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Genetic analysis and molecular mapping of the early flowering gene(s) introgressed from the late flowering species *Brassica oleracea* into *B. napus*

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Contents

1. Background

Earliness of flowering is an important trait in *Brassica napus* (AC genome, *n* = 19) canola due to short growing season in the Canadian prairies. Traditionally, *B. rapa* (A genome, *n* = 10) has been grown in Alberta, the Peace River region of British Columbia and northern Saskatchewan where crop growing season is relatively short. This crop species was generally favored by the growers in the Prairie Provinces, especially in the short growing season zones, primarily due to its early maturity. With the development of early maturing *B. napus*, the acreage of *B. rapa* gradually declined to <2% of the total canola acreage in Canada. This is a huge change for *B. rapa* acreage when compared with the acreage in late 1980's; at that time, this species equally shared the acreage with *B. napus*. This reduction in *B. rapa* acreage was primarily due to the development of relatively early maturing herbicide tolerant *B. napus* cultivars that fitted to some extent in the short growing season areas; as well as due to yield advantage associated with early maturing *B. napus* when compared with *B. rapa*. However, the level of earliness achieved so far in *B. napus* does not exclude a predisposition of this crop to the risk of frost damage. Frost damage not only reduces seed yield but also affects the seed oil quality (Daun 1980). Therefore, further improvement of earliness in *B. napus* would be desired. Currently, hybrid *B. napus* cultivars has captured more than 90% canola acreage in Canada and this type of cultivars tend to be slightly late maturing as compared to the open-pollinated cultivars. In this regard, improvement of earliness in hybrid canola cultivars would be desired.

Days to flowering in spring type *B. napus* is a quantitative trait, and is governed by both additive and dominance types of genes (Ringdahlet al. 1986) and exhibits high heritability (Cruz et al. 2007, Ringdahl et al. 1986, Schranz et al, 2002). In Brassica oilseed crop, this trait correlates well with days to maturity (Mahmood et al, 2007, Miller 2001), and flowering data from greenhouse generally agree with field data (Cruz et al. 2007). Therefore, selection for earliness of flowering will reflect earliness of maturity, and it is possible to made selection under greenhouse conditions. Thus knowledge on the gene(s) controlling earliness of flowering will facilitate the development of early flowering/maturing canola cultivars.

Flowering time is a complex trait, regulated by different pathways. Genetic and molecular analysis of the model plant *Arabidopsis* has unveiled this trait to a great extent (reviewed in Jung

and Müller 2009, Kim et al. 2009, Turck et al. 2008, Putterill et al. 2004). Different pathways, such as photoperiod, vernalization, and autonomous, and their interactions are involved in the control of this trait. The key regulator in the photoperiod pathway is the *CONSTANS* (*CO*) gene. This gene under long-day condition and at the end of the light phase accumulates CO protein which activates the transcription of the *FLOWERING LOCUS T* (*FT*) gene to initiate flowering. The *FT* is a central integrator gene, receive signals from different pathways. Protein of this gene interact physically with *FLOWERING LOCUS D* (*FD*) protein in the meristem; and this complex activate the third floral integrator gene *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and the floral meristem identity genes *SEPALATA* (*SEP*), *FRUITFUL* (*FUL*) and *APETALA 1* (*AP1*) and triggers flowering. The CO protein does not accumulate under short day; therefore, activation of *FT* transcription does not occur and delay flowering. In case of the vernalization pathway, *FLOWERING LOCUS C* (*FLC*) is the key regulator gene which represses the expression of the floral integrator genes *FT*, *FD* and *SOC1* and result failure of flowering. However, *FLC* is down-regulated by vernalization and thus enable promotion of flowering by the floral integrator genes. There are evidences to support that the *FLC*'s is also involved in autonomous flowering (Kole et al. 2001, Michaels and Amasino 2001). The *FLC* is activated by its upstream regulator *FRIGIDA* (*FRI*) gene; these two genes (*FRI* and *FLC*) are therefore the major determinants for natural variation of flowering.

Quantitative trait loci (QTL) mapping of flowering time in *Brassica,* based on population derived from crossing of genotypes of different growth habit, have detected several loci with variable effect on the trait. The A genome chromosomes A2, A3, A7 and A10 of *B. rapa* (Osborn et al. 1997, Axelsson et al. 2001, Kole et al. 2001, Schranz et al. 2002, Lou et al. 2007, Li et al. 2009, Kitamoto et al. 2014) and the C genome chromosomes C1, C2, C3, C5 and C9 of *B. oleracea* (Bohuon et al 1998, Rae et al. 1999, Axelsson et al. 2001, Okazaki et al. 2007) reported to carry major QTL affecting flowering time. Among these, the QTL on A10 (Osborn et al. 1997, Schranz et al. 2002) and C2 (Okazaki et al. 2007) play greatest role in vernalization-responsive flowering. Some of the important flowering genes were also identified in the QTL regions affecting flowering. For example, in case of *B. rapa*, the major photoperiod-responsive gene *CO* was identified in the QTL region of A2 (Axelsson et al. 2001); and duplicated copies of the vernalization-responsive gene *FLC*, viz. *FLC1*, *FLC2*, *FLC3* and *FLC5*, were detected in the

QTL regions of A10, A2, A3 and A3, respectively (Schranz et al. 2002, Kim et al. 2006, Yang et al. 2006, Lou et al. 2007, Li et al. 2009, Xiao et al. 2013, Kitamoto et al. 2014). These *FLC* gene copies found to be located near the telomeric region of the chromosomes. Molecular analysis of the *FLC1* of *B. rapa* revealed that a natural mutation of G → A at the first nucleotide (5') site in 6th intron results aberrant splicing products which is associated with early flowering (Yuan et al. 2009). In case of the C genome of *B. oleracea*, the *CO* gene was identified in the QTL region of C3 and C9 (Axelsson et al. 2001), and the *FLC1*, *FLC2*, *FLC3* and *FLC5* were identified in the QTL region of C9, C2, C3 and C3, respectively. Theoretically, all these flowering time loci of the A and C genomes of *B. rapa* and *B. oleracea* are accumulated in *B. napus*. Indeed, all these chromosomes as well as the chromosomes A4, A6, C6 and C8 carrying flowering time QTL have been identified in *B. napus* by different researchers (Ferreira et al. 1995, Osborn et al. 1997, Schranz et al. 2002, Long et al. 2007, Mei et al. 2009, Wang et al. 2009, Raman et al. 2013, Luo et al. 2014). Zou et al. (2014) also identified flowering time QTL on the C genome linkage groups C2, C3, C4, C6, C7 and C8 of *B. carinata*.

Wang et al. (2009) reported that the C genome chromosome C6 carry a FT gene for flowering time. The genomic region carrying the FT gene corresponds to the block E in chromosome 1 of *Arabidopsis thaliana*; however, this block is inversely duplicated in the C genome chromosome C6.

Robert et al. (1998) isolated and characterized four *B. napus* homologues of the Arabidopsis *CO* gene and mapped to the linkage groups A10 and C9. Lou et al. (2014) also mapped a photoperiod-responsive QTL on C9. Cai et al. (2008) reported four photosensitive QTL on the chromosomes A3, A10, C4 and C8 of *B. napus*. Thus, the genomic regions of three A genome chromosomes, A2, A3 and A10, and four C genome chromosomes, C3, C4, C8 and C9, may carry genes involved photoperiod pathway affecting flowering time in *B. napus* (Robert et al. 1998, Axelsson et al. 2001, Cai et al. 2008, Lou et al. 2014).

Very little information is available in case of mapping of flowering time gene involved in autonomous pathway. Based on a DH population derived from crossing of two spring type parents, Lou et al. (2014) provided evidence that the chromosome C8 carry a QTL which control flowering time by autonomous pathway in *B. napus*.

In case of the *FLC*, nine homologues, four from the A genome and five from the C genome were cloned from *B. napus*, and these genes are mapped on the chromosomes A2, A3, A10, C2, C3 and C9 (Tadege et al. 2001, Zou et al. 2012). The coding sequences of these *FLC* homologues are relatively conserved; however, show polymorphism primarily in the intronic and promotor regions (Zou et al. 2012). Seven of the nine *B. napus FLC* homologues located in the collinear region of *FLC* in Arabidopsis genome block R (at one end of chromosome V, Schranz et al. 2006) while two *FLC*'s from A3 and C3 located in the genome block J (at one end of chromosome II). The chromosome A10 of *B. napus* carry the largest effect vernalization responsive flowering time QTL *qFT10-4* (Long et al. 2007). Hou et al. (2012) fine mapped the *qFT10-4* and cloned an ortholog of *FLC* (*BnFLC.A10*) from this region. Sequence comparison (approximately 7 kb) of two alleles of this *FLC* disclosed that a miniature inverted-repeat transposable element (MITE) upstream of *BnFLC.A10* (not in coding region) is strongly associated with vernalization requirement. The MITEs are short (< 500 bp) AT-rich sequence, bear terminal inverted repeats (TIRs; 10–15 bp), and inserted predominantly in gene-rich regions and can result phenotypic variation (for review see Fattash et al. 2013). The MITE sequence that detected in winter type was absent in *BnFLC.A10* allele from spring type (Hou et al. 2012). Thus, the MITE insertion/deletion in *BnFLC.A10* gene seems to be one of the major causes of differentiation between winter and spring growth habit type.

The *FRI* gene, the key regulator of *FLC*, was identified on the *B. napus* chromosome A3 and resides in the chromosome region where a major flowering time QTL was mapped (Wang et al. 2011). This genomic region also found to carry a *FLC* (*BnFLC.A3b*) gene (Zou et al. 2012). In case of the C genome, *FRI* was mapped on the chromosomes C3 and C9 of *B. oleracea* (Irwin et al. 2012). This gene in *A. thaliana* is located at one end of the chromosome IV (Irwin et al. 2012).

Thus, it is apparent that several gene loci are involved in the control of flowering time variation in *B. napus*; and allelic variation for these genes does exist (Yuan et al. 2009, Wang et al. 2011). Given that very limited variants of the diploid parental species *B. rapa* and *B. oleracea* were involved in the origin of *B. napus*, it is highly unlikely that all allelic variation of these loci that result earliness of flowering has been included in *B. napus* during its evolution. Therefore,

further improvement of earliness in *B. napus* is possible through exploitation of the allelic variants of its parental species.

The oilseed *B. napus* is considered a long-day plant. According to King and Kondra (1986), the minimum optimum photoperiod (MOP) for the Canadian *B. napus* cultivars is about 18 hrs. The natural day length for majority of the canola growing areas in Canada is about 15 to 17 hrs prior to flowering; this indicate that MOP may not be reached under this growth condition. Therefore, identification of gene(s) controlling photo insensitivity and reduced basic vegetative period (juvenile to before flowering) would be important for the development of early flowering/maturing *B. napus* canola cultivars for the Canadian prairies. QTL mapping studies so far have identified the chromosomes A2, A3, A10, C3, C4, C5 and C9 carry photosensitivity genes affecting flowering time (Robert et al. 1998, Axelsson et al. 2001, Cai et al. 2008, Lou et al. 2014).

The early flowering genotypes generally produce low seed yield. This might be due to the negative effect of at least some (if not all) of the early flowering allele(s) on physiology of the plant. Leaf area index prior to flowering generally correlates well with seed yield in *B. napus* (Allen and Morgan 1975, Chongo and McVetty 2001). Higher leaf area index can be achieved in relatively late flowering cultivars which would have limited value for the short growing season areas. Alternatively, this can be achieved in early flowering type with faster expansion of leaves. Our knowledge of the genes controlling earliness and their effect on physiology of the plant and seed yield in canola is limited. By use of molecular markers linked to the flowering time gene, it would be possible to generate isogenic lines for the individual flowering allele(s) to understand the effect of these genes at the physiological level in the plant. For this, identification of molecular markers linked to these genes would be needed.

Among the six *Brassica* species of U's triangle, the *B. rapa* flowers and matures earlier than any other species. On the other hand, *B. oleracea*, the other parental species of *B. napus*, flowers and matures much later than both *B. napus* and *B. rapa*. Therefore, several researchers utilized earliness of *B. rapa* for the improvement of earliness in *B. napus* (Kubik et al. 1999, Miller 2001). *Brassica oleracea* generally flowers and matures much later than any other Brassica species. However, considerable variations for days to flower exist in this species, where it's earliest flowering variant flowers 3 to 4 weeks later than spring type *B. napus*. To our

knowledge, no research has been conducted so far to understand the early flowering gene(s) of *B. oleracea* and its implication for the improvement of earliness in *B. napus*. In a research project, previously funded by Natural Sciences and Engineering Research Council (NSERC) and Alberta Canola Producers Commission (ACPC), we conducted interspecific hybridization between *B. napus* (cv. Hi-Q) and *B. oleracea* var. *alboglabra* (hereafter referred to as *B. alboglabra*) for the improvement of earliness in *B. napus* canola. From this project we developed a *B. napus* line which flowers about a week earlier than the *B. napus* parent Hi-Q through reconstitution of its C genome with the C genome of *B. alboglabra* (Rahman et al. 2011).

Objectives: The long-term goal of this research project is to develop early flowering and maturing *B. napus* canola germplasm and cultivars without sacrificing seed yield for the Canadian prairies. In the short-term and during this project period, the following objectives were laid out:

(i) understand the genetic control of flowering and maturity in the early flowering *B. napus* line where earliness was introgressed from *B. alboglabra*;

(ii) map this earliness gene(s) and identify molecular markers for use in marker assisted breeding; and

(iii) study the effect of this introgressed early flowering gene(s) on physiology of the plant.

Knowledge gained from this research can be used by canola breeders for the development of early maturing and high yielding canola cultivars for the Canadian prairies.

2. Methodology

2.1. Plant Materials

The early flowering *B. napus* inbred line (RIL-144) used in this research was developed from an interspecific cross of *B. napus* cv. Hi-Q × *B. alboglabra*. Theoretically, the A genome of this line will be exactly same as the C genome of Hi-Q, while its C genome will carry allele(s) from *B. alboglabra* contributing to the earliness of flowering. Any segregating population generated from crossing of this line with Hi-Q would therefore obviously reflect the genetic variation for the C genome allele's introgressed from *B. alboglabra*. With this hypothesis, the early flowering *B. napus* line RIL-144 was crossed to the *B. napus* parent Hi-Q, and doubled haploid (DH) lines were produced from F_1 through microspore culture. The detail of the technique is reported elsewhere (Kebede et al. 2010). The F_1 plants were also self-pollinated for F_2 seeds, as well as backcrossed to both parents for backcross seeds.

2.2. Evaluation for Days to Flower

The DH lines were evaluated for days to flower (DTF) under controlled environmental conditions in growth chamber under 18, 14 and 10 h photoperiod with 20 ºC and 18 ºC constant temperature as well as under 16 h photoperiod with 20/16 ºC and 18/8 ºC (day/night) temperature. The detail of the growth chamber experiments is summarized in Table 1. Each of these growth chamber experiments were repeated 3 to 5 times, except the 10 h experiment which was conducted two times, and these constituted the 2-5 replications. In each replication, 2 to 4 plants of each DH line were grown and the average value of these plants was used in statistical analysis. The two parents and their F_1, as well as an early flowering *B. napus* cv. Peace were included in the experiments for comparison. Peace is an early flowering and maturing *B. napus* cultivar developed at the University of Alberta, and registered in Canada in 2001. For all these experiments, except the 16 h photoperiod and 18/8 ºC experiment, the plants were grown in 32-cell tray with pot of 7 cm × 7 cm × 9 cm (L × W × D) size filled with Sunshine Professional Growing Mix (Sunshine Horticulture, 15831 N.E. Bellevue, USA). In case of the 16 h

photoperiod and 18/8 ºC experiment, plants were grown in 10 cm × 10 cm × 10 cm (L × W × D) size pots. Data on days to flower was recorded at three-open-flower stage.

The parental, F_1, F_2 and backcross populations were evaluated for days to flower under 10 and 18 h photoperiod and 18 ºC constant temperature; and the plants were grown in 32-cell tray with pot of 7 cm × 7 cm × 9 cm (L × W × D) size. Due to limited space in the growth chamber, these populations were grown in two to three times in the same growth chamber.

In addition to the growth chamber experiments, the DH lines were evaluated in replicated field trials in South Campus (Edmonton Research Station) and St. Albert Research Farm of the University of Alberta in 2010, 2011, 2012 and 2013 for days to flower. A summary of the field trials is presented in Table 2. Seeding was done either in single row plots of 2.0 m long with plot to plot distance of 0.6 m or in full plots of 5.0 m × 1.3 m or 5.0 m × 1.1 m size. Self-pollinated seeds were used for this study. Days to flower were recorded on a plot basis as the number of days from seeding to approximately 50% plants in the plot having at least one open flower.

2.3. Evaluation for Seed Yield

For yield trials, open-pollinated seeds of the DH lines and the parents collected from the flowering time trial conducted in 2010 in field (South Campus) were used. Yield trials conducted in Alberta in 2011, 2012 and 2013; a summary of the trials is presented in Table 3. Due to large number of entries in the trials, a modified randomized complete block design, as described by Mahmood et al. (2007), was used. For this, the 97 DH lines, RIL-144, and the F_1 (generated from additional Hi-Q × RIL-144 crosses) were nested into three blocks and randomized within each block. Two checks, Hi-Q and Peace, were included in each block.

2.4. Study of the Effect of Earliness on Plant Physiology

To study the effect of the earliness, introgressed from *B. alboglabra* into *B. napus*, on physiology of the plant and seed yield, a set of near-isogenic lines for the genes contributing to this earliness would be needed. For this, mapping of the flowering time and identification of molecular markers linked to these genes/alleles would be needed. Therefore, within the scope of this

research project, a preliminary investigation was carried out by analyzing the DH lines for dry matter (biomass); and the relationship of flowering time and dry matter with seed yield in this DH population. For this, the DH lines were grown in a growth chamber at 16 h photoperiod and 18/8 ºC (day/night) temperature [this is 30 years average of high and low temperature in Edmonton region (the canola mid-season zone) in May to June (http://www.theweathernetwork.com/statistics/CL3012209/caab0103/)] and were evaluated for leaf, root and total dry matter. The plants were grown in 10 cm × 10 cm × 10 cm (L × W × D) size pots filled with compost-free soil with added micronutrients. Uses of this type of soil in the experiment ease washing of the roots for estimation of root dry matter. The parents, F_1 and the early-flowering *B. napus* cv. Peace were included in the experiments for comparison. The experiments were conducted in October 2011, March 2012, October 2012, March 2013 and May 2013. In all experiment's, except the March 2012 experiment, two plants of each DH line were grown; while in the March 2012 experiment, three plants of each DH line were grown. Mean data of these plants were used for statistical analysis, and the different seeding dates constituted the number of replications.

At three-open-flower stage, plants were cut at the soil level and the leaves were detached from the stem and put separately in paper bags. These plant parts were dried at 50 ºC for four weeks and weighed for leaf and stem (which also included the inflorescence) dry matter. The roots were washed under running water and dried at 50 ºC for four weeks and weighed for dry matter. Total dry matter was calculated by summing the leaf, stem and root dry matters.

Table 1. Summary of the experiments conducted in growth chambers for days to flower and dry matter (biomass) content of the doubled haploid (DH), F_2 and backcross populations of Hi-Q × RIL-144 of *Brassica napus*. Earliness of flowering in the line RIL-144 introgressed from *B. oleracea* var. *alboglabra*.

Photo-period	Temp. (day/ night)	Popu-lation[1]	No. DH lines/ plants	No. plants /DH	Pot size[2]	Soil type[3]	Seedin time	Growth chamber
Days to flower:								
18 h	18 ºC	DH	95	3	32-cell	Sun Gro	Oct 2011	R105-A06
18 h	18 ºC	DH	93	3	32-cell	Sun Gro	Jun 2012	R105-A13
18 h	18 ºC	DH	92	3	32-cell	Sun Gro	Jun 2013	R105-A06
16 h	20 ºC	DH	85	2	32-cell	Sun Gro	May 2009	GC163
16 h	20 ºC	DH	85	2	32-cell	Sun Gro	May 2009	GC160
16 h	20 ºC	DH	85	2	32-cell	Sun Gro	Jan 2010	GC16
16 h	20 ºC	DH	85	2	32-cell	Sun Gro	Jan 2010	GC17
16 h	20 ºC	DH	85	4	32-cell	Sun Gro	May 2010	GC
14 h	18 ºC	DH	92	3	32-cell	Sun Gro	Jun 2012	R105-A06
14 h	18 ºC	DH	95	3	32-cell	Sun Gro	Mar 2013	R105-A06
14 h	18 ºC	DH	90	3	32-cell	Sun Gro	Sep 2013	R105-A06
10 h	18 ºC	DH	93	3	32-cell	Sun Gro	Jun 2012	R105-A05
10 h	18 ºC	DH	93	3	32 -cell	Sun Gro	May 2013	R105-A06
16h	18/8 ºC	DH	94	2	10 cm	Soil + micro nutrients	Oct 2012	R105-A06
16h	18/8 ºC	DH	92	2	10 cm	Soil + micro nutrients	Mar 2013	GC 162-N
16h	18/8 ºC	DH	94	2	10 cm	Soil + micro nutrients	May 2013	GC 162-N
16 h	20/16 ºC	DH	94	4	32-cell	Sun Gro	Jan 2011	GC
18 h	18 ºC	F_2	174		32-cell	Sun Gro	Mar 2012	R105-A11
18 h	18 ºC	F_2	111		32-cell	Sun Gro	Feb 2012	R105-A13
18 h	18 ºC	F_2	197		32-cell	Sun Gro	Feb 2012	R105-A06
10 h	18 ºC	F_2	185		32-cell	Sun Gro	Oct 2011	R105-A05
10 h	18 ºC	F_2	206		32-cell	Sun Gro	Oct 2011	R105-A05
18 h	18 ºC	B_1	160		32-cell	Sun Gro	Nov 2011	R105-A06
18 h	18 ºC	B_2	96		32-cell	Sun Gro	Nov 2011	R105-A06
10 h	18 ºC	B_1	93		32-cell	Sun Gro	Nov 2011	R109-A13
10 h	18 ºC	B_2	113		32-cell	Sun Gro	Jun 2012	R105-A06
Leaf dry matter:								
16h	18/8 ºC	DH	85	2	10 cm	Soil + micro nutrients	Oct 2011	R105-A06
16h	18/8 ºC	DH	94	3	10 cm	Soil + micro nutrients	Mar 2012	R109-A29
16h	18/8 oC	DH	94	2	10 cm	Soil + micro nutrients	Oct 2012	R105-A06
16h	18/8 ºC	DH	92	2	10 cm	Soil + micro nutrients	Mar 2013	GC 162-N
Root dry matter:								
16h	18/8 ºC	DH	95	3	10 cm	Soil + micro nutrients	Mar 2012	R109-A29
16h	18/8 ºC	DH	91	2	10 cm	Soil + micro nutrients	Oct 2012	R105-A06
16h	18/8 ºC	DH	91	2	10 cm	Soil + micro nutrients	Mar 2013	GC 162-N
16h	18/8 ºC	DH	95	2	10 cm	Soil + micro nutrients	May 2013	GC 162-N
Total dry matter:								

16h	18/8 °C	DH	95	3	10 cm	Soil + micro nutrients	Mar 2012	R109-A29
16h	18/8 °C	DH	95	2	10 cm	Soil + micro nutrients	Oct 2012	R105-A06
16h	18/8 °C	DH	92	2	10 cm	Soil + micro nutrients	Mar 2013	GC 162-N
16h	18/8 °C	DH	95	2	10 cm	Soil + micro nutrients	May 2013	GC 162-N

[1]B_1 = backcross of (Hi-Q × RIL-144) × Hi-Q; B_2 = backcross of (Hi-Q × RIL-144) × RIL-144.
[2]32-cell = Tray with pot size of 7 cm × 7 cm × 9 cm (L × W × D); 10 cm = pot size of 10 cm × 10 cm × 10 cm (L × W × D).
[3]Sun Grow = Sunshine professional Growing Mix (Sunshine Horticulture, 15831 N.E. Bellevue, USA); Soil + micro nutrients = Compost-free soil with added micronutrients.

