to the low temperatures necessary to induce vernalization (Milford 2006); therefore, the main factor that might cause the loss of vernalization requirement is daylength. There was a large difference in daylength between the two growth conditions. The outdoor daylength at Memuro (42.9°N, 143.1°E), where the survey was conducted, was between 9.1 h (late December) and 15.4 h (late June), whereas in the greenhouses, light supplementation at night extended the length of the day to 24 h.

The importance of daylength has been demonstrated by conducting experiments under artificial light conditions in an incubator (Hoft et al. 2017). The results of these experiments identified at least three beet strains that did not initiate bolting (0%) under a 16-h daylength condition but did so (100%) under a 22-h daylength. One of these was a fodder beet (seed code: 080396) and the other was a leaf beet (seed code: 081845), the same species as the sugar beet, which exhibited relatively short (approximately 40 days) time to bolting. The third beet was wild (seed code: 080538); however, the number of days to bolting was relatively high (approximately 70 days). Although outdoor field tests were not conducted, the flowering characteristics of these strains were similar to those of the BLOND strains used in the current study.

In contrast to the experiment conducted in the incubator using only incandescent lamps (100 W) as the light source, this study showed that even under the same 24-h daylength conditions, the two BLOND strains exhibited very different bolting rates. Bolting rate and stem length of 'NK-420 mm-O (BLOND)'

and 'TA-33BB-O (annual)' tended to increase with daylength, whereas 'NK-422 mm-O (BLOND)' did not bolt under any daylength condition. However, all strains, including 'NK-422 mm-O', exhibited rapid bolting induction under sunlight and night supplemental light using an incandescent lamp (200 W). Because the intensity of natural light (~ 100,000 lx) is much higher than that of incandescent light (300 lx), and an incandescent lamp has a relatively high proportion of the infrared spectrum compared to sunlight, the light intensity and spectrum are also considered to be related to bolting development.

Major genetic factors are presumed to be few: proposal of the responsible gene (Bd)

Although this study did not provide much information on the estimation of related genes or loci, the following arguments can be made: First, in the two generations of F_1 and BC_1F_1 obtained from crosses between BLOND and biennial plants, the model of a single dominant gene fit well, suggesting that a single major gene controls the flowering characteristics of BLOND. There may be other minor genes involved, as the segregation ratio of F_2 exhibited a tendency to be higher in summer and lower in winter than the 75% segregation ratio expected from the model.

Second, the flowering characteristics of BLOND cannot be explained solely by the key (*B*) locus, which controls the response to vernalization among several loci involved in flowering without vernalization treatments (Hohmann et al. 2005; Buttner et al. 2010; Hoft et al. 2017). The *BvBTC1* gene, which is the causative gene of the *B* locus, of the BLOND strains used in this study is known to be haplotype 'g'; nevertheless, there are many strains with haplotype 'g' that do not exhibit BLOND characteristics (Kuroda et al. 2019). Therefore, it is unlikely that the flowering characteristics of BLOND are determined solely by the *B* locus and that other related loci are involved.

Third, although *B'* (Owen et al. 1940) has many features in common with BLOND (described below), the flowering characteristics of BLOND cannot be explained by *B'*, which is allelic to *B*. (1) *B'* is dominant over *b*. (2) Plants with *B* are strictly annual, whereas plants with *B'* remain vegetative under field conditions, and plants with *B'* bolt as quickly as plants with *B* under relatively low-temperature greenhouse conditions. If *B'* were indeed identical to BLOND, then *B* and BLOND would be allelic (i.e., linked to each other) and the *BvBTC1* haplotype would not explain the flowering characteristics of BLOND as described in the second point. Therefore, *B'* is not the locus of BLOND, and the locus of BLOND exists elsewhere. We propose '*Bd* (daylength)' as the gene responsible for BLOND in this paper (Table 5).

Future research directions

Future research should focus on the following two aspects. The first is the identification of the genomic regions that control BLOND. BLOND is thought to function in a manner that releases the switch of vernalization requirements by daylength, which is an interesting characteristic because daylength and vernalization not only determine the timing of flowering in crops (Blumel et al. 2015; Jung and Muller et al. 2009) but also play a major role in plant adaptation and evolution (Amasino 2010; Ream et al. 2012). We have already started to map populations between BLOND and biennials and have begun to identify genomic regions of *Bd* through molecular biological approaches. The second objective was to clarify the effect of BLOND on the bolting tolerance. Owen et al. (1940) reported that 'Clone70', which exhibits similar characteristics to BLOND lines, has a low vernalization requirement and weak tolerance to bolting. This is termed 'easy bolting' and is a trait that should be improved for sugar production, as it is prone to bolting initiation in the first year of growth (Kuroda et al. 2019). Therefore, it is necessary to investigate whether BLOND functions in a direction that directly weakens the bolting tolerance.

Conclusions

The results of this study indicated that the two BLOND strains have characteristics that can suppress bolting during sugar production and promote flowering during seed production. In other words, from a practical breeding standpoint, BLOND strains could be used for field evaluations of important agronomic traits, such as yield characteristics outdoors, and for quickly generating plants in greenhouses by adding supplemental light at night. In particular, the BLOND strains identified in this study exhibited extremely

rapid emergence in response to bolting in the greenhouse, similar to annual strains. Thus, the time required for one-seed production could be effectively reduced from approximately one year to one-third of that required for conventional lines (approximately four months), similar to that of annual beets. We believe that this method will accelerate the development of new breeding strains and varieties.

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Declarations

Conflict of interests The authors declare that they have no conflict of interest.

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