Table 2. Summary of experiments conducted in field trails from 2010 to 2013 for days to flower of the doubled haploid (DH) lines from Hi-Q × RIL-144 cross of *Brassica napus*. Earliness of flowering in the line RIL-144 introgressed from *B. oleracea* var. alboglabra.

No. DH lines	No. replication	Plot size	Seeding year	Location
96	2	1 row (2m)	2010	South Campus
90	2	full plot (5m × 1.3 m)	2011	South Campus
96	2	1-row (2m)	2011	South Campus
85	2	full plot (5m × 1.3 m)	2011	St. Albert
96	2	1-row (2m)	2011	St. Albert
91	2	full plot (5m × 1.1 m)	2012	South Campus
92	2	1-row	2012	South Campus
90	2	full plot (5m × 1.1 m)	2012	St. Albert
93	3	1-row	2013	South Campus
92	2	full plot (5m × 1.1 m)	2013	South Campus
92	2	full plot (5m × 1.1 m)	2013	St. Albert

Table 3. Summary of the experiments conducted in field trails for seed yield of the doubled haploid (DH) lines of Hi-Q × RIL-144 cross of *Brassica napus*. Earliness of flowering in the line RIL-144 introgressed from *B. oleracea* var. *alboglabra*.

No. DH lines	No. replication	Plot size	Seeding year	Location
90	2	full plot (5m × 1.3 m)	2011	South Campus
85	2	full plot (5m × 1.3 m)	2011	St. Albert
91	2	full plot (5m × 1.1 m)	2012	South Campus[a]
90	2	full plot (5m × 1.1 m)	2012	St. Albert[a]
92	2	full plot (5m × 1.1 m)	2013	South Campus[b]
92	2	full plot (5m × 1.1 m)	2013	St. Albert
92	2	full plot (5m × 1.1 m)	2013	Killam

[a]Seed yield data was not included due to heil damage.
[b]Seed yield data was not included due to damage caused by root maggot.

2.5. Molecular Mapping of Flowering Time and Other Traits

Genomic DNA was extracted from leaf samples of the parents and the DHs using Sigma DNA extraction Kit (Sigma-Aldrich, St Louis) following manufacturer's instruction. Simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers, as well as SSR markers developed based on flowering time gene sequence were used to construct the genetic linkage map and to identify chromosomal location of the flowering genes. The SSR markers were collected from Agriculture and Agri-Food Canada (AAFC) through a material transfer agreement, and from the papers published by Lowe et al. (2004), Piquemal et al. (2005), Suwabe et al. (2006), Cheng et al. (2009), and Biotechnology and Biological Science Research Council, UK, (http://www.brassica.bbsrc.ac.uk/BrassicaDB). In case of AFLP, 15 AFLP primer combinations were used.

Forty SSR primer pairs were designed based on 13 key flowering time genes of *Arabidopsis thaliana* as reported by Ehrenreich et al. (2009). For this, DNA sequence alignment information from *A. thaliana* (Ehrenreich et al. 2009, File S1) was used to perform a BLAST search on The National Center for Biotechnology Information (NCBI) database, and primers were designed from publicly available homologous sequences in *B. napus*, *B. oleracea*, and *B. rapa*. Where no *Brassica* homologous sequences were found, sequence information from *A. thaliana* was used directly. In this case, the exon regions were identified using the software GENSCAN (Burge and Karlin 1997) and primers were designed from these regions. In addition to this, six SSR primer pairs were designed from *A. thaliana FLOWERING LOCUS T (FT)* gene by the use of sequence information from the Arabidopsis Information Resource (TAIR) (https://www.arabidopsis.org/), and 20 primers were designed from the *FLOWERING LOCUS C (FLC1, FLC2, FLC3, FLC4*), *FLOWERING LOCUS T (FT), CONSTANS (CO)* and *FRIGIDA* (*FRI*) gene sequences of *B. oleracea* and *B. rapa* obtained from NCBI database. All these primers were designed using the software Primer3 (Rozen and Skaletsky 2000). Ten flowering time gene based markers published by Razi et al. (2008) and Wang et al. (2009), and four *FRIGIDA* gene based markers obtained from Dr. Christian Jung, University of Kiel, Germany were also used. Thus, a total of 80 flowering time gene based markers were used in this study.

All the above-mentioned markers were evaluated for parental polymorphism, and the polymorphic markers were used to genotype the DH population. DNA fragments were detected using four multiplexed fluorescent dyes by Applied Bio-systems (ABI) system.

The genotypic data of SSR and AFLP marker loci were arranged in a scoring matrix using Microsoft Excel file where the alphabet 'A' was assigned for the alleles derived from Hi-Q and 'B' for the alleles from RIL144. Segregation of the marker alleles to the expected 1:1 ratio (segregation pattern for doubled haploid) was examined (Chi-square test, $P = 0.05$). Markers showing this simple segregation were included for construction of the framework map; markers that showed distorted segregation were added later to this map. Linkage analysis and map construction were done using Mapmaker/EXP 3.0 (Lincoln et al. 1992). A log of the odds (LOD) threshold of 4 and a maximum recombination frequency of 0.4 was used in linkage analysis and to construct the genetic linkage map. Kosambi mapping function (Kosambi 1944) was used to calculate map distances (centimorgans, cM). A linkage map was constructed by comparing the likelihoods of all permutations using the "compare" command. To determine the most likely order of the marker loci within a linkage group, multi-point analyses were performed. The command "ripple" was used to compare the likelihoods of all permutations while constructing the linkage map. The markers loci showing less than 0.01% recombination were initially excluded from the linkage map until the order of the triplets in the linkage map was at least 1000 times greater than any other possible triplet orders. The command "genotype" was used to examine double crossovers. The whole genetic map was re-checked for potential errors and correction made as appropriate. Markers that were initially excluded from the framework map were later added as accessory markers onto the map. For this, 'try' and 'near' commands were used to position the markers onto the linkage map.

QTL analysis of the traits was done using the software QTL cartographer ver. 2.5 (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm) (Wang et al. 2006), and composite interval mapping (CIM) and single marker (SM) analysis methods were followed. In CIM method, the likelihood of a QTL and its effect at every 1 cM intervals with 10 cM windows was estimated, and the flanking markers were identified. To declare a QTL, permutation tests were done (n = 1000 permutations for each) to determine the threshold for LOD or likelihood-ratio (LR) scores using an auto cofactor (type I error rate) of $P = 0.05$. The average of the QTL additive effect and

percent phenotypic variation (R^2) explained by the putative QTL were obtained from the CIM model within the QTL peak area. The whole procedure of QTL mapping was repeated at least four times. Digenic epistasis analysis for additive × additive gene interaction was done using QTLNetwork 2.0 (http://ibi.zju.edu.cn/software/qtlnetwork/ download.htm) (Yang et al. 2008).

2.6. Other Statistical Analysis

Analysis of variance, and different descriptive statistics, such as mean, standard deviation and variances, as well as Spearman's correlation coefficients were calculated using the SAS software program of version 9.3 (SAS Institute, 2004).

3. Results

3.1. Development of Doubled Haploid (DH) Lines

A total of 110 DH lines (Table 4) were developed from the F_1 plants of Hi-Q × RIL-144 through application of microspore culture technique. Of 516 microspore-derived embryos transferred from liquid media to solid media, 110 (21.3%) gave DH plants. Seed increase of these DHs was done in greenhouse.

Table 4. Success rate for obtaining doubled haploid lines of Hi-Q × RIL-144 of *Brassica napus* through microspore culture

Step	Total number	% success from previous step
Transfer of embryos to solid media	516	
Transfer of plantlets to soilless media	364	70.5
Evaluation of mature plants	269	73.9
No. spontaneous diploids	110	40.9

3.2. Evaluation of the DH Lines in Growth Chamber for Days to Flower

Analysis of variance was conducted using PROC MIXED procedure of SAS (SAS Institute 2003) where the effect of genotype was nested in different photoperiods (10h, 14h, 16h and 18h) using the following model: $Yij = \mu + G_i + P_j + G(P)_{ij} + e_{ij,}$ in which *Yij* is observation of genotype *i* in environment (photoperiod) *j*, μ is the general grand mean, G_i is genotype *i* in photoperiod *j*, *j* is photoperiod (either 10h, 14h, 16h or 18h) and $G(P)_{ij}$ is the nested effect of genotype *i* in photoperiod *j*, and *eij* is the residual error of genotype *i* in photoperiod *j.* The variance analysis showed significant effect of genotype and photoperiod on days to flower. The effect of genotype nested in photoperiod was also significant (Table S1). This implies that photoperiod had profound influence on the genes controlling days to flower.

3.2.1. Evaluation under 18 hrs photoperiod and 18 ºC constant temperature

Frequency distribution of the DH lines, evaluated in October 2011, June 2012 and June 2013, for days to flower is presented in Fig. 1a, 1b and 1c, respectively; and least square means (LS means) data of the three experiments is presented in Fig. 1d. Flowering was much delayed in the October 2011 experiment as compared to the experiments in June 2012 and June 2013; however, correlation between the three experiments for days to flower of the DH lines was statistically significant (Table 6). This suggests that some non-genetic factors may have delayed flowering in October 2011; however, relative flowering date of the DH lines was fairly same in these experiments. The distribution of the DH lines in all cases was continuous suggesting that multiple gene loci be involved in the control of flowering time variation in this population. The early flowering line RIL-144 flowered about three days earlier than Hi-Q, and the F_1 plants flowered similar to RIL-144. This indicates that the earliness of flowering exhibited dominance effect (Table 5a, 5b, 5c, 5d). This is also evident from the skewed distribution of the DH population toward the earliness; the mean of the DH population was also closer to RIL-144 than to the parent Hi-Q. Some of the DH lines flowered later than the late-flowering parent Hi-Q suggesting that the RIL-144 may carry some alleles which cause lateness of flowering when combined with the Hi-Q allele(s).

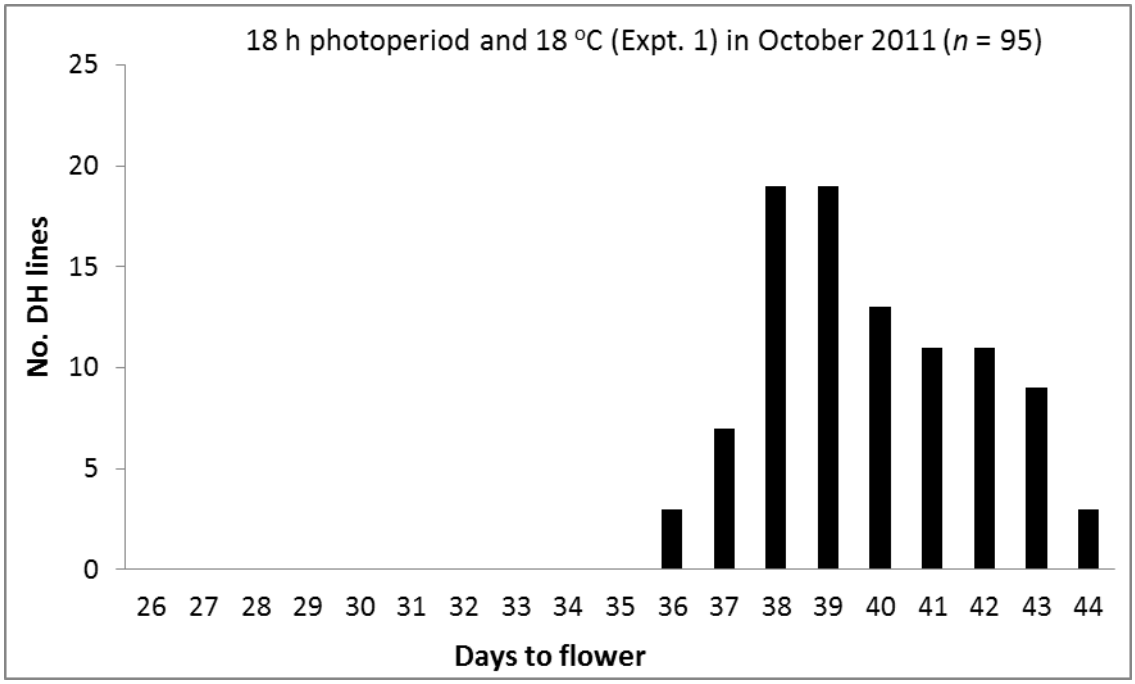

Figure 1a. Frequency distribution of the DH lines of Hi-Q × RIL-144 of *Brassica napus* grown under 18 h photoperid and 18 ºC constant temperature for days to flower. Experiment conducted in October 2011.

Table 5a. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q **×** RIL-144 cross of *Brassica napus* grown under 18 h photoperid and 18 ºC constant temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in October 2011.

Population	No.	Mean ± S.D.	Range
DH lines	95	39.2 ± 2.4	36 - 44
Hi-Q	3	42.3 ± 1.5	41 - 44
RIL-144	3	38.0 ± 0.0	38 - 38
F_1 (Hi-Q × RIL-144)	3	39.0 ± 1.0	38 - 40
Peace	3	39.0 ± 1.0	38 - 40

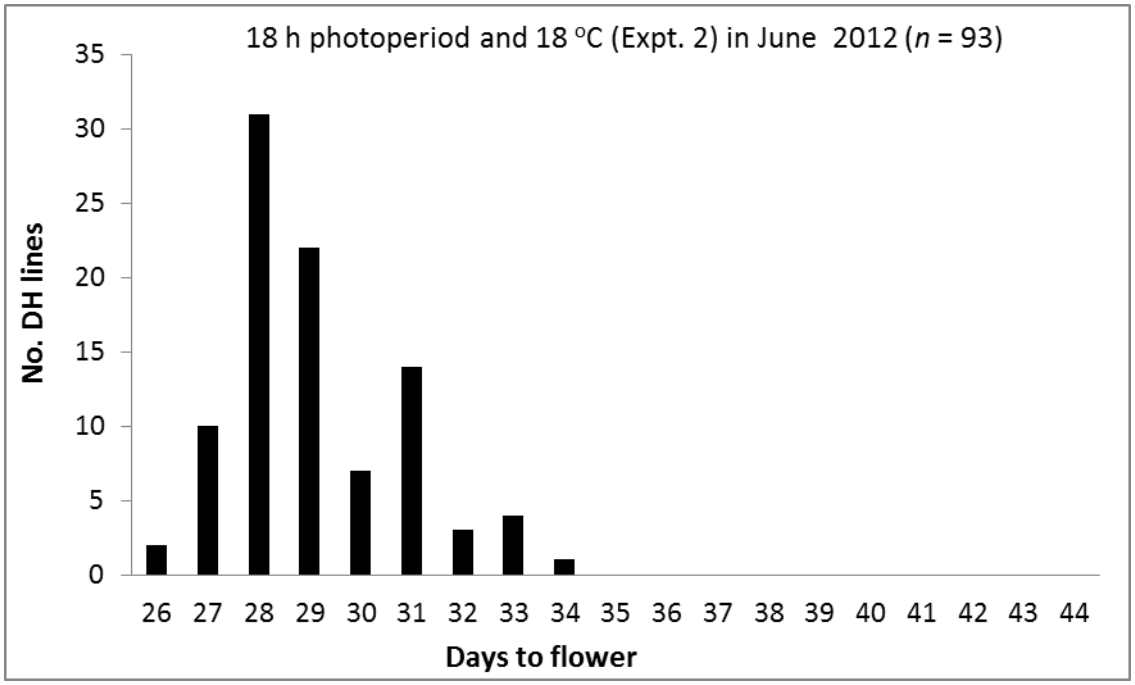

Figure 1b. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 18 h photoperid and 18 ºC constant temperature for days to flower. Experiment conducted in June 2012.

Table 5b. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 18 h photoperid and 18 ºC constant temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in June 2012.

Description	No.	Means ± SD	Range
DH lines	93	28.8 ± 2.0	26 - 34
Hi-Q	6	31.0 ± 0.9	30 - 32
RIL-144	6	28.5 ± 1.1	27 - 30
F_1 (Hi-Q × RIL-144)	4	28.0 ± 0.0	28 - 28
Peace	5	29.0 ± 1.6	27 - 31

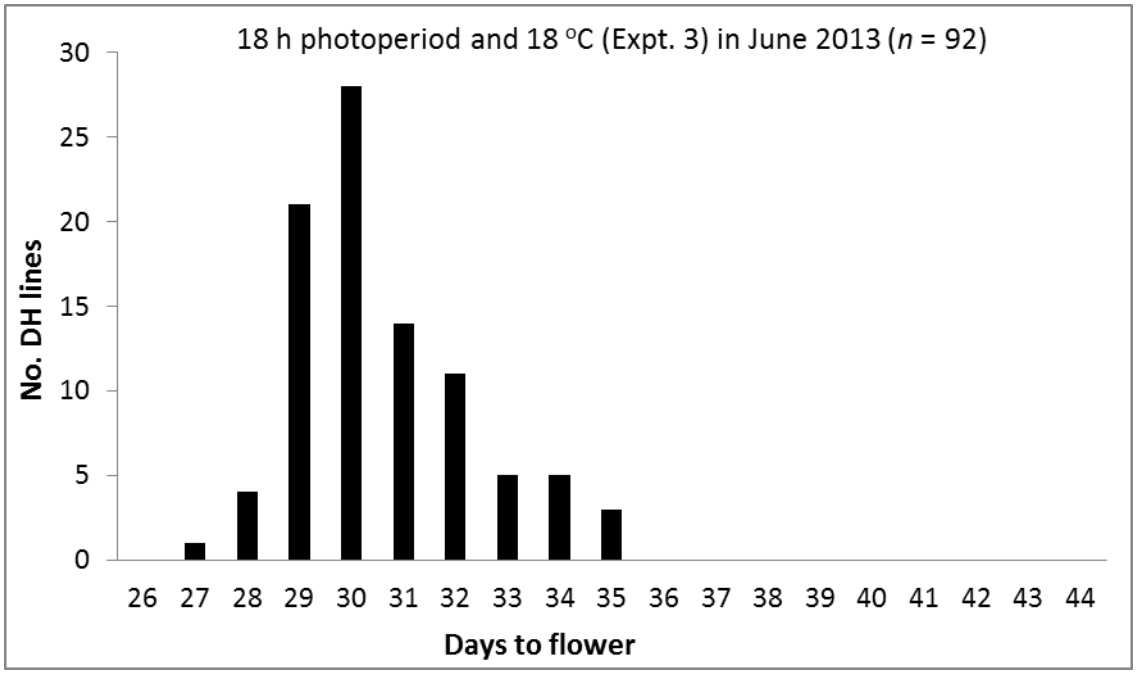

Figure 1c. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 18 h photoperid and 18 ºC constant temperature for days to flower. Experiment conducted in June 2013.

Table 5c. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 18 h photoperid and 18 ºC constant temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in June 2013.

Description	No.	Mean ± SD	Range
DH lines	92	30.2 ± 1.7	27 - 35
Hi-Q	4	32.8 ± 1.0	32 - 34
RIL-144	4	30.0 ± 0.8	29 - 31
F_1 (Hi-Q × RIL-144)	4	30.0 ± 0.0	30 - 30
Peace	4	30.5 ± 1.3	29 - 32

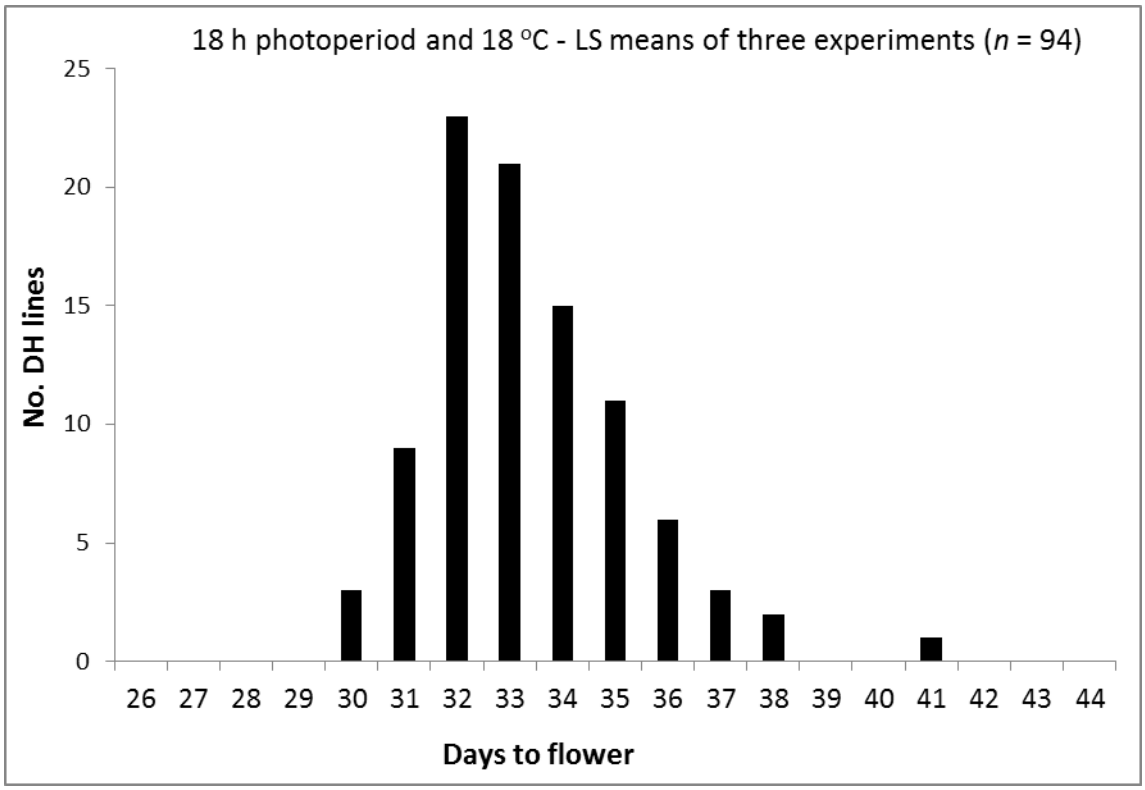

Figure 1d. Frequency distribution of LS mean (October 2011, June 2012 and June 2013 experiments) of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 18 h photoperid and 18 ºC constant temperature for days to flower.

Table 5d. Descriptive statistics based on LS means of three experiments (October 2011, June 2012 and June 2013) for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 18 h photoperid and 18 ºC constant temperature. The early flowering *B. napus* cv. Peace is included as check.

Description	No.	Mean ± SD	Range
DH lines	94	33.0 ± 1.9	30 - 41
Hi-Q	13	35.3 ± 1.0	30 - 44
RIL-144	13	32.3 ± 1.0	27 - 38
F_1 (Hi-Q × RIL-144)	11	32.3 ± 1.0	28 - 40
Peace	12	32.9 ± 1.0	27 - 40

Table 6. Spearman's correlation coefficients among the October 2011, June 2012 and June 2013 experiments for days to flower of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* 18 h photoperid and 18 ºC constant temperature.

	June 2012	June 2013
October 2011	0.496*** (90)[a]	0.498*** (90)
June 2012		0.920*** (90)

*** p<0.001; [a]In brackets, *df*

3.2.2. Evaluation under 16 h photoperiod and 20 ºC constant temperature

Distribution of the DH lines evaluated under 16 h photoperiod and 20 ºC constant temperature was very similar to the distribution observed under 18 h photoperiod and 18 ºC constant temperature (Fig. 2a, 2b, 2c, 2d, 2e and 2f). In this case also, Hi-Q flowered about three days later than the early flowering line RIL-144, the F_1 plants flowered similar to RIL-144 (Table 7a, 7b, 7c, 7d, 7e, 7f), and some of the DH lines flowered later than Hi-Q (Fig. 2a, 2b, 2c, 2d, 2e and 2f) suggesting that the RIL-144 may carry some alleles which cause lateness of flowering when combined with the Hi-Q allele(s).

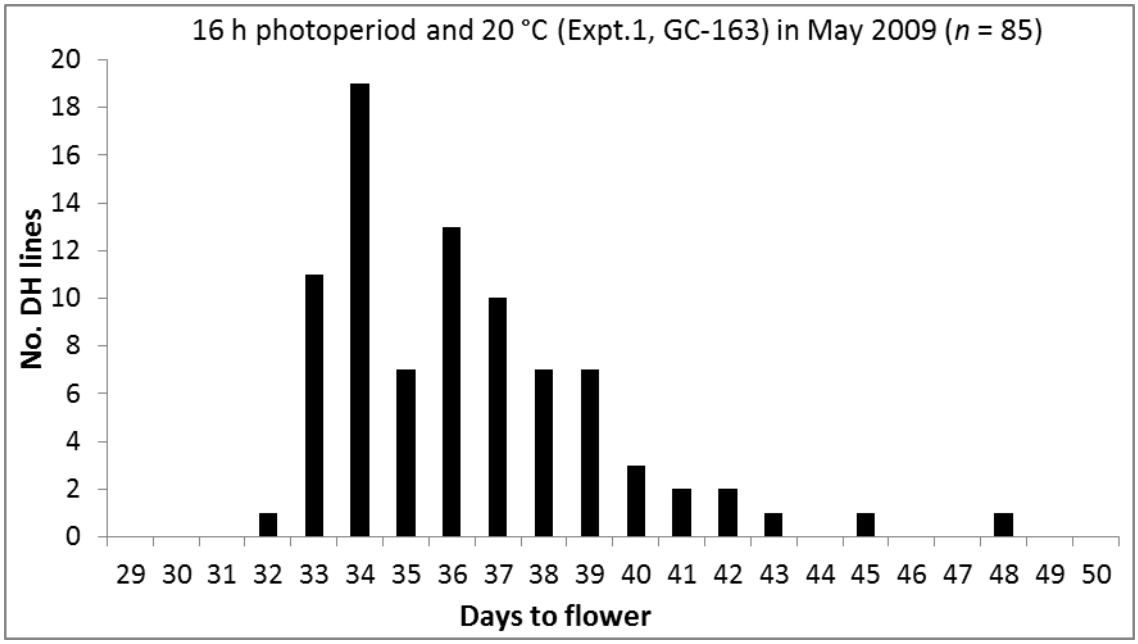

Figure 2a. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 20 ºC constant temperature for days to flower. Experiment conducted in May 2009 (GC-163).

Table 7a. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 20 ºC constant temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in May 2009 (GC-163).

Description	No.	Mean ± SD	Range
DH lines	85	36.1 ± 3.0	32 - 48
Hi-Q	4	35.5 ± 1.0	35 - 36
RIL-144	2	33.0 ± 0.0	33 - 33
Peace	2	36.0 ± 0.0	36 - 36

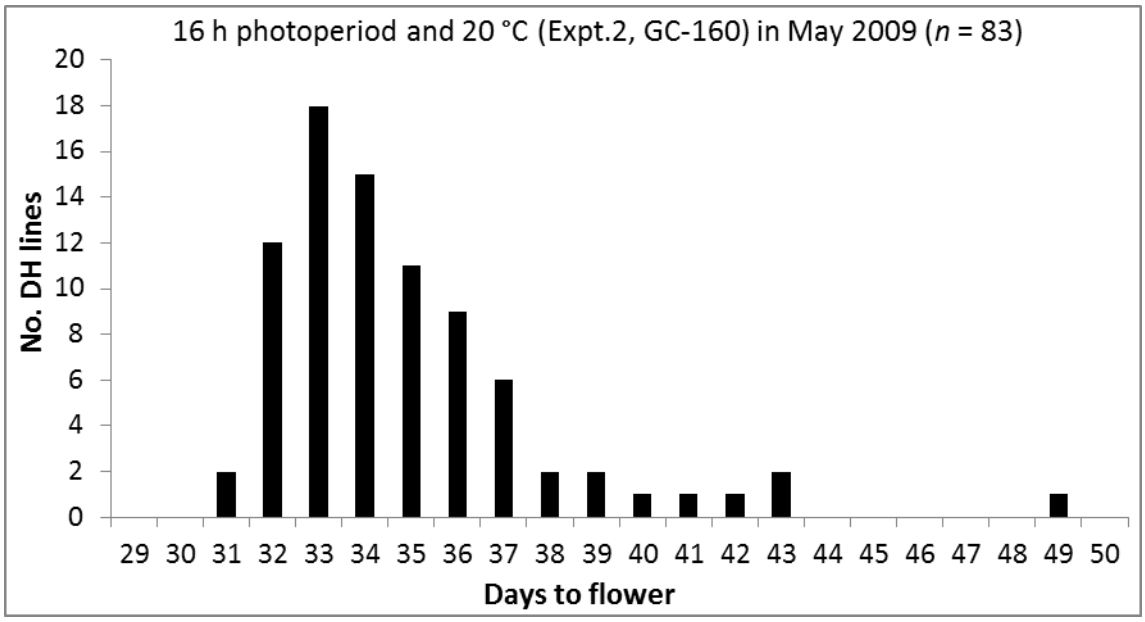

Figure 2b. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 20 ºC constant temperature for days to flower. Experiment conducted in May 2009 (GC-160).

Table 7b. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 20 ºC constant temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in May 2009 (GC-160).

Description	No.	Mean ± SD	Range
DH lines	83	34.7 ± 3.1	31 - 49
Hi-Q	4	34.3 ± 0.5	34 - 35
RIL-144	2	32.6 ± 0.7	32 - 33
Peace	2	35.0 ± 4.2	32 - 38

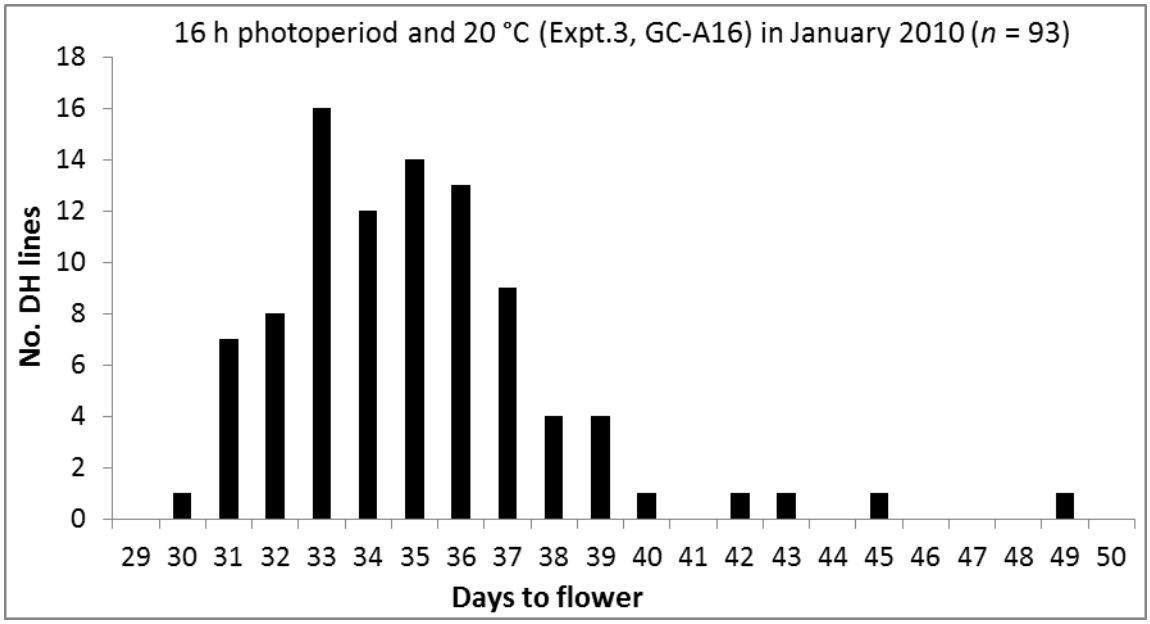

Figure 2c. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 20 ºC constant temperature for days to flower. Experiment conducted in January 2010 (GC-A16).

Table 7c. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 20 ºC constant temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in January 2010 (GC-A16).

Description	No.	Mean ± SD	Range
DH lines	93	34.8 ± 3.1	30 - 49
Hi-Q	2	37.0 ± 0.0	37 - 37
RIL-144	2	33.0 ± 0.0	33 - 33
F_1 (Hi-Q × RIL-144)	2	33.0 ± 1.4	32 - 34
Peace	2	37.0 ± 1.4	36 - 38

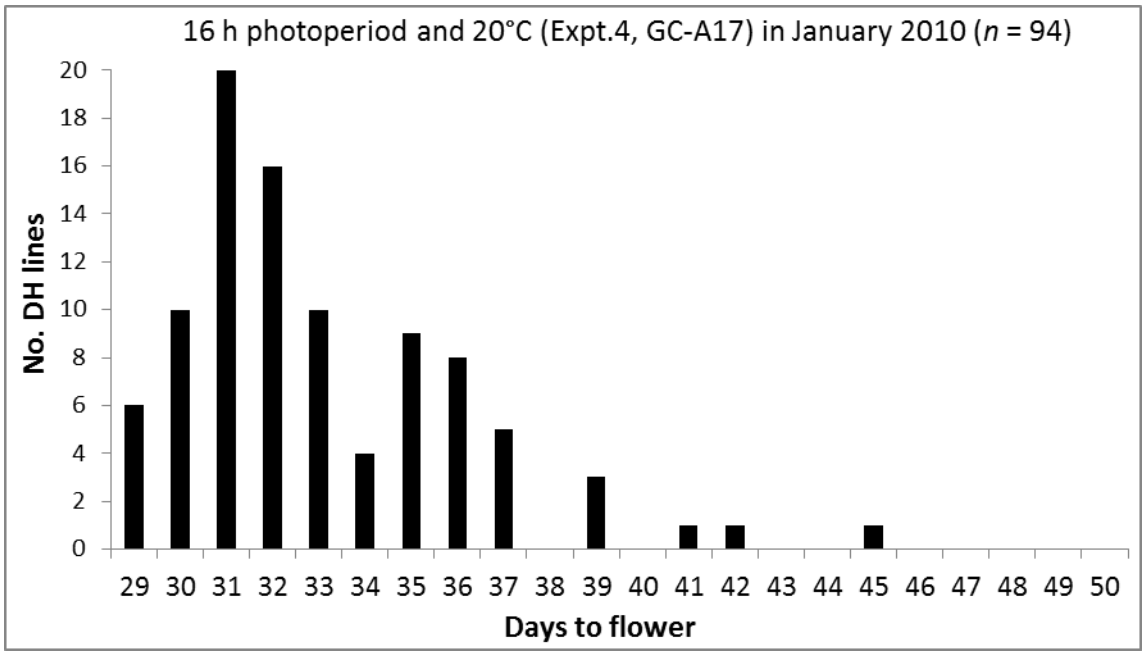

Figure 2d. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 20 ºC constant temperature for days to flower. Experiment conducted in January 2010 (GC-A17).

Table 7d. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 20 ºC constant temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in January 2010 (GC-A17).

Description	No.	Mean ± SD	Range
DH lines	94	32.8 ± 3.0	29 - 45
Hi-Q	2	34.5 ± 0.7	34 - 35
RIL-144	2	34.0 ± 0.0	34 - 34
F_1 (Hi-Q × RIL-144)	2	31.5 ± 0.7	31 - 32
Peace	2	34.5 ± 2.1	33 - 36

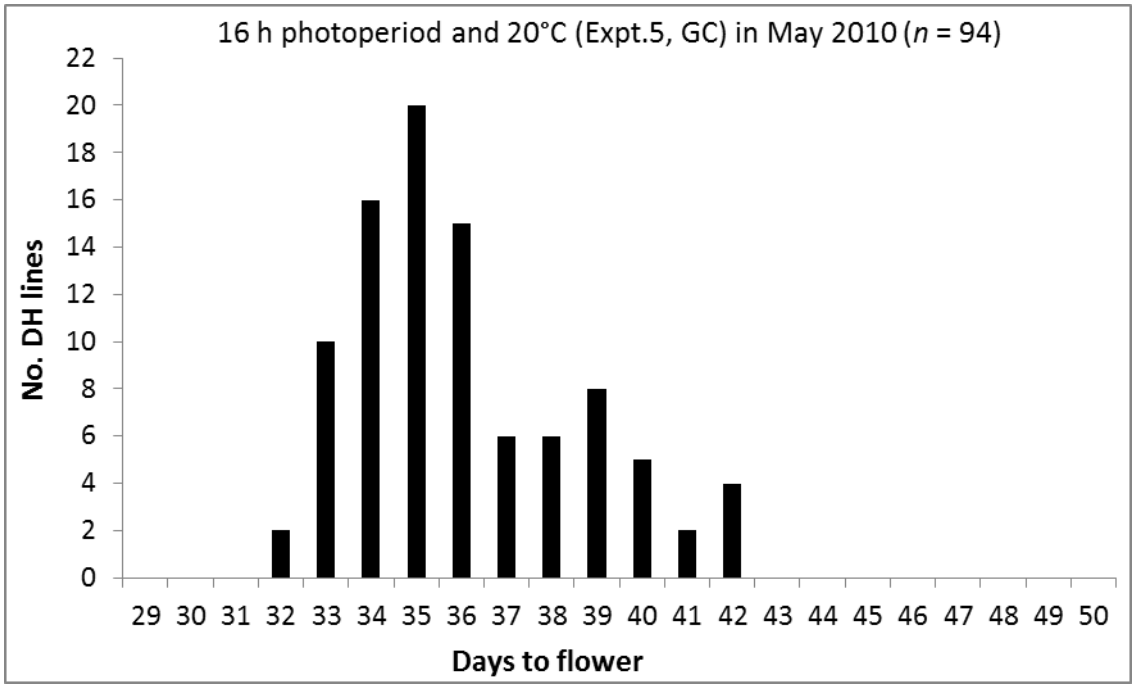

Figure 2e. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 20 ºC constant temperature for days to flower. Experiment conducted in May 2010.

Table 7e. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 20 ºC constant temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in May 2010.

Description	No.	Mean ± SD	Range
DH lines	94	35.6 ± 2.5	32 - 42
Hi-Q	4	39.0 ± 0.0	39 - 39
RIL-144	4	34.0 ± 1.2	33 - 35
F_1 (Hi-Q × RIL-144)	4	35.3 ± 0.5	35 - 36
Peace	4	40.3 ± 2.9	38 - 44

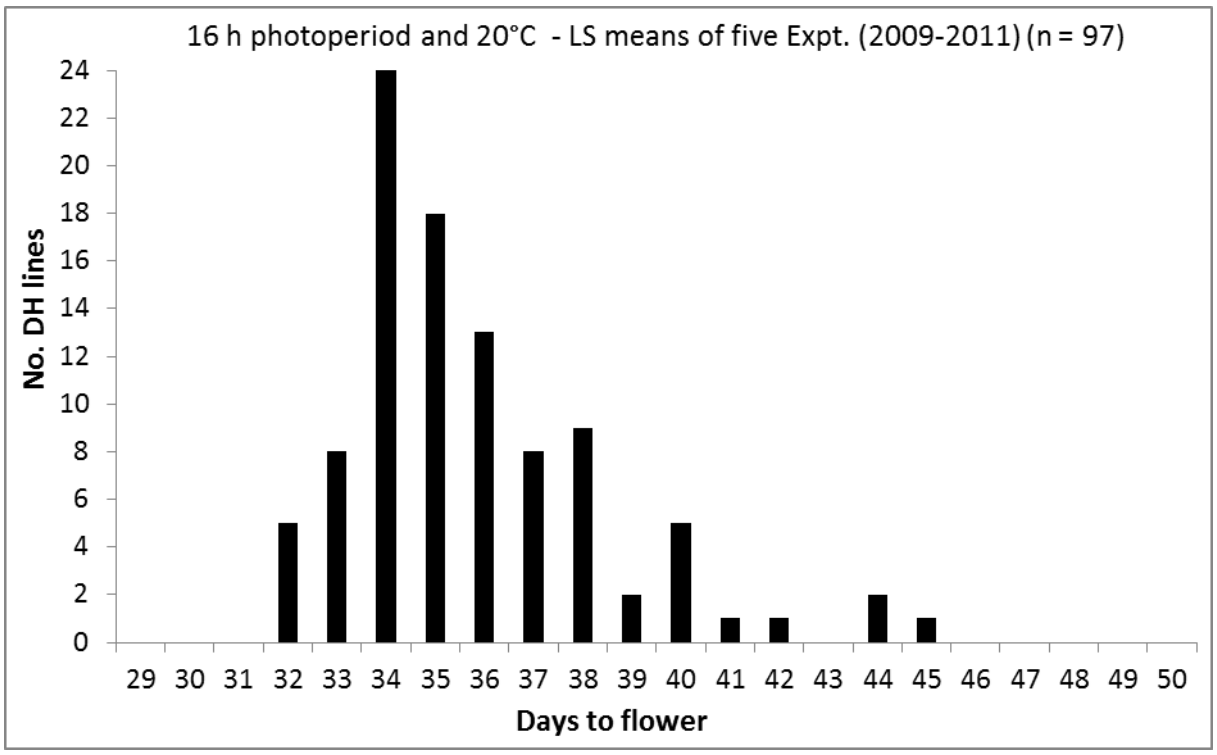

Figure 2f. Frequency distribution of LS means (five experiments of May 2009, January 2010 and May 2010) of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 18 h photoperid and 20 ºC constant temperature for days to flower.

Table 7f. Descriptive statistics based on LS means of five experiments (May 2009, January 2010 and May 2010) for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 18 h photoperid and 20 ºC constant temperature. The early flowering *B. napus* cv. Peace is included as check.

Description	No.	Mean ± SD	Range
DH lines	97	34.8 ± 2.9	32 - 45
Hi-Q	16	36.1 ± 0.4	34 - 39
RIL-144	12	33.3 ± 0.4	32 - 35
F_1 (Hi-Q × RIL-144)	8	33.3 ± 0.9	31 - 36
Peace	12	36.6 ± 2.1	32 - 40

Table 8. Spearman's correlation coefficients among the five experiments conducted in May 2009, January 2010 and May 2010 for days to flower of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* 18 h photoperid and 20 ºC constant temperature.

	May 2009 GC-160	Jan 2010 GC-A16	Jan 2010 GC-A17	May 2010
May 2009 GC-163	0.744*** (76)	0.498*** (76)	0.575*** (76)	0.562*** (73)
May 2009 GC-160		0.552*** (74)	0.615*** (74)	0.554*** (72)
Jan 2010 GC-A16			0.785*** (90)	0.751*** (86)
Jan 2010 GC-A17				0.746*** (88)

*** $p<0.001$; [a]In brackets, *df*

3.2.3. Evaluation under 14 h photoperiod and 18 ºC constant temperature

The DH lines were evaluated in a growth cabinet in June 2012, March 2013 and September 2013. The distribution of the DH population for days to flower was continuous in all three experiments and skewed towards the earliness (Fig. 3a, 3b, 3c and 3d). Correlation between the three experiments for the DH lines was statistically significant (Table 6) suggesting that the DH lines behaved similarly in all three experiments for days to flower; thus, pooling data of the three experiments could be justified. The distribution of LS means data was also similar to the individual experiments. The F_1 value was close to the early flowering line RIL-144 (Table 9a, 9b, 9c and 9d) – as was observed under 18 h photoperiod. The DH and F_1 data suggests that multiple genes to be involved in flowering variation in this DH population, where some of the genes exhibit dominance effect towards the earliness.

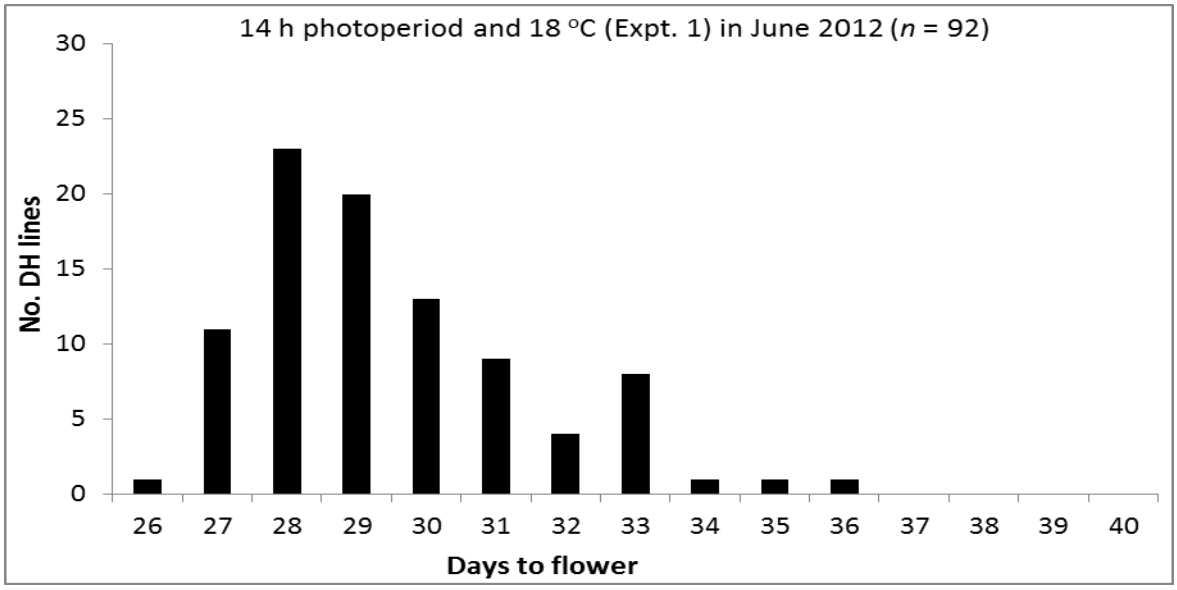

Figure 3a. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 14 h photoperid and 18 ºC constant temperature for days to flower. Experiment conducted in June 2012.

Table 9a. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 14 h photoperid and 18 ºC constant temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in June 2012.

Description	No.	Mean ± SD	Range
DH lines	92	29.2 ± 1.9	26 - 36
Hi-Q	8	31.4 ± 0.5	31 - 32
RIL-144	6	28.0 ± 0.7	27 - 29
F_1 (Hi-Q × RIL-144)	5	27.8 ± 1.6	26 - 30
Peace	8	28.6 ± 1.4	27 - 31

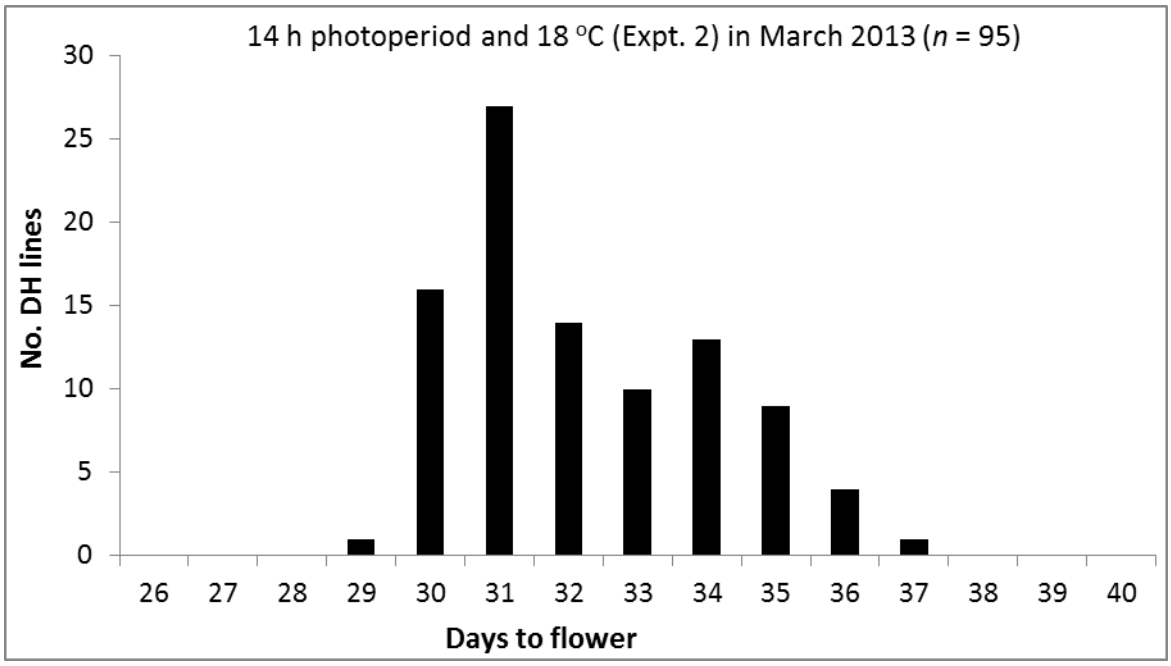

Figure 3b. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 14 h photoperid and 18 ºC constant temperature for days to flower. Experiment conducted in March 2013.

Table 9b. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 14 h photoperid and 18 ºC constant temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in March 2013.

Description	No.	Mean ± SD	Range
DH lines	95	32.2 ± 1.9	29 - 37
Hi-Q	3	35.3 ± 1.5	34 - 37
RIL-144	4	29.8 ± 0.5	29 - 30
F_1 (Hi-Q × RIL-144)	4	30.8 ± 0.5	30 - 31
Peace	3	31.3 ± 0.6	31 - 32

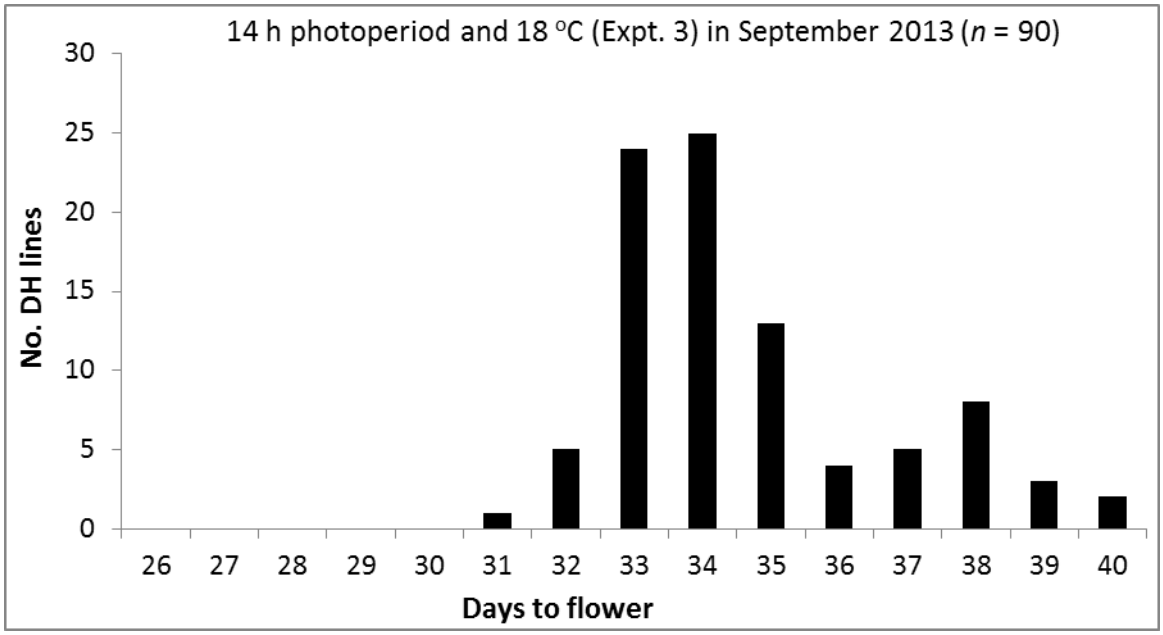

Figure 3c. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 14 h photoperid and 18 ºC constant temperature for days to flower. Experiment conducted in September 2013.

Table 9c. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 14 h photoperid and 18 ºC constant temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in September 2013.

Description	No.	Mean ± SD	Range
DH lines	90	34.4 ± 2.1	31 - 40
Hi-Q	4	35.3 ± 0.5	35 - 37
RIL-144	3	31.7 ± 0.6	31 - 32
F_1 (Hi-Q × RIL-144)	4	33.0 ± 1.2	32 - 34
Peace	3	33.0 ± 1.0	32 - 34

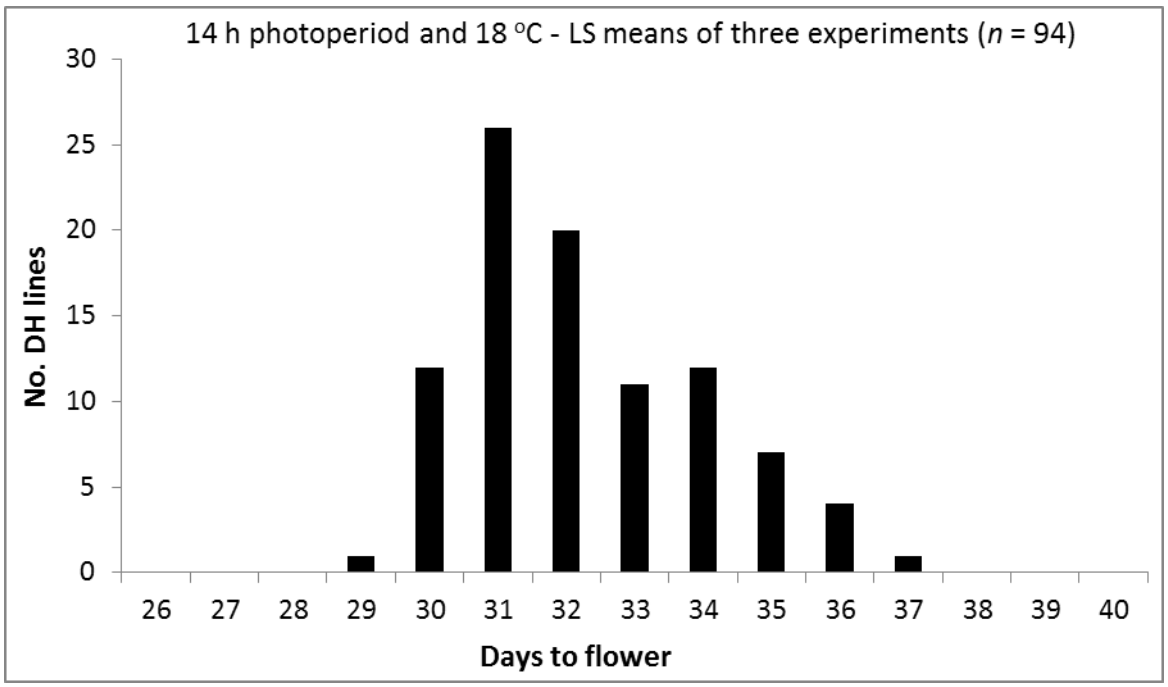

Figure 3d. Frequency distribution of LS means (June 2012, March 2013 and September 2013 experiments) of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 14 h photoperid and 18 ºC constant temperature for days to flower.

Table 9d. Descriptive statistics based on LS means of three experiments (June 2012, March 2013 and September 2013) for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 14 h photoperid and 18 ºC constant temperature. The early flowering *B. napus* cv. Peace is included as check.

Description	No.	Mean ± SD	Range
DH lines	94	31.9 ± 1.7	29 - 37
Hi-Q	15	33.9 ± 1.1	31 - 37
RIL-144	13	29.9 ± 1.1	27 - 34
F_1 (Hi-Q × RIL-144)	13	30.5 ± 1.0	26 - 34
Peace	14	31.0 ± 1.1	27 - 34

Table 10. Spearman's correlation coefficients among the June 2012, March 2013 and September 2013 experiments for days to flower of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* under 14 h photoperid and 18 ºC constant temperature.

	March 2013	September 2013
June 2012	0.637*** (89)[a]	0.614*** (88)
March 2013		0.631*** (85)

*** p<0.001; [a]in brackets, *df*

3.2.4. Evaluation under 10 h photoperiod and 18 ºC constant temperature

The experiment was conducted in June 2012 and May 2013. The early flowering parent RIL-144, on an average, required 57 days to flower while Hi-Q required 92 days to flower (Table 11a, 11b and 11c); this was much longer than the time required to flower under 14, 16 and 18 h photoperiod. The cultivar Hi-Q, which was bred under long photoperiod condition, was affected to a greater extent than the early flowering line RIL-144.

Frequency distribution of the DH lines for days to flower of the June 2012 and May 2013 experiments are presented in Fig. 4a and 4b, respectively, and LS means data of the two experiments in Fig. 4c. The DH population showed wider variation when compared with the extent of variation observed under 18, 16 and 14 h photoperiod. The distribution was nearly a bi-modal in case of the experiment conducted in May 2013 as well as based on LS means data (Fig. 4b and 4c). The mean of the DH population was similar to the mid-parent value.

Correlation between the two experiments for days to flower was statistically significant (Table 12). This indicates that the DH lines behaved similarly for days to flower in these two experiments.

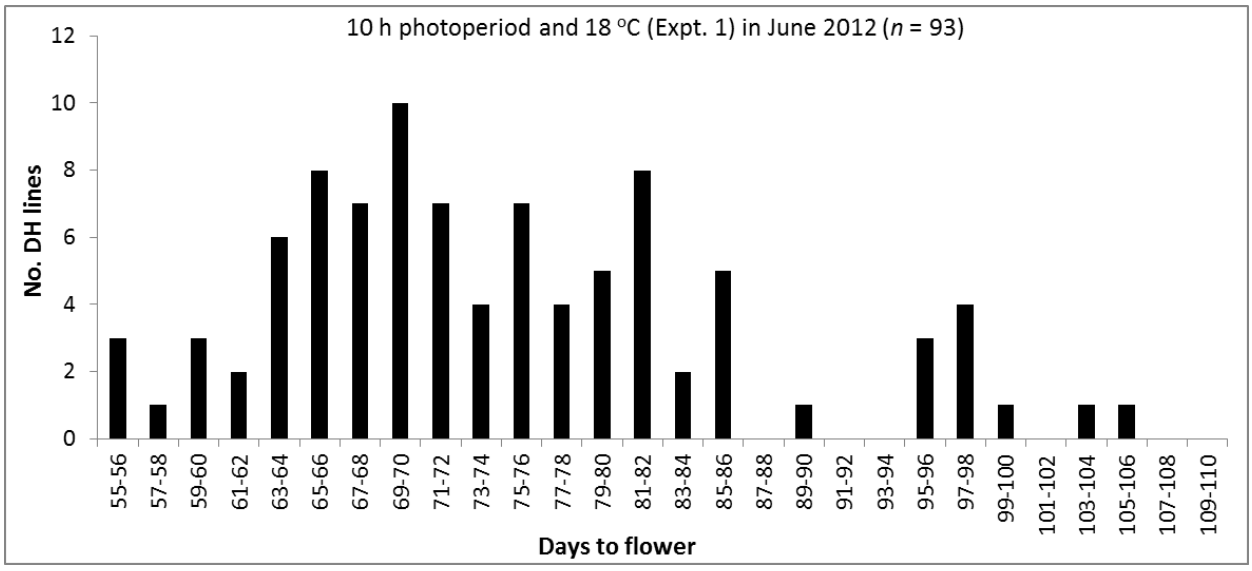

Figure 4a. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 10 h photoperid and 18 ºC constant temperature for days to flower. Experiment conducted in June 2012.

Table 11a. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 10 h photoperid and 18 ºC constant temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in June 2012.

Description	No.	Mean ± SD	Range
DH lines	93	73.3 ± 11.6	55 - 105
Hi-Q	3	87.3 ± 2.1	85 - 89
RIL-144	4	55.3 ± 1.5	53 - 56
F_1 (Hi-Q × RIL-144)	3	90.0 ± 1.0	89 - 91
Peace	5	83.7 ± 2.3	81 - 85

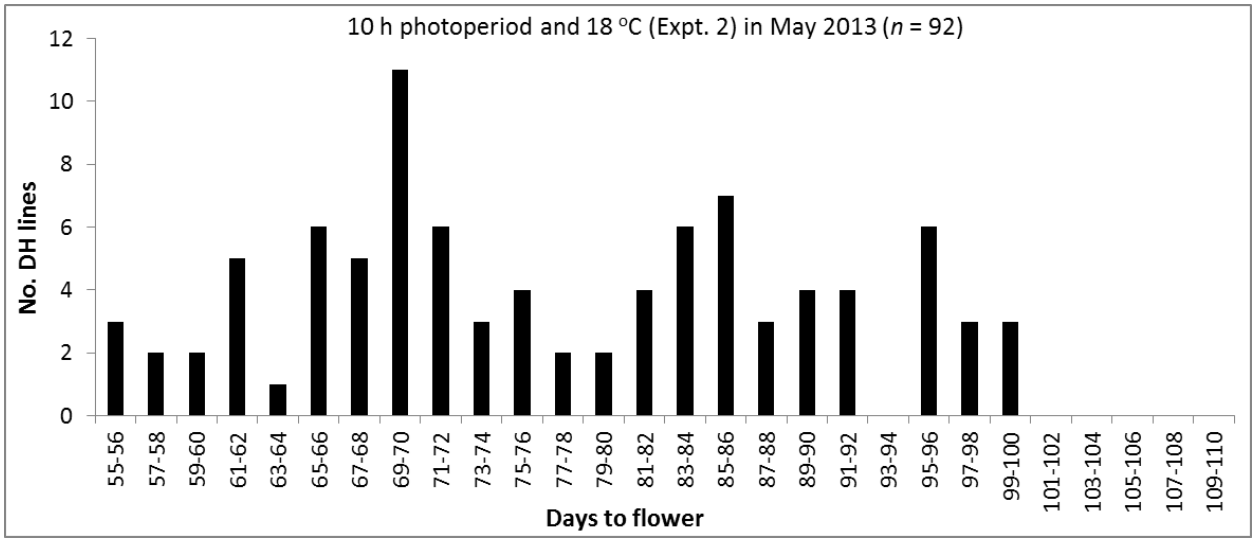

Figure 4b. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 10 h photoperid and 18 ºC constant temperature for days to flower. Experiment conducted in May 2013.

Table 11b. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 10 h photoperid and 18 ºC constant temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in May 2013.

Description	No.	Mean ± SD	Range
DH lines	92	76.0 ± 12.3	55 - 100
Hi-Q	3	97.0 ± 2.6	95 - 100
RIL-144	4	58.5 ± 1.5	57 - 60
F_1 (Hi-Q × RIL-144)	3	72.0 ±1.0	71 - 73
Peace	4	60.5 ± 1.3	59 - 62

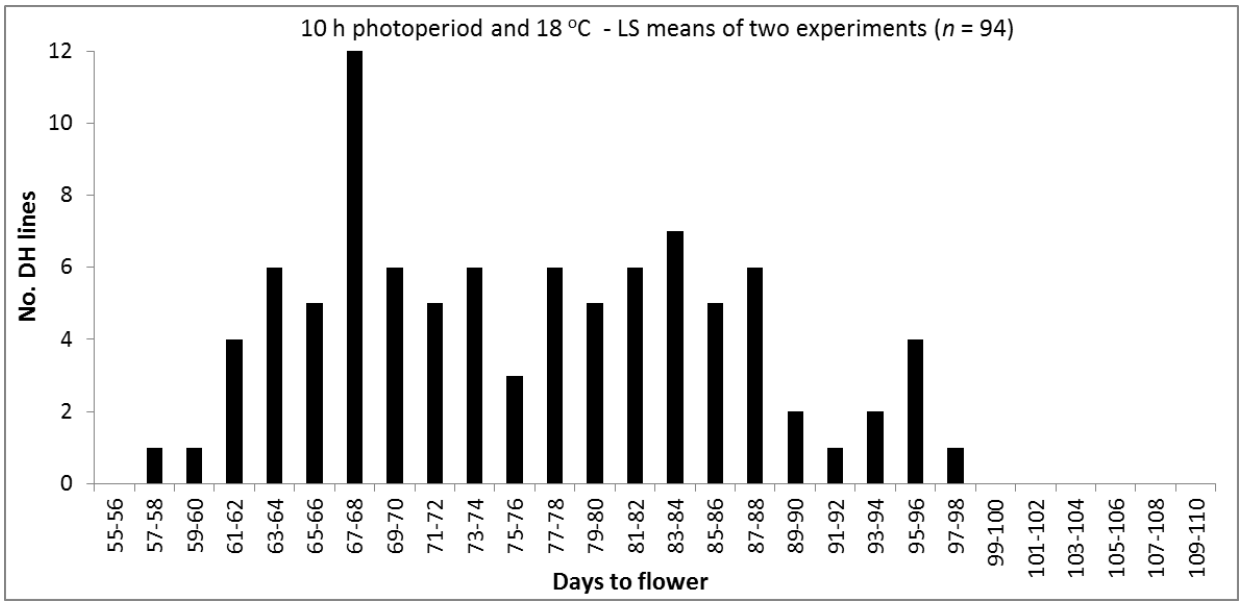

Figure 4c. Frequency distribution of LS means (June 2012 and May 2013 experiments) of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 10 h photoperid and 18 ºC constant temperature for days to flower.

Table 11c. Descriptive statistics based on LS means of two experiments (June 2012 and May 2013) for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 10 h photoperid and 18 ºC constant temperature. The early flowering *B. napus* cv. Peace is included as check.

Description	No	Mean ± SD	Range
DH lines	94	74.8 ± 10.0	56 - 97
Hi-Q	6	92.2 ± 7.6	85 -100
RIL-144	8	56.9 ± 7.6	53 - 59
F_1 (Hi-Q × RIL-144)	6	80.7 ± 7.6	60 - 90
Peace	9	73.0 ± 7.6	59 - 75

Table 12. Spearman's correlation coefficients among the June 2012 and May 2013 experiments for days to flower of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* under 10 h photoperid and 18 ºC constant temperature.

	May 2013
June 2012	0.413*** (88)[a]

***p<0.001; [a]in brackets, *df*

3.2.5. Evaluation under 16 h photoperiod and 18/8 ^{0}C (day/night) temperature

The DH lines were evaluated in October 2012, March 2013 and May 2013. In case of the October 2012 and March 2013 experiments, the mean of the DH population and F_1 fall in between the two parents and the values were close to the early flowering line RIL-144 than to Hi-Q (Table 13a and 13b) – a trend similar to 18, 16 and 14 h experiments was evident. In the case of the May 2013 experiment, the two Hi-Q plants flowered only three days later than RIL-144 (Table 11c); this was unexpectedly small difference when compared the difference between the two parents in the other two experiments. However, correlation between the experiments for days to flower of the DH lines was highly significant (Table 14); this suggests that the DH lines behaved similarly in all three experiments. In the May 2013 experiment, the parent Hi-Q for some unknown reason flowered much earlier; however, flowering of the other populations in this experiment agreed well with the other experiments.

The variation for flowering time in the DH population was continuous; however, a trend of weak bimodal distribution is evident in this population (Fig. 5a, 5b, 5c and 5d). QTL analysis might be able to disclose whether a locus exhibiting major effect on flowering time is involved in this population.

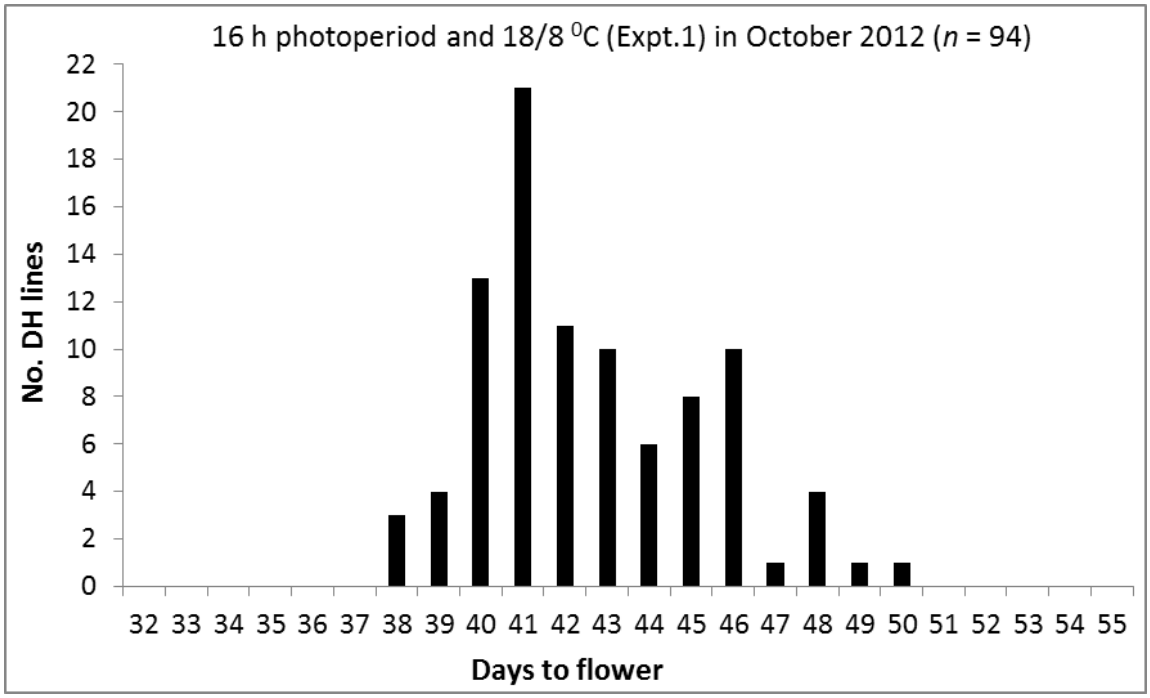

Figure 5a. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature for days to flower. Experiment conducted in October 2012.

Table 13a. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in October 2012.

Description	No.	Mean ± SD	Range
DH lines	94	42.4 ± 3.0	38 - 50
Hi-Q	5	46.6 ± 1.3	45 - 48
RIL-144	4	39.5 ± 1.3	38 - 41
F_1 (Hi-Q × RIL-144)	5	41.4 ± 0.5	41 - 42
Peace	5	39.8 ± 1.1	38 - 41

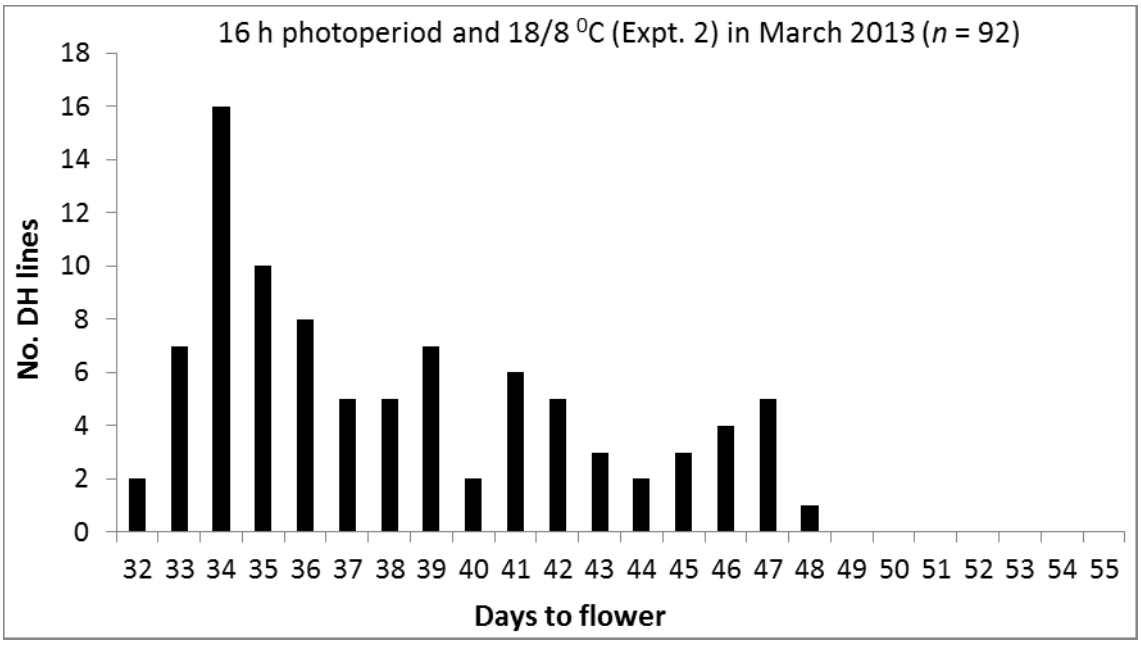

Figure 5b. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature for days to flower. Experiment conducted in March 2013.

Table 13b. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in March 2013.

Description	No.	Mean ± SD	Range
DH lines	92	38.0 ± 4.5	32 - 48
Hi-Q	3	48.0 ± 1.0	47 - 49
RIL-144	4	32.8 ± 1.2	31 - 34
F_1 (Hi-Q × RIL-144)	2	35.5 ± 0.7	35 - 36
Peace	4	31.3 ± 2.6	29 - 35

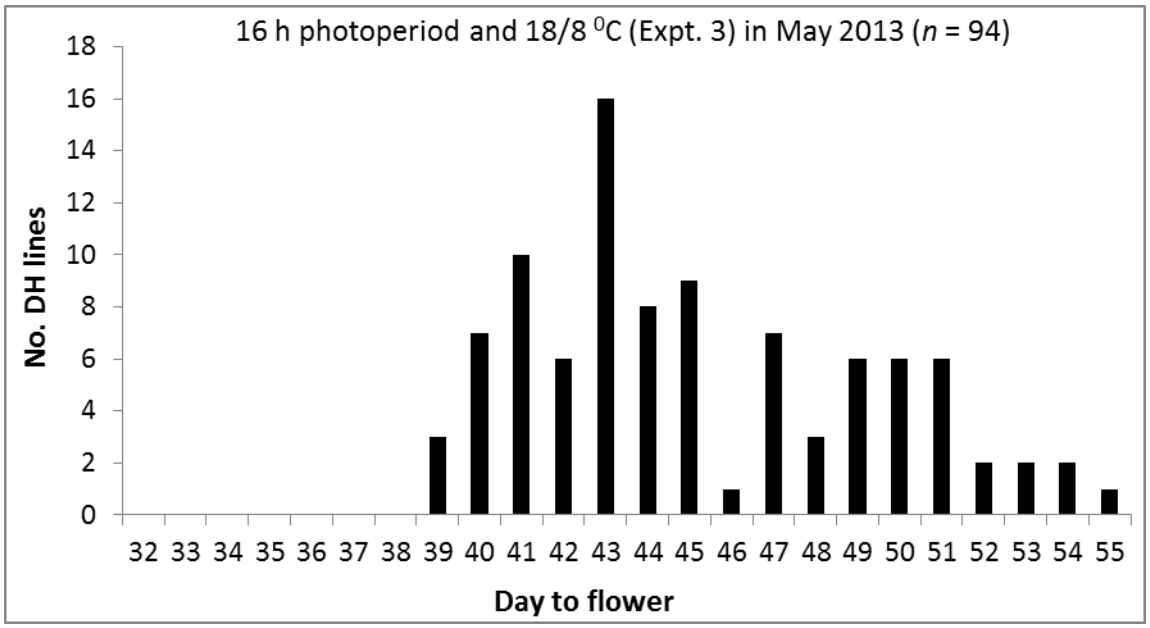

Figure 5c. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature for days to flower. Experiment conducted in May 2013.

Table 13c. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in May 2013.

Description	No.	Mean ± SD	Range
DH lines	94	45.0 ± 4.1	39 - 55
Hi-Q	2	44.0 ± 0.0	44 - 44
RIL-144	2	41.0 ± 0.0	41 - 41
F_1 (Hi-Q × RIL-144)	3	43.3 ± 3.1	40 - 46
Peace	3	41.3 ± 0.6	41 - 42

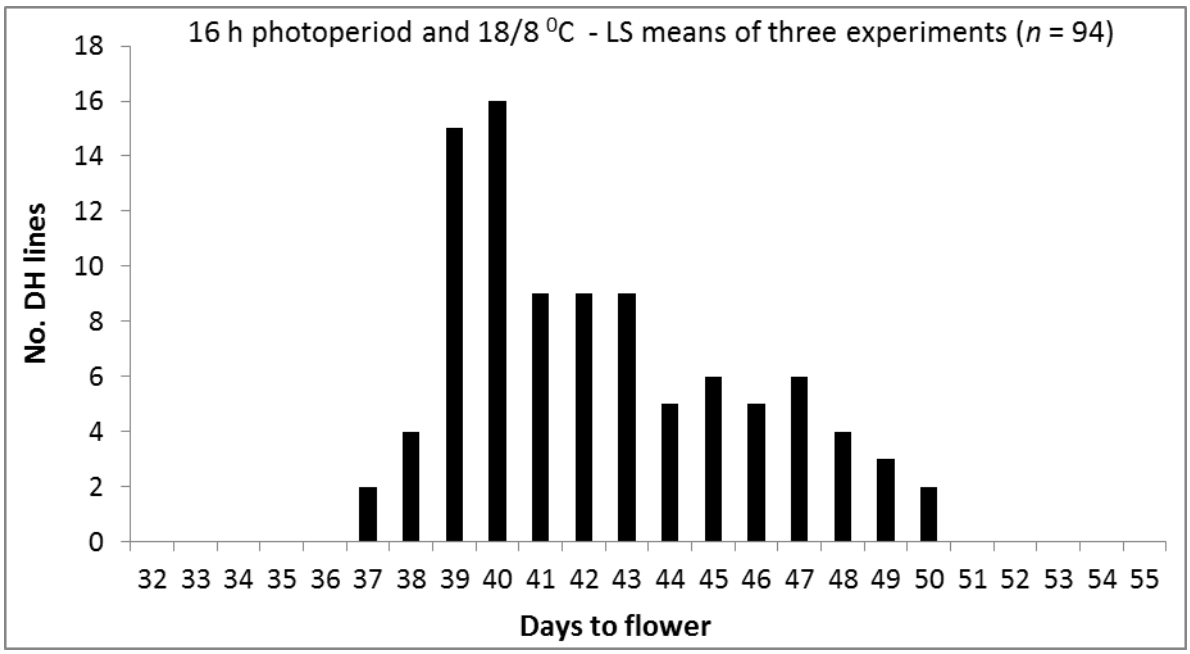

Figure 5d. Frequency distribution of LS means (October 2012, March 2013 and May 2013 experiments) of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature for days to flower.

Table 13d. Descriptive statistics based on LS means of three experiments (October 2012, March 2013 and May 2013) for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature. The early flowering *B. napus* cv. Peace is included as check.

Description	No.	Mean ± SD	Range
DH lines	94	42.0 ± 3.4	37 - 50
Hi-Q	10	46.5 ± 2.7	44 - 49
RIL-144	10	37.6 ± 2.7	31 - 41
F_1 (Hi-Q × RIL-144)	10	40.2 ± 2.7	35 - 46
Peace	12	37.4 ± 3.8	29 - 42

Table 14. Spearman's correlation coefficients among the October 2012, March 2013 and May 2013 experiments for days to flower of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature.

	March 2013	May 2013
October 2012	0.541*** (88)[a]	0.541*** (92)
March 2013		0.760*** (88)

*** p<0.001; [a]in brackets, *df*

3.2.6. Evaluation under 16 h photoperiod and 20/16 ^{0}C (day/night) temperature

Under 16 h photoperiod and 20/16 ^{0}C temperature, the parent Hi-Q flowered about six days later than RIL-144, and flowering in F_1's was intermediate of the two parents (Table 15). A continuous distribution for days to flower was observed in the DH population where majority of the plants flowered before Hi-Q and some flowered after Hi-Q (Fig. 6).

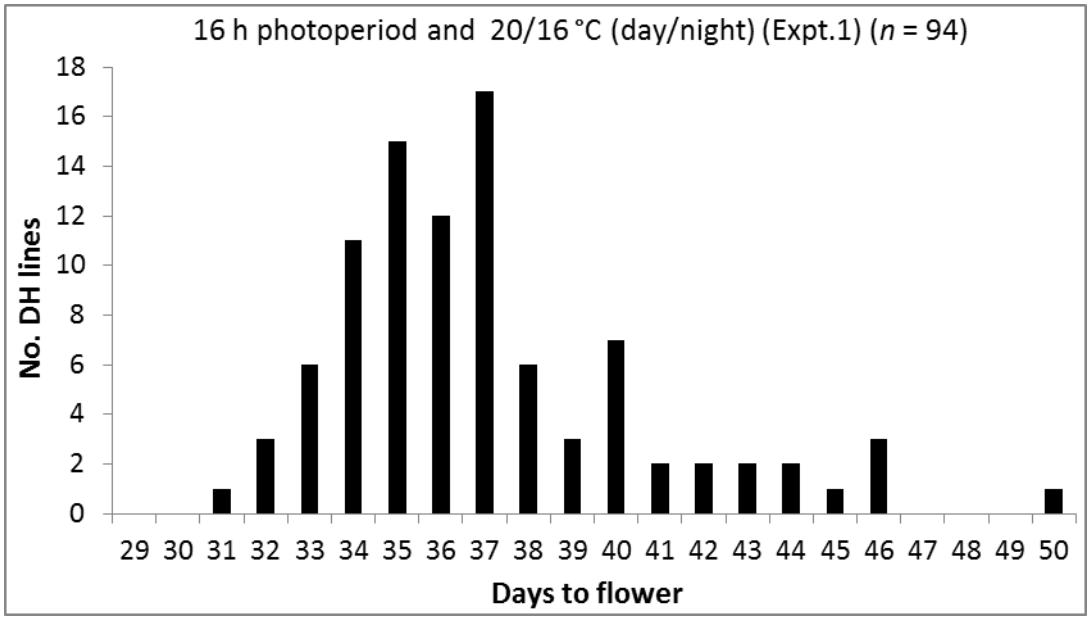

Figure 6. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of Brassica napus grown under 16 h photoperid and 20/16 0C (day/night) temperature for days to flower. Experiment conducted in January 2011.

Table 15. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 20/16 ^{0}C (day/night) temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in January 2011.

Description	No.	Mean ± SD	Range
DH lines	94	36.7 ± 3.6	31 - 50
Hi-Q	4	41.0 ± 1.4	40 - 43
RIL-144	4	35.3 ± 0.5	35 - 36
F_1 (Hi-Q × RIL-144)	4	38.3 ± 1.0	37 - 39
Peace	4	36.8 ± 2.2	34 - 39

3.2.7. Correlation between the growth chamber experiments for days to flower

The DH lines showed significant correlation for days to flower when grown under different photoperiod (Table 16) suggesting that the trait is highly heritable. In general, the coefficients of correlations between the 14, 16 and 18 h experiments were stronger than the coefficients of correlations between 10 h and the 14, 16 and 18 h experiments. This might be due to different genetic control of flowering under short day (10 h) condition. QTL analysis expected to provide further insight of this.

Table 16. Spearman's correlation coefficients among the growth chamber experiments, conducted under different photoperiod and temperature, for days to flower of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus*. LS means data used to estimate the correlations.

	DTF16h-20C	DTF14h-18C	DTF10h-18C	DTF16h-18/8C	DTF16h-20/16C
DTF18h-18C	0.612[a]	0.751	0.467	0.560	0.537
	<.0001[b]	<.0001	<.0001	<.0001	<.0001
	92[c]	92	93	92	92
DTF16h-20C		0.631	0.488	0.695	0.776
		<.0001	<.0001	<.0001	<.0001
		92	94	92	92
DTF14h-18C			0.536	0.681	0.640
			<.0001	<.0001	<.0001
			92	92	92
DTF10h-18C				0.476	0.580
				<.0001	<.0001
				92	92
DTF16h-18/8C					0.618
					<.0001
					92

[a]Spearman correlation coffcient, [b]probablty of the correlation coffcient ($p \leq 0001$ is highly significan correlation), [c]degree of freedom
Definition for the variables:
DTF10h-18C: Least square means of days to flowering under 10 h photoperiod and 18 °C constant temperature in growth chamber
DTF14h-18C: Least square means of days to flowering under 14 h photoperiod and18 °C constant temperature in growth chamber
DTF16h-18/8C: Least square means of days to flowering under 16 h photoperiod and 18/8 °C (day/night) temperature in growth chamber
DTF18h-18C: Least square means of days to flowering under 18 h photoperiod and 18 °C constant temperature in growth chamber
DTF16h-20/16C: Least square means of days to flowering under 16 h photoperiod and 20/16 °C (day/night) temperature in growth chamber
DTF16h-20C: Least square means of days to flowering under 16 h photoperiod and 20 °C constant temperature in growth chamber

As mentioned earlier, days to flower was significantly affected by photoperiod (Table S1); therefore, LSmean data from pairs of environments (photoperiods) were tested for significant difference (Dunnett's test) using adjusted *p*-value. In this analysis, we exclude 16 h photoperiod data as this experiment was conducted in different size pots. The difference for days to flower between 14 and 18 h photoperiod was not statistically significant; however, a significant difference ($p < 0.0001$) was found between 10 and 14 h, and 10 and 18 h photoperiod

growth conditions (Table S2). Flowering in the DH lines was significantly delayed at 10 h photoperiod compared to 14 or 18 h photoperiod.

3.3. Evaluation of the F_2 and Backcross Populations in Growth Chamber for Days to Flower

3.3.1. Evaluation under 18 h photoperiod and 18 ^{0}C constant temperature

A total of 308 F_2 plants were grown in two growth chambers in February 2012 and 174 in one growth chamber in March 2012. Variation for days to flower, and the two mean values of the F_2 data from the two growth chambers of the February 2012 experiment was not statistically different ($p<0.05$); therefore, flowering data from these two growth chambers were pooled and presented. Flowering was delayed in case of the February 2012 experiment as compared to the March 2012 experiment (Fig. 7a and 7b). This was apparently due to the effect of some non-genetic factors other than the photoperiod and temperature in the growth chambers. However, the distribution of the F_2 population was continuous in all cases (Fig. 7a and 7b) suggesting quantitative nature of the trait – as was found in other experiments with the DH population. In contrast to the DH population, which is homozygous lines, the F_2 population mean was close to the late flowering parents Hi-Q.

The distribution of the backcross populations, the B_1 [(Hi-Q × RIL-144) × Hi-Q] (Fig. 8) and B_2 [(Hi-Q × RIL-144) × RIL-144] (Fig. 9), was also continuous.

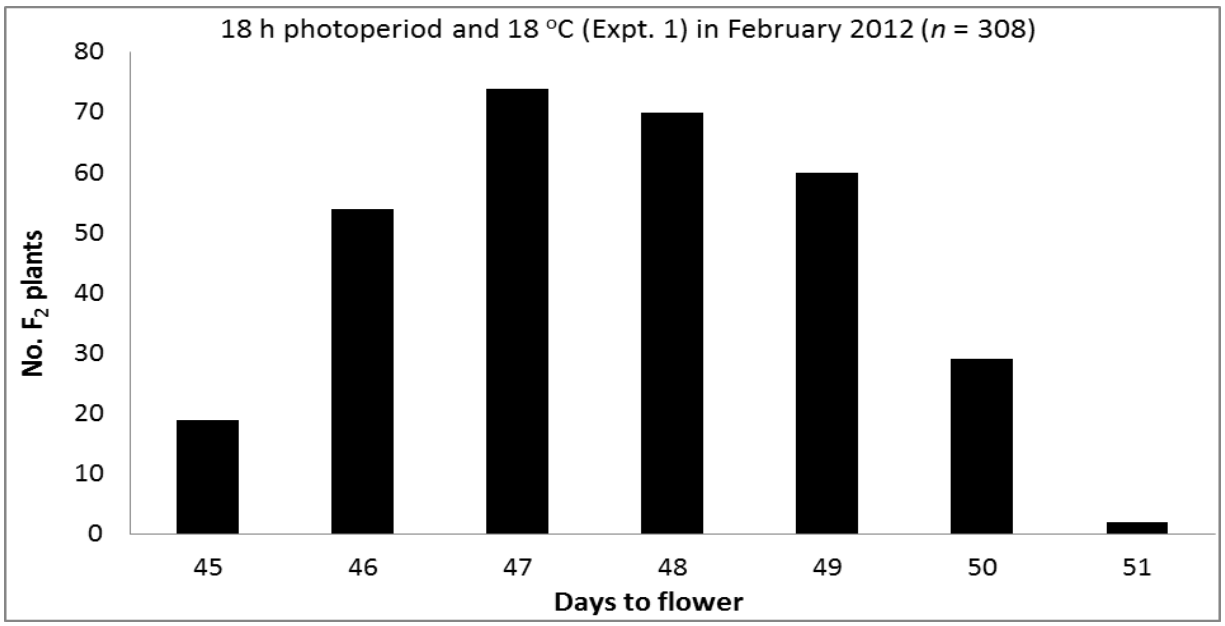

Figure 7a. Frequency distribution of the F_2 population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 18 h photoperid and 18 ^{0}C constant temperature for days to flower. Experiment conducted in February 2012.

Table 17a. Descriptive statistics for days to flower of the parents and the F_2 population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 18 h photoperid and 18 ^{0}C constant temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in February 2012.

Lines	No.	Mean ± SD	Range
F_2	308	47.6 ± 1.4	45 - 51
Hi-Q	5	47.8 ± 0.8	47 - 49
RIL-144	6	45.5 ± 0.6	45 - 46
F_1 (Hi-Q × RIL-144)	4	46.3 ± 0.5	46 - 47
Peace	4	46.3 ± 0.5	46 - 47

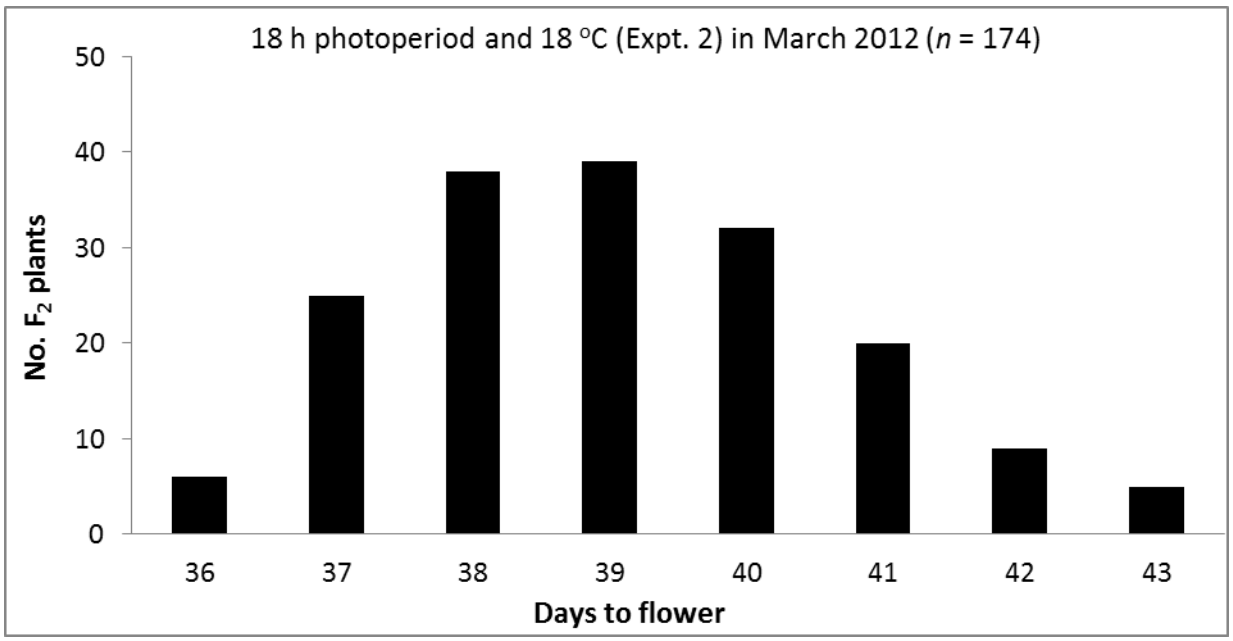

Figure 7b. Frequency distribution of the F_2 population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 18 h photoperid and 18 ^{0}C constant temperature for days to flower. Experiment conducted in March 2012.

Table 17b. Descriptive statistics for days to flower of the parents and the F_2 population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 18 h photoperid and 18 ^{0}C constant temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in March 2012.

Populations	No.	Mean ± SD	Range
F_2	174	39.1 ± 1.6	36 - 43
Hi-Q	4	39.3 ± 0.8	38 - 40
RIL-144	4	36.7 ± 0.5	36 - 37
F_1 (Hi-Q × RIL-144)	3	37.0 ± 0.0	37 - 37
Peace	3	37.3 ± 0.6	37 - 38

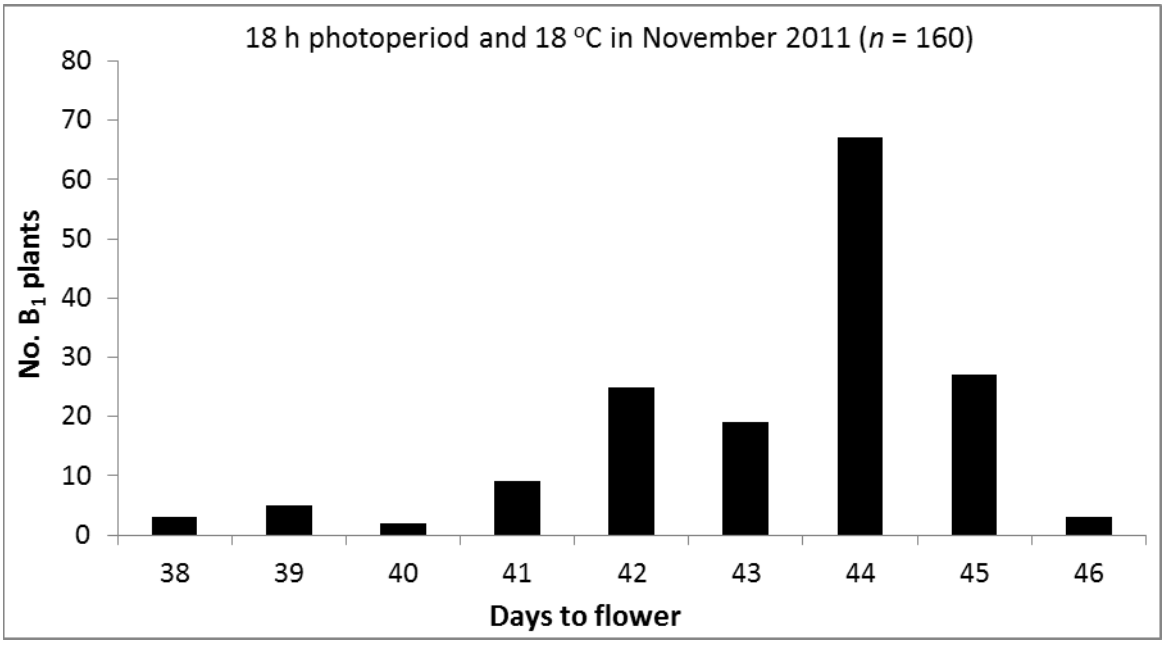

Figure 8. Frequency distribution of the B_1 population [(Hi-Q × RIL-144) × Hi-Q] of *Brassica napus* grown under 18 h photoperid and 18 ^{0}C constant temperature for days to flower. Experiment conducted in November 2011.

Table 18. Descriptive statistics for days to flower of the parents and the B_1 population [(Hi-Q × RIL-144) × Hi-Q] of *Brassica napus* grown under 18 h photoperid and 18 ^{0}C constant temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in November 2011.

Lines	No.	Mean ± SD	Range
B_1	160	43.0 ± 1.6	38 - 46
Hi-Q	4	42.3 ± 1.5	41 - 44
RIL-144	4	38.0 ± 0.0	38 - 38
F_1 (Hi-Q × RIL-144)	4	39.0 ± 1.0	38 - 40
Peace	4	39.0 ± 1.0	38 - 40

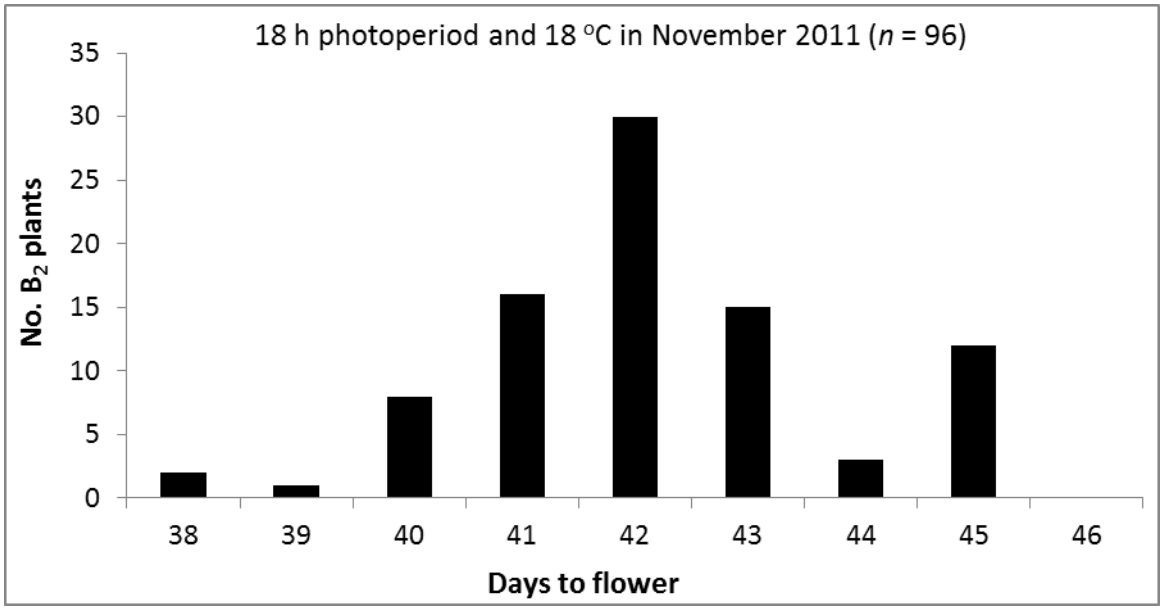

Figure 9. Frequency distribution of the B_2 population [(Hi-Q × RIL-144) × RIL-144] of *Brassica napus* grown under 18 h photoperid and 18 ^{0}C constant temperature for days to flower. Experiment conducted in November 2011.

Table 19. Descriptive statistics for days to flower of the parents and the B_2 population [(Hi-Q × RIL-144) × RIL-144] of *Brassica napus* grown under 18 h photoperid and 18 ^{0}C constant temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in November 2011.

Lines	No.	Mean ± SD	Range
B_2	96	42.6 ± 1.8	38 - 45
Hi-Q	4	42.3 ± 1.5	41 - 44
RIL-144	4	38.0 ± 0.0	38 - 38
F_1 (Hi-Q × RIL-144)	4	39.0 ± 1.0	38 - 40
Peace	4	39.0 ± 1.0	38 - 40

3.3.2. Evaluation under 10 h photoperiod and 18 ^{0}C constant temperature

A total of 390 F_2 plants were grown in two growth chambers in October 2011, and pooled data from these two chambers presented in Fig. 10 and Table 20. The F_2 population took significantly longer time to flower when compared with the same segregating population grown under longer (18 h) photoperiod. About 8% of the F_2 plants failed to flower within 112 days after seeding; this is unlike the experiment with the DH population where all flowered within 105 days of seeding. The DH lines are homozygous; on the other hand, extreme heterozygosity is the general feature of an F_2 population. Thus, heterozygosity might have played a role on the observed difference for days to flower. Investigation of the exact cause of this difference between the F_2 and DH population could not be made during this project period.

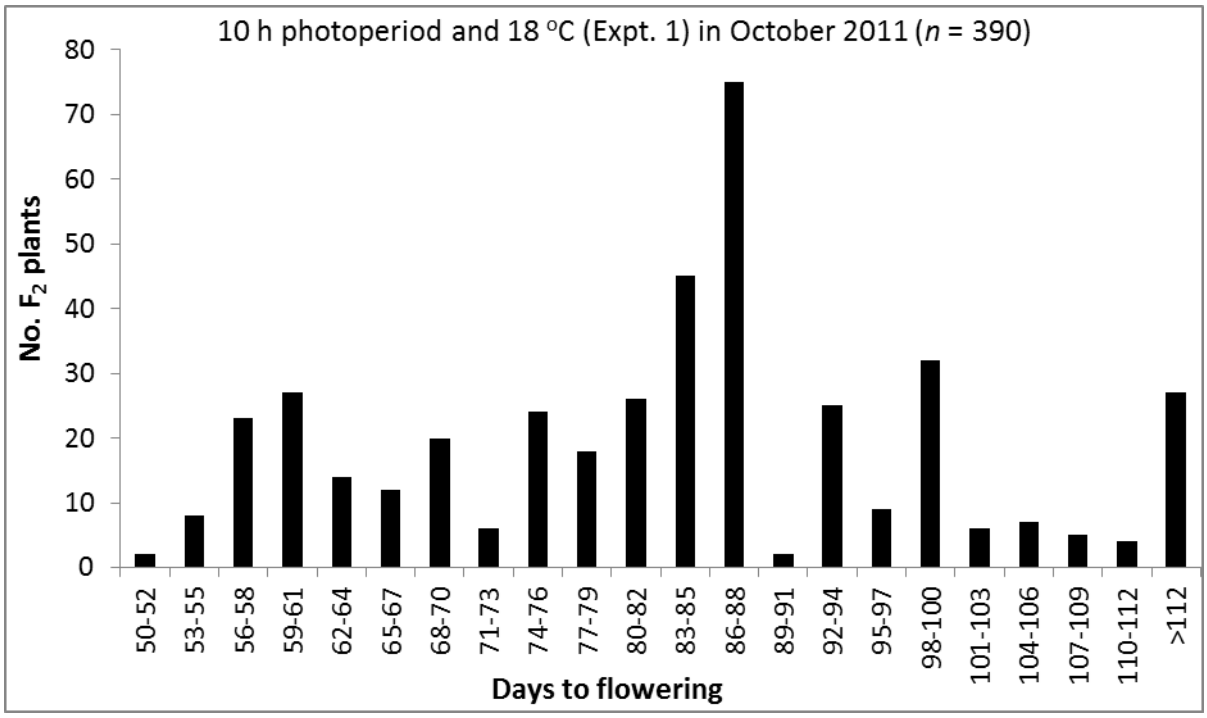

Figure 10. Frequency distribution of the F_2 population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 10 h photoperid and 18 ^{0}C constant temperature for days to flower. Experiment conducted in October 2011.

Table 20. Descriptive statistics for days to flower of the parents and the F_2 population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 10 h photoperid and 18 ^{0}C constant temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in October 2011. Flowering time data of the 358 plants that flowered within 112 days after seeding presented; 32 plants did not flowered at the time of termination of the experiment at 112 days of seeding.

Lines	No.	Mean ± SD	Range
F_2*	358	78.6 ± 14.0	50 - 110
Hi-Q	6	77.2 ± 2.2	76 - 80
RIL-144	5	69.8 ± 1.1	69 - 71
F_1 (Hi-Q × RIL-144)	4	78.8 ± 1.5	78 - 81
Peace	4	67.5 ± 0.6	67 - 68

*F_2 seeds of the F_1 plant from where the DH lines DH002 to DH091 produced.

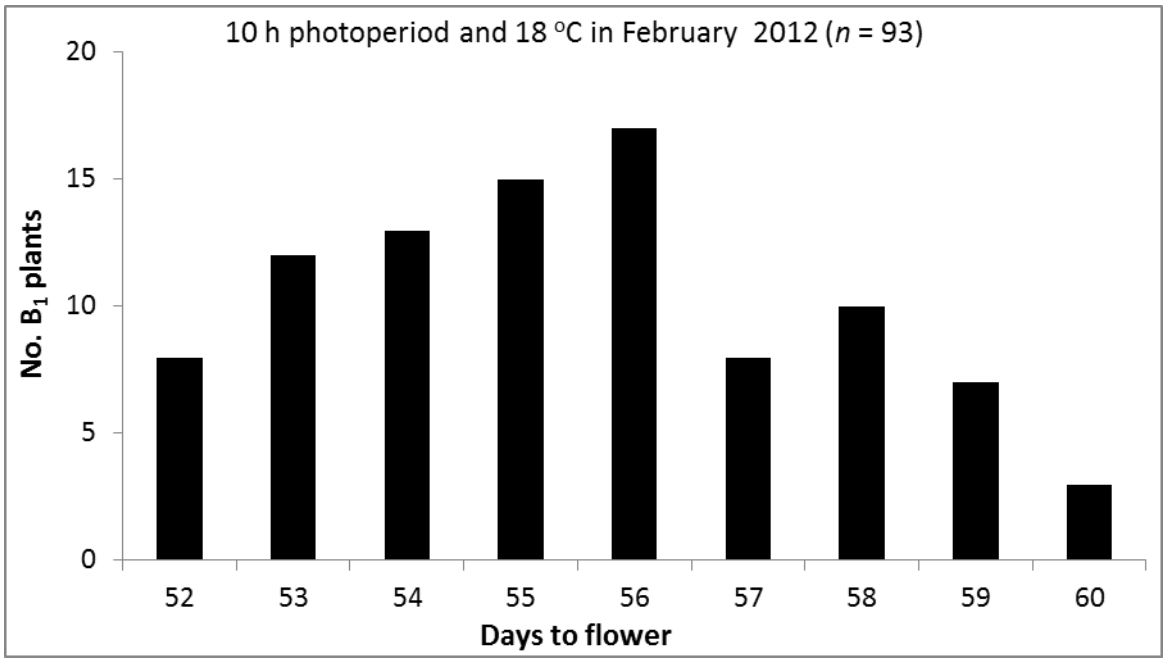

Figure 11. Frequency distribution of the B_1 population [(Hi-Q × RIL-144) × Hi-Q] of *Brassica napus* grown under 10 h photoperid and 18 ^{0}C constant temperature for days to flower. Experiment conducted in February 2012.

Table 21. Descriptive statistics for days to flower of the parents and the B_1 population [(Hi-Q × RIL-144) × Hi-Q] of *Brassica napus* grown under 10 h photoperid and 18 ^{0}C constant temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in February 2012.

Lines	No.	Mean ± SD	Range
B_1	93	55.5 ± 2.2	52 - 60
Hi-Q	3	57.0 ± 1.0	56 - 58
RIL-144	3	53.0 ± 1.0	52 - 54
F_1 (Hi-Q × RIL-144)	3	55.0 ± 1.0	54 - 56
Peace	3	53.0 ± 1.0	52 - 54

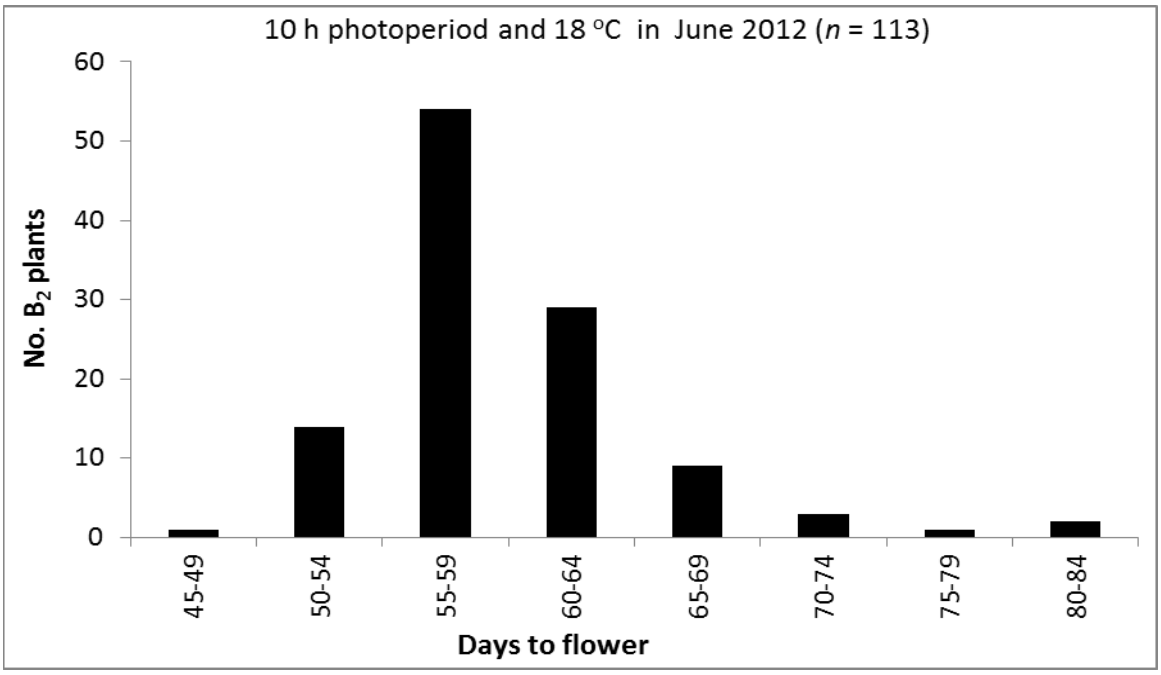

Figure 12. Frequency distribution of the B_2 population [(Hi-Q × RIL-144) × RIL-144] of *Brassica napus* grown under 10 h photoperid and 18 ^{0}C constant temperature for days to flower. Experiment conducted in June 2012.

Table 22. Descriptive statistics for days to flower of the parents and the B_2 population [(Hi-Q × RIL-144) × RIL-144] of *Brassica napus* grown under 10 h photoperid and 18 ^{0}C constant temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in June 2012.

Lines	No.	Mean ± SD	Range
B_2	113	59.9 ± 4.6	48 - 84
Hi-Q	3	59.3 ± 3.1	56 - 62
RIL-144	3	56.7 ± 1.2	56 - 58
F_1 (Hi-Q × RIL-144)	3	66.7 ± 0.6	66 - 67
Peace	3	55.0 ± 1.0	54 - 56

3.4. Evaluation of the DH lines for Dry Matter (Biomass)

3.4.1. Leaf dry matter

Despite we maintained the same photoperiod and temperature in growth chamber in all four experiments, variation between the experiments for leaf dry matter was quite high. Moreover, among the four experiments, the March 2013 experiment did not show significant correlation with the October 2011 and March 2012 experiments (Table 24). This was unlike the flowering time experiments where correlation among the experiments in all cases was statistically significant. This suggests that, leaf dry matter is more sensitive to genotype × environment interaction as compared to days to flower. However, on an average, the parent Hi-Q produced greater leaf dry matter as compared to the early flowering parent RIL-144. In all four experiments, the DH population showed continuous variation for this trait (Fig. 13a, 13b, 13c, 13d).

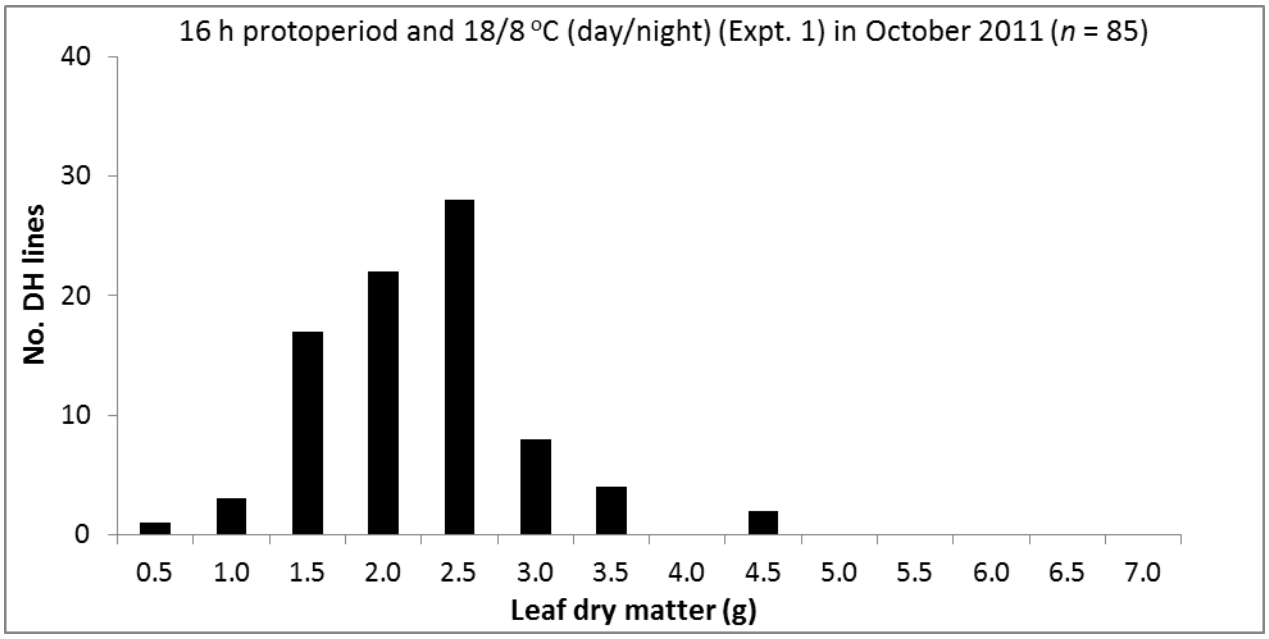

Figure 13a. Frequency distribution of the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature for leaf dry matter. Experiment conducted in October 2011.

Table 23a. Descriptive statistics for leaf dry matter of the parents and the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in October 2011.

Population	No.	Mean ± SD (g)	Range (g)
DH	85	2.01 ± 0.67	0.5 - 4.4
Hi-Q	4	1.58 ± 0.55	0.9 - 2.4
RIL-144	4	1.30 ± 1.09	0.1 - 2.8
Peace	4	0.95 ± 0.33	0.5 - 1.0

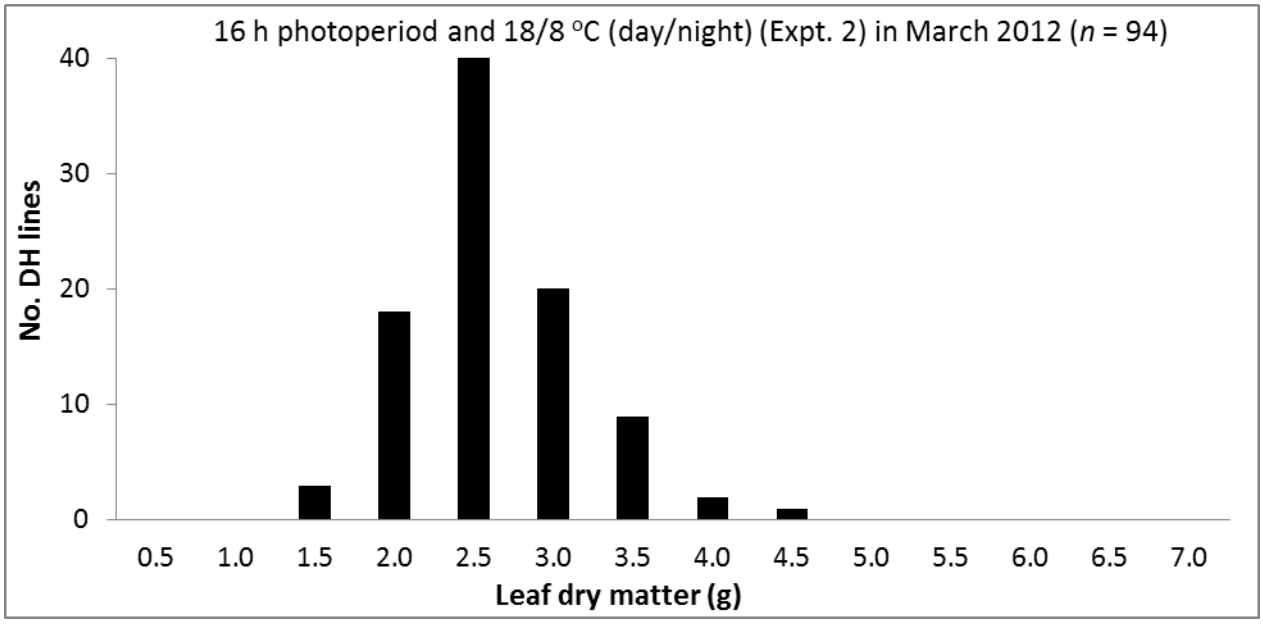

Figure 13b. Frequency distribution of the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature for leaf dry matter. Experiment conducted in March 2012.

Table 23b. Descriptive statistics for leaf dry matter of the parents and the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in March 2012.

Population	No.	Mean ± SD (g)	Range (g)
DH	94	2.39 ± 0.54	1.4 - 4.4
Hi-Q	2	2.15 ± 0.35	1.9 - 2.4
RIL-144	3	2.00 ± 0.36	1.7 - 2.4
F_1 (Hi-Q × RIL-144)	3	2.53 ± 0.65	1.9 - 3.2
Peace	2	1.90 ± 0.57	1.5 - 2.3

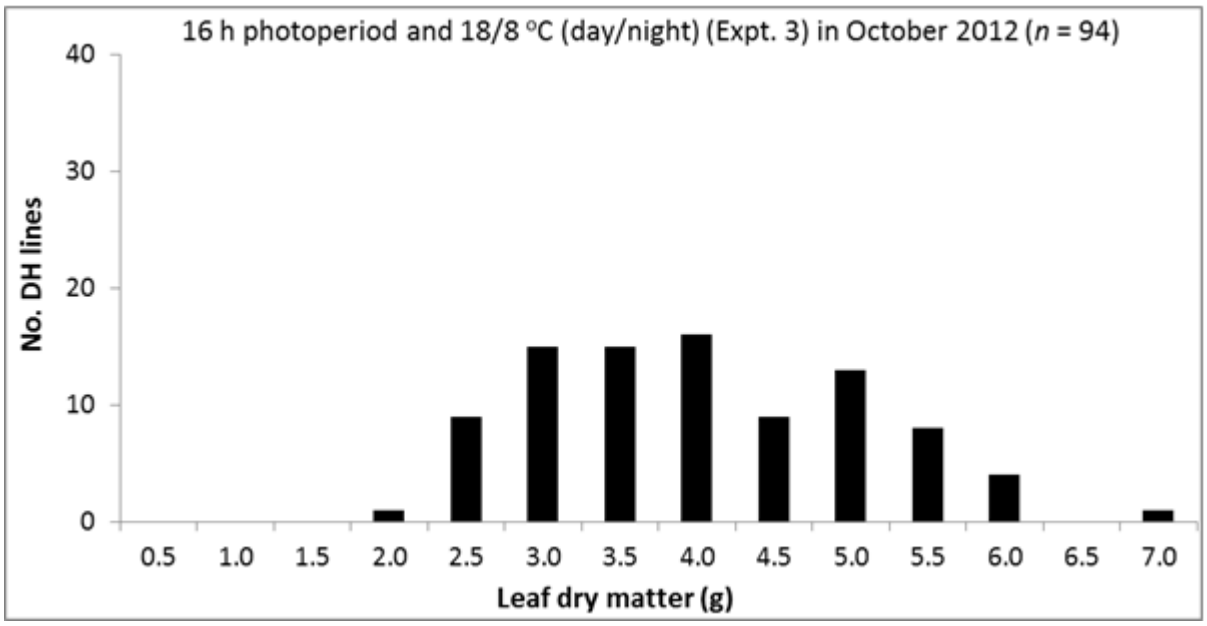

Figure 13c. Frequency distribution of the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature for leaf dry matter. Experiment conducted in October 2012.

Table 23c. Descriptive statistics for leaf dry matter of the parents and the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in October 2012.

Population	No.	Mean ± SD (g)	Range (g)
DH	94	3.90 ± 1.20	1.9 - 7.4
Hi-Q	5	5.30 ± 1.54	3.4 – 7.3
RIL-144	5	3.25 ± 1.11	1.9 - 4.5
F_1 (Hi-Q × RIL-144)	5	5.38 ± 1.55	3.4 – 6.8
Peace	5	2.68 ± 0.75	2.2 – 3.8

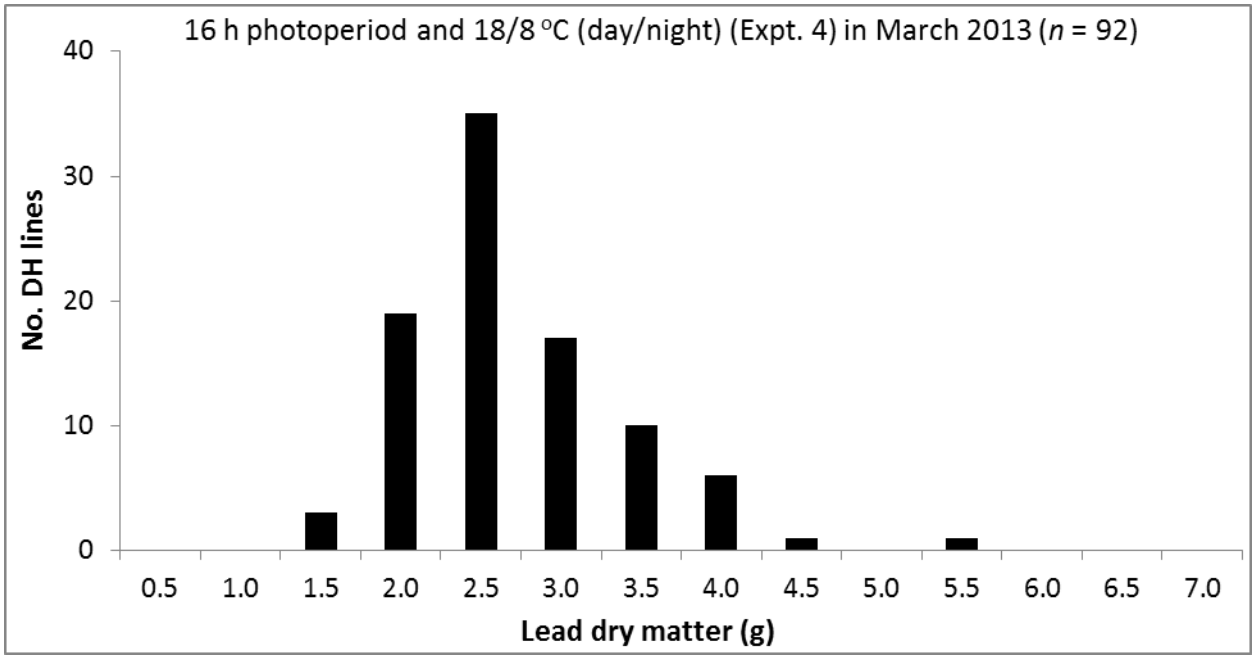

Figure 13d. Frequency distribution of the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature for leaf dry matter. Experiment conducted in March 2013.

Table 23d. Descriptive statistics for leaf dry matter of the parents and the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in March 2013.

Population	No.	Mean ± SD (g)	Range (g)
DH	92	2.49 ± 0.65	1.2 - 5.5
Hi-Q	4	3.25 ± 0.42	2.8 – 3.7
RIL-144	4	2.41 ± 0.48	1.8 – 3.0
F_1 (Hi-Q × RIL-144)	2	3.03 ± 0.31	2.7 – 3.3
Peace	4	1.84 ± 0.69	1.0 – 2.5

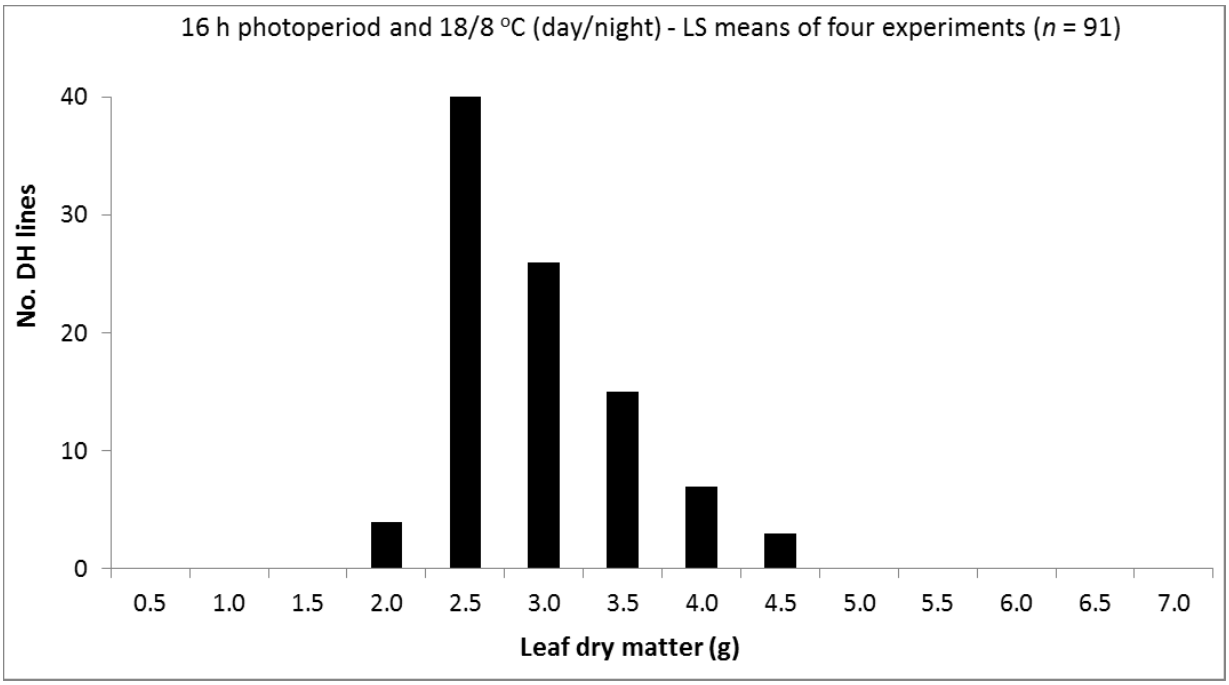

Figure 13e. Frequency distribution of the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature for leaf dry matter. LS means data based on four experiments conducted during October 2011 to March 2013.

Table 23e. Descriptive statistics for leaf dry matter of the parents and the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature. The early flowering *B. napus* cv. Peace is included as check. LS means data based on four experiments conducted during October 2011 to March 2013.

Population	No.	Mean ± SD (g)	Range (g)
DH	91	2.70 ± 0.60	1.7 - 4.5
Hi-Q	15	2.72 ± 0.88	0.9 - 7.3
RIL-144	16	1.96 ± 0.83	0.1 - 4.5
F_1 (Hi-Q × RIL-144)	10	3.07 ± 1.03	1.8 - 6.8
Peace	15	1.49 ± 0.90	0.5 - 3.8

Table 24. Spearman's correlation coefficients among the October 2011, March 2012, October 2012 and March 2013 experiments for leaf dry matter of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature.

	Oct 2011	Mar 2012	Oct 2012
Mar 2012	0.258* (81)[a]		
Oct 2012	0.260* (81)	0.339*** (89)	
Mar 2013	0.175 (79)	0.131 (88)	0.295** (88)

*p<0.05, **p<0.01; ; [a]in brackets, *df*

3.4.2. Root dry matter

Unlike leaf dry matter, root dry matter showed significant correlation among the four experiments conducted in March 2012, October 2012, March 2013 and May 2013 (Table 26). This suggests that the DH lines behaved similarly for root dry matter in these experiments. On an average, Hi-Q produced more than double amount of root dry matter than the early flowering line RIL-144. The F_1 and DH population mean fell in between the two parents. Variation in the DH population was continuous (Fig. 14a, 14b, 14c, 14d and 14e); QTL mapping may disclose genetic control of this trait.

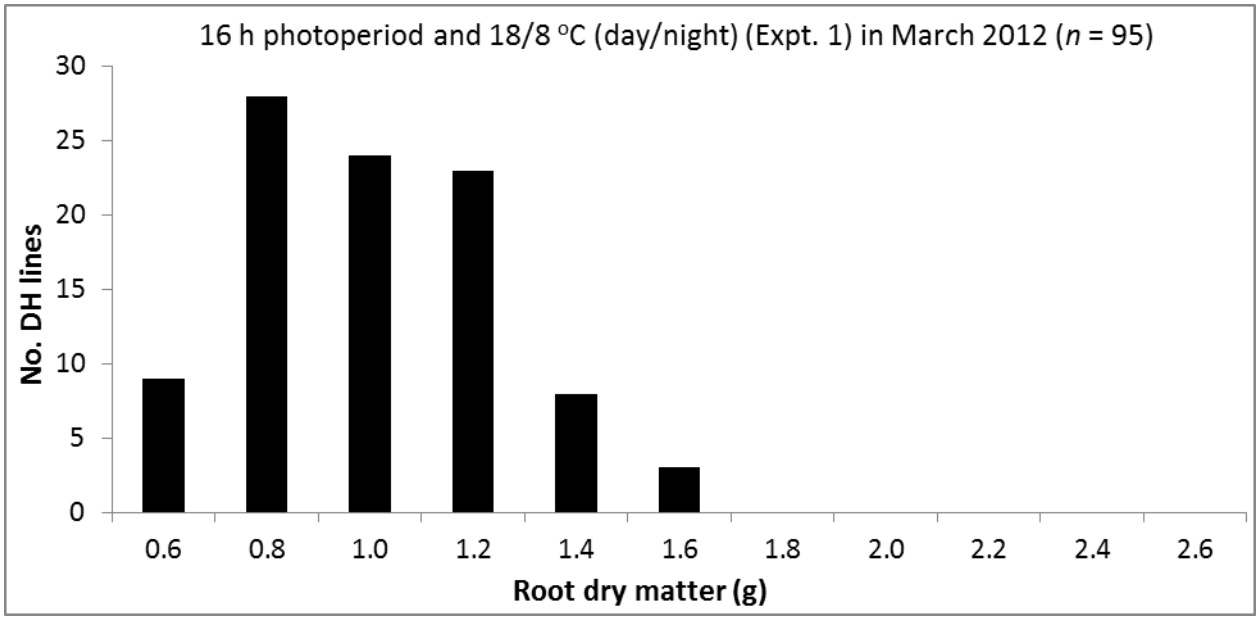

Figure 14a. Frequency distribution of the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature for root dry matter. Experiment conducted in March 2012.

Table 25a. Descriptive statistics for root dry matter of the parents and the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in March 2012.

Lines	No.	Mean ± SD (g)	Range (g)
DH	93	0.91 ± 0.25	0.6 - 1.6
Hi-Q	4	1.17 ± 0.14	1.0 - 1.3
RIL-144	4	0.74 ± 0.34	0.5 - 1.2
F_1 (Hi-Q × RIL-144)	4	1.10 ± 0.11	1.0 - 1.2
Peace	4	1.03 ± 0.34	0.7 - 1.4

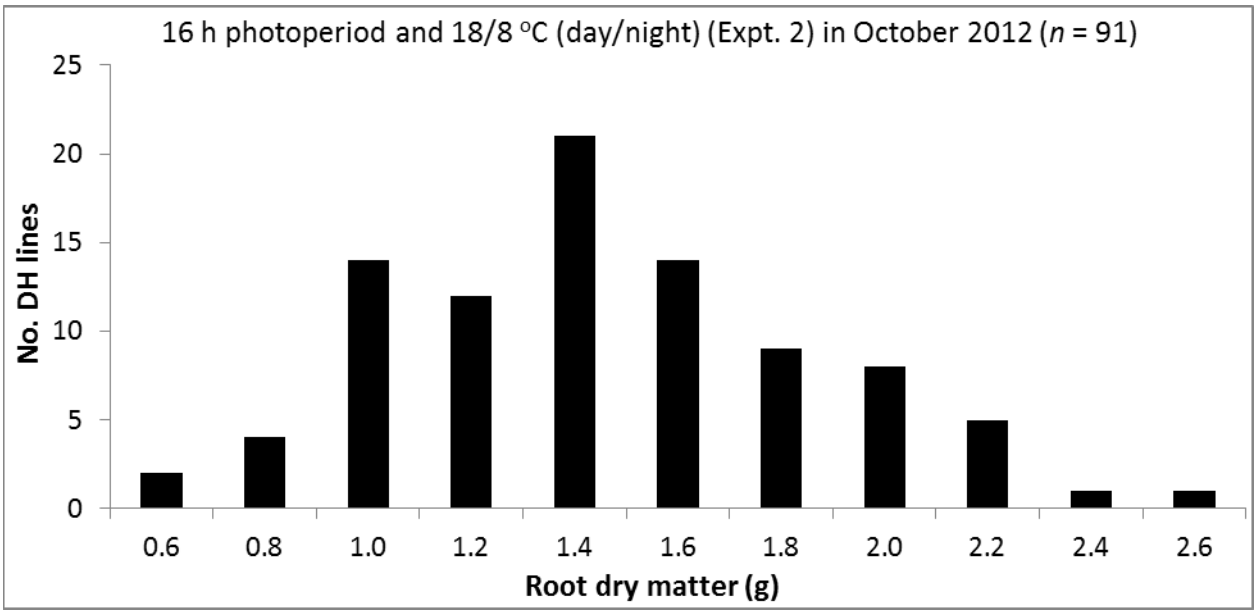

Figure 14b. Frequency distribution of the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature for root dry matter. Experiment conducted in October 2012.

Table 25b. Descriptive statistics for root dry matter of the parents and the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in October 2012.

Lines	No.	Means ± SD (g)	Range (g)
DH	91	1.41 ± 0.41	0.6 - 2.6
Hi-Q	5	2.20 ± 0.7	1.6 - 3.4
RIL-144	5	0.66 ± 0.4	0.3 - 1.2
F_1 (Hi-Q × RIL-144)	5	1.80 ± 0.2	1.6 - 2.0
Peace	5	1.44 ± 0.54	0.6 - 2.0

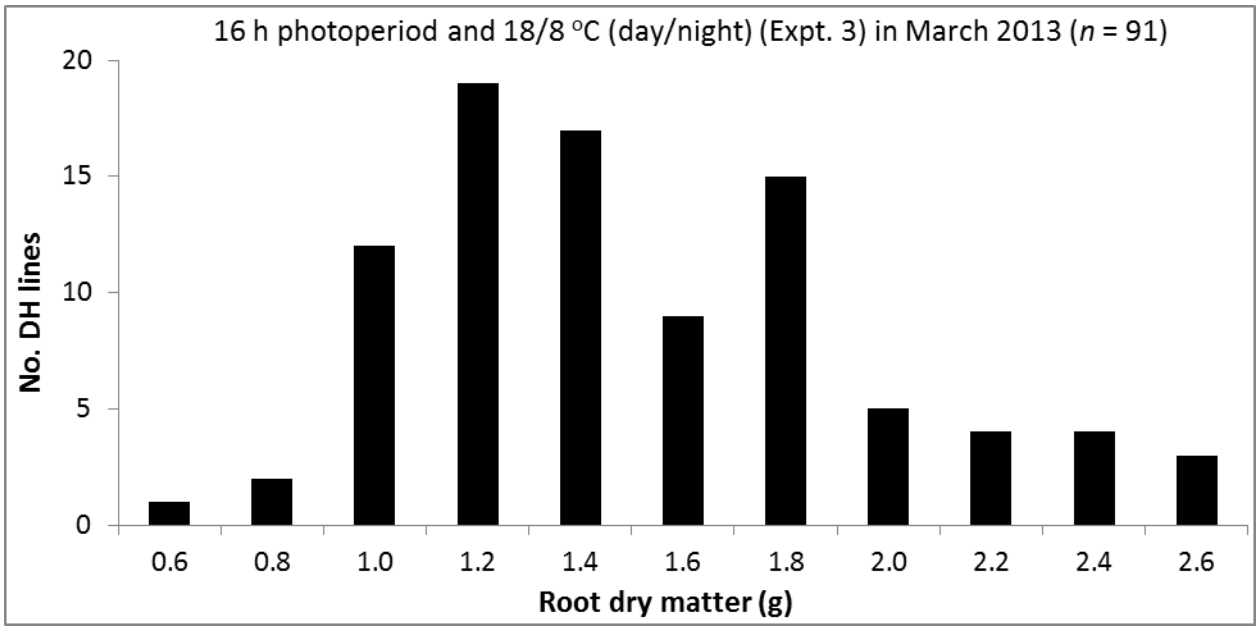

Figure 14c. Frequency distribution of the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature for root dry matter. Experiment conducted in March 2013.

Table 25c. Descriptive statistics for root dry matter of the parents and the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in March 2013.

Lines	No	Mean ± SD	Range
DH	91	1.42 ± 0.44	0.6 - 2.6
Hi-Q	4	2.84 ± 0.24	2.6 - 3.1
RIL-144	4	0.82 ± 0.04	0.8 - 0.9
F_1 (Hi-Q × RIL-144)	2	1.71 ± 0.08	1.6 - 1.8
Peace	4	1.00 ± 0.34	0.7 - 1.4

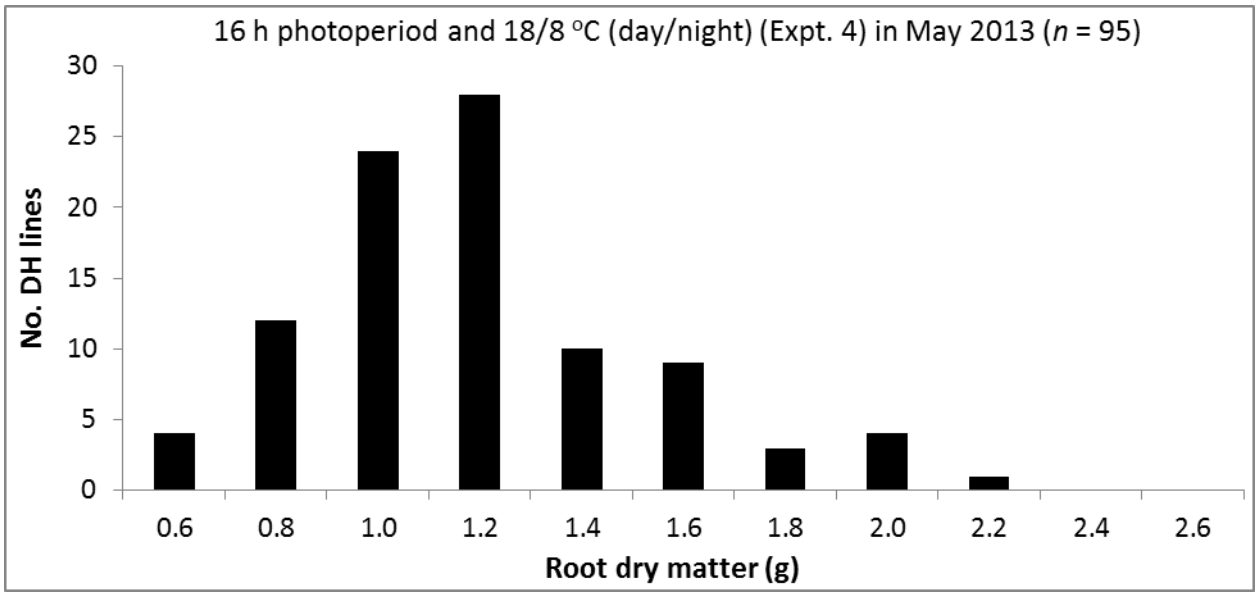

Figure 14d. Frequency distribution of the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature for root dry matter. Experiment conducted in May 2013.

Table 25d. Descriptive statistics for root dry matter of the parents and the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in May 2013.

Lines	No	Mean ± SD	Range
DH	95	1.10 ± 0.33	0.6 - 2.2
Hi-Q	5	1.12 ± 0.12	0.9 - 1.2
RIL-144	3	0.55 ± 0.13	0.4 - 0.7
F_1 (Hi-Q × RIL-144)	2	1.02 ± 0.13	0.9 - 1.1
Peace	1	0.81 ± 0.0	0.8 - 0.8

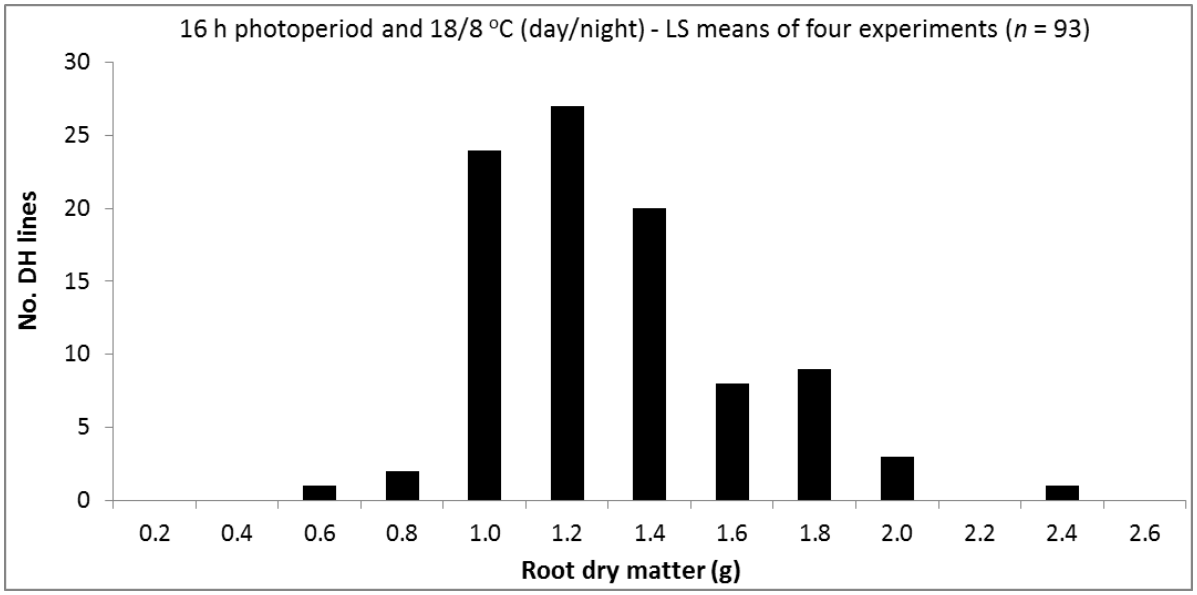

Figure 14e. Frequency distribution of the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature for root dry matter. LS means data based on four experiments conducted during March 2012 to May 2013.

Table 25e. Descriptive statistics for root dry matter of the parents and the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature. The early flowering *B. napus* cv. Peace is included as check. LS means data based on four experiments conducted during March 2012 to May 2013.

Lines	No	Mean ± SD	Range
DH	93	1.20 ± 0.30	0.6 - 2.4
Hi-Q	18	1.60 ± 0.54	0.9 - 3.4
RIL-144	16	0.64 ± 0.53	0.3 - 1.2
F_1 (Hi-Q × RIL-144)	13	1.30 ± 0.41	0.9 - 2.0
Peace	14	0.98 ± 0.51	0.6 - 1.8

Table 26. Spearman's correlation coefficients among the March 2012, October 2012, March 2013 and May 2013 experiments for root dry matter of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature.

	Mar 2012	Oct 2012	Mar 2013
Oct 2012	0.454*** (87)		
Mar 2013	0.460*** (90)	0.587*** (90)	
May 2013	0.452*** (90)	0.569*** (90)	0.642*** (92)

*p<0.05, **p<0.01; ; [a]in brackets, *df*

3.4.3. Total dry matter

Like root dry matter, total dry matter also showed significant correlation among the four experiments (Table 28). This suggests that the DH lines behaved similarly for both root and total dry matter in these experiments.

On an average, the parent Hi-Q produced about 70% greater amount of total dry matter as compared to the early-flowering line RIL-144 (Tables 27e). The F_1 plants had almost same amount of total dry matter as Hi-Q. The distribution of the DH population for total dry matter was continuous (Fig. 15a, 15b, 15c, 15d, 15e) suggesting quantitative nature of this trait.

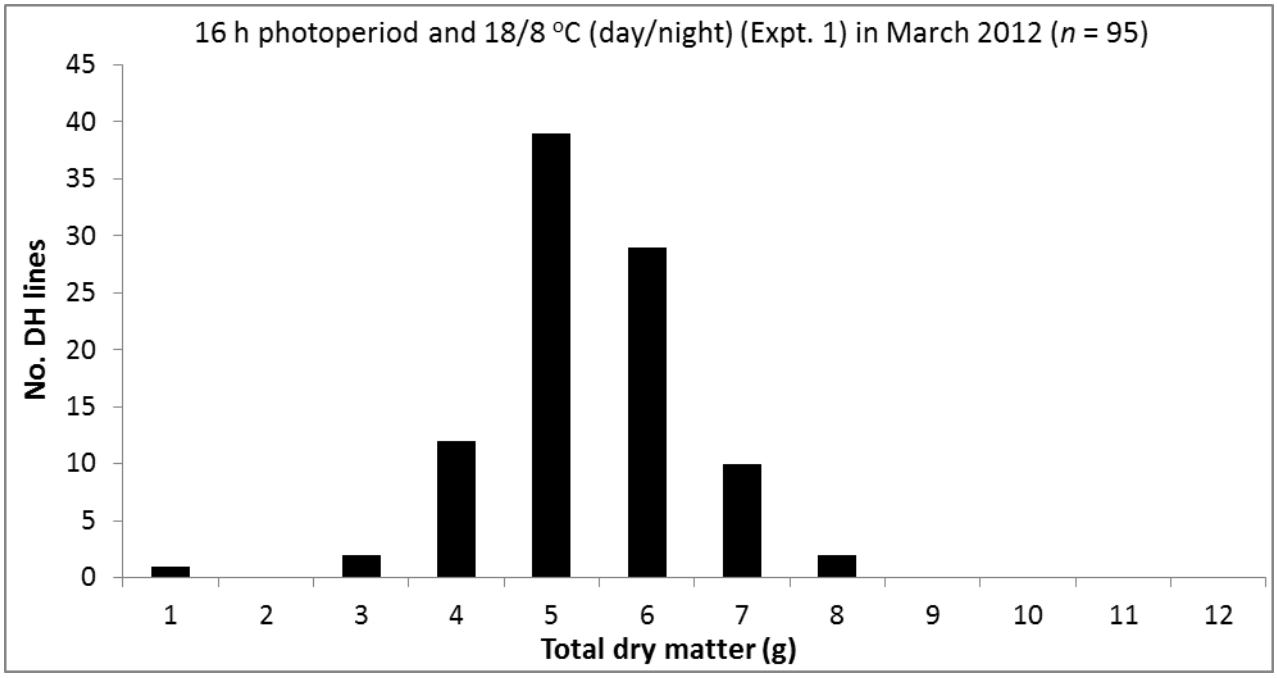

Figure 15a. Frequency distribution of the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature for total dry matter. Experiment conducted in March 2012.

Table 27a. Descriptive statistics for total dry matter of the parents and the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in March 2012.

Lines	No.	Mean ± SD (g)	Range (g)
DH	95	4.9 ± 1.0	0.6 - 7.8
Hi-Q	2	5.0 ± 0.0	5.0 - 5.0
RIL-144	3	3.9 ± 0.5	3.4 - 4.4
F_1 (Hi-Q × RIL-144)	3	5.3 ± 1.0	4.5 - 6.4
Peace	2	4.4 ± 1.4	3.4 - 5.3

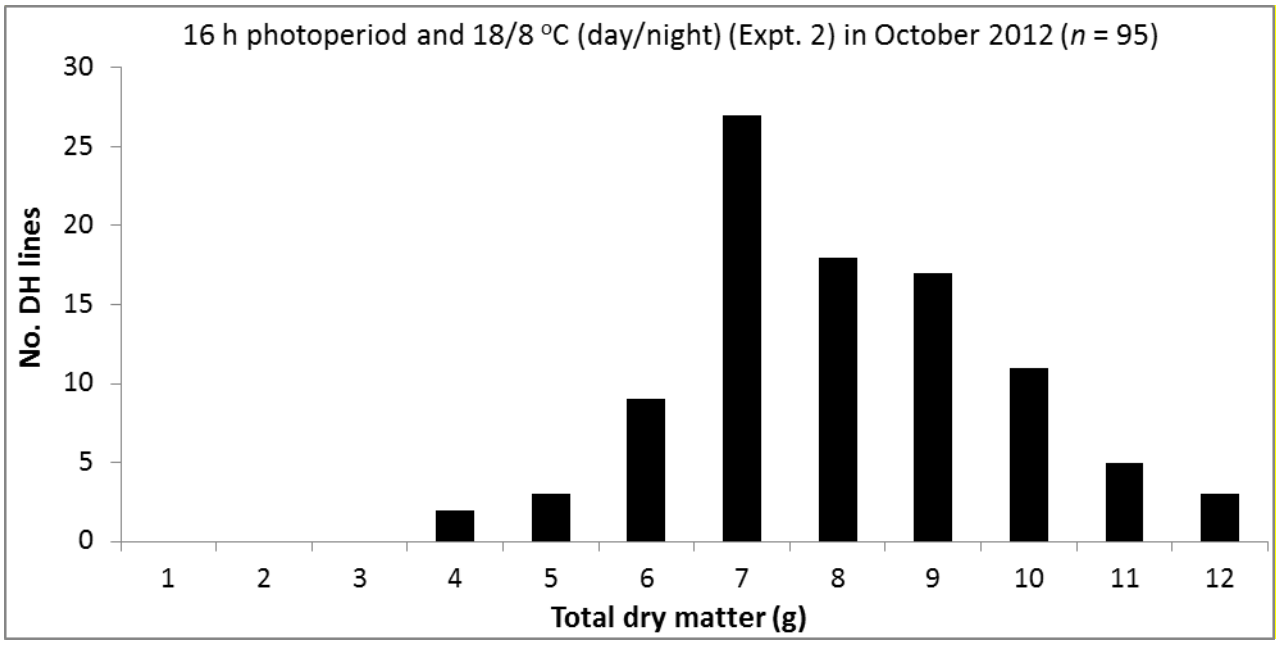

Figure 15b. Frequency distribution of the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature for total dry matter. Experiment conducted in October 2012.

Table 27b. Descriptive statistics for total dry matter of the parents and the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in October 2012.

Lines	No.	Mean ± SD (g)	Range (g)
DH	95	7.6 ± 1.7	3.6 - 11.5
Hi-Q	5	11.7 ± 2.7	9.8 - 15.3
RIL-144	5	5.7 ± 2.0	3.9 - 8.5
F_1 (Hi-Q × RIL-144)	5	10.3 ± 2.0	7.9 - 12.3
Peace	5	6.3 ± 1.8	4.8 - 8.9

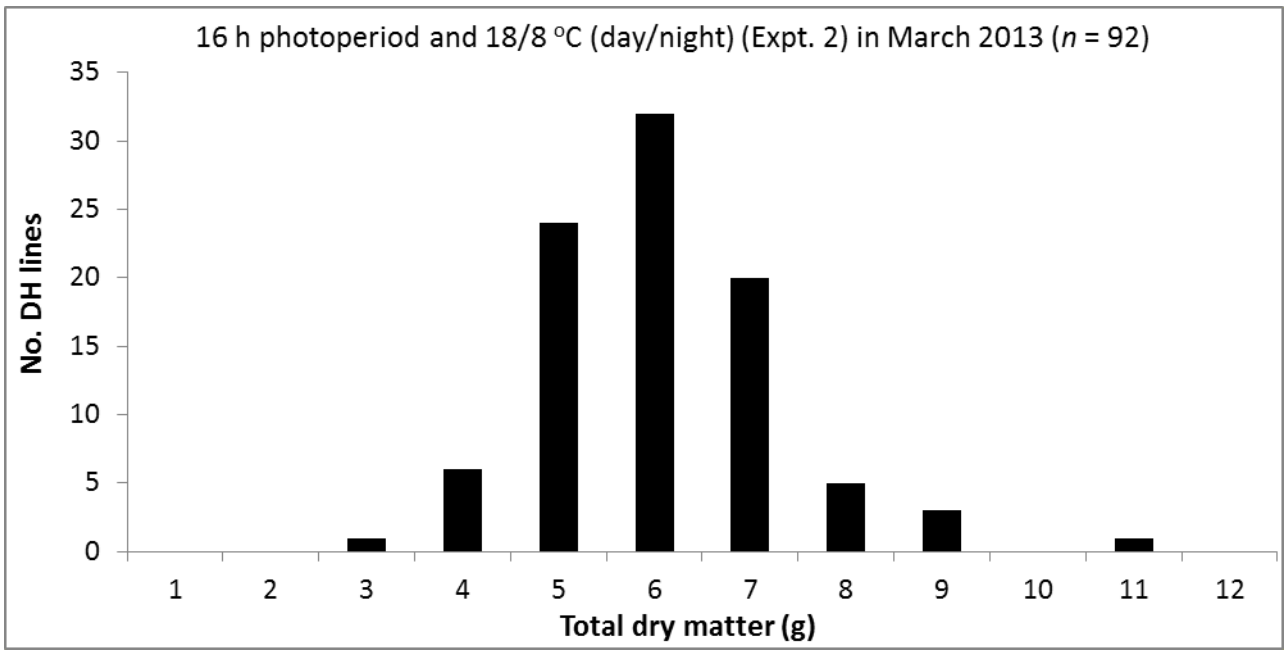

Figure 15c. Frequency distribution of the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature for total dry matter. Experiment conducted in March 2013.

Table 27c. Descriptive statistics for total dry matter of the parents and the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in March 2013.

Lines	No.	Mean ± SD (g)	Range (g)
DH	92	5.5 ± 1.2	3.0 - 10.5
Hi-Q	4	8.9 ± 0.5	8.4 - 9.5
RIL-144	4	4.8 ± 0.7	3.8 - 5.5
F_1 (Hi-Q × RIL-144)	3	6.6 ± 0.6	6.0 - 7.1
Peace	4	4.2 ± 1.2	2.7 - 5.3

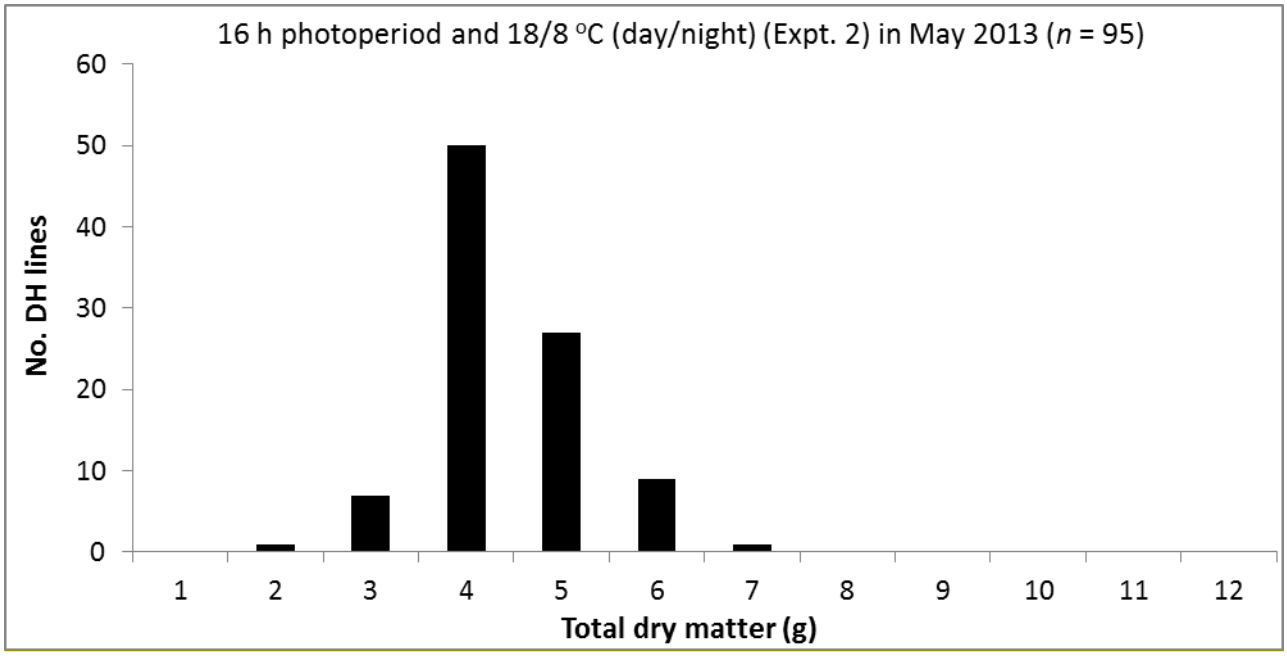

Figure 15d. Frequency distribution of the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature for total dry matter. Experiment conducted in May 2013.

Table 27d. Descriptive statistics for total dry matter of the parents and the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in May 2013.

Lines	No.	Mean ± SD (g)	Range (g)
DH	95	3.9 ± 0.8	1.6 - 6.8
Hi-Q	5	3.9 ± 0.2	3.7 - 4.1
RIL-144	3	2.8 ± 0.2	2.7 - 3.1
F_1 (Hi-Q × RIL-144)	2	4.5 ± 0.3	4.2 - 5.7
Peace	1	3.1 ± 0.0	3.1 - 3.1

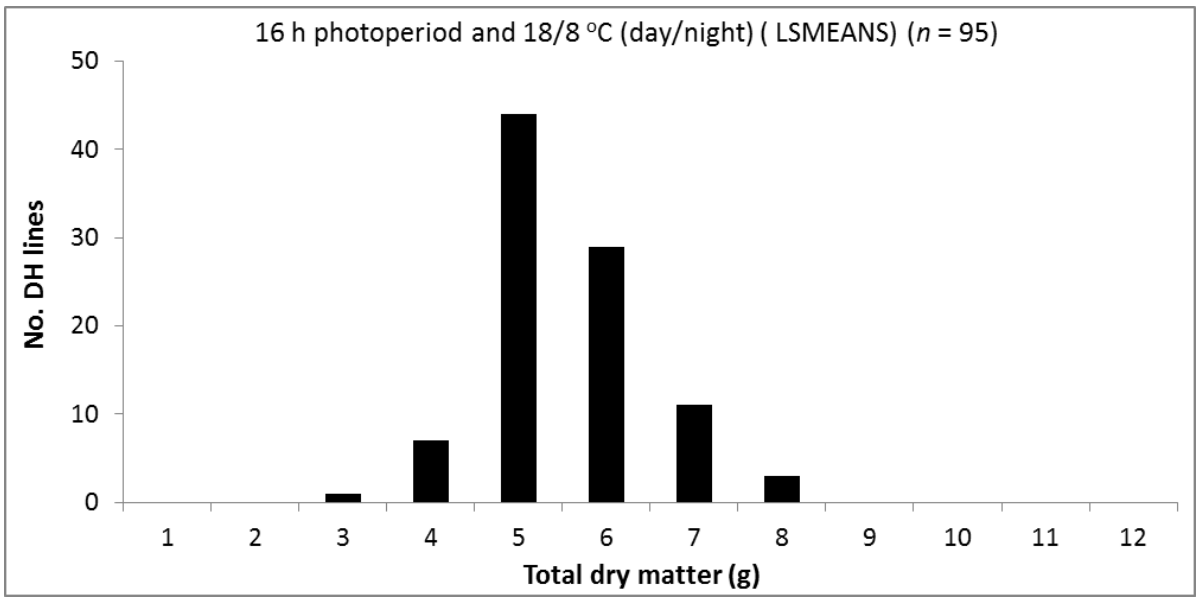

Figure 15e. Frequency distribution of the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature for root dry matter. LS means data based on four experiments conducted during March 2012 to May 2013.

Table 27e. Descriptive statistics for root dry matter of the parents and the DH population of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature. The early flowering *B. napus* cv. Peace is included as check. LS means data based on four experiments conducted during March 2012 to May 2013.

Lines	No	Mean ± SD	Range
DH	95	5.0 ± 0.9	2.8 - 7.7
Hi-Q	16	6.5 ± 1.6	3.5 - 15.2
RIL-144	15	3.8 ± 1.6	1.9 - 7.8
F_1 (Hi-Q × RIL-144)	13	6.3 ± 2.0	4.2 - 12.3
Peace	12	3.7 ± 1.8	2.8 - 8.9

Table 28. Spearman's correlation coefficients among the March 2012, October 2012, March 2013 and May 2013 experiments for root dry matter of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown under 16 h photoperid and 18/8 ^{0}C (day/night) temperature.

	Mar 2012	Oct 2012	Mar 2013
Oct 2012	0.223* (93)		
Mar 2013	0.226* (90)	0.252* (90)	
May 2013	0.279* (93)	0.322** (93)	0.555*** (90)

*p<0.05, **p<0.01, ***p<0.001; [a]in brackets, *df*

3.5. Field Evaluation of the DH lines for Days to Flower

Analysis of variance was conducted using PROC MIXED procedure of SAS (SAS Institute, 2003) in which genetic, environment, and interaction between genotype and environment was analyzed using the following model: $Yij = \mu + gi + ej + geij + eij$, where *Yij* is observation of genotype *i* in environment *j* ; μ is the general mean; *gi* is the effect of genotype *i*; *ej* is the effect of environment *j, geij* is the genotype × environment interaction of genotype *i* in environment *j*; and e*ij* is the residual error of genotype *i* in environment *j*. Environment and genotype × environment interaction factors were considered as random. In both 1-row and full plot field trials, significance variation between the genotypes for days to flower was found; however, no genotype × environment interaction could be detected (Table S3).

Like the growth chamber experiments, the parent RIL-144 flowered earlier than Hi-Q; flowering in F_1 was intermediate of the two parents (Tables 29a to 29l). The distribution of the DH population for days to flower was continuous (Fig. 16a to 16l), as was found in most of the growth chamber experiments conducted under long photoperiod (≥14 h).

Correlation between the experiments for days to flower was significant in 54 of the 55 combinations (Table 30). This suggests that the DH lines behaved similarly in most of the field trials, i.e. flowering time is a highly heritable trait and least vulnerable to genotype × environment interaction.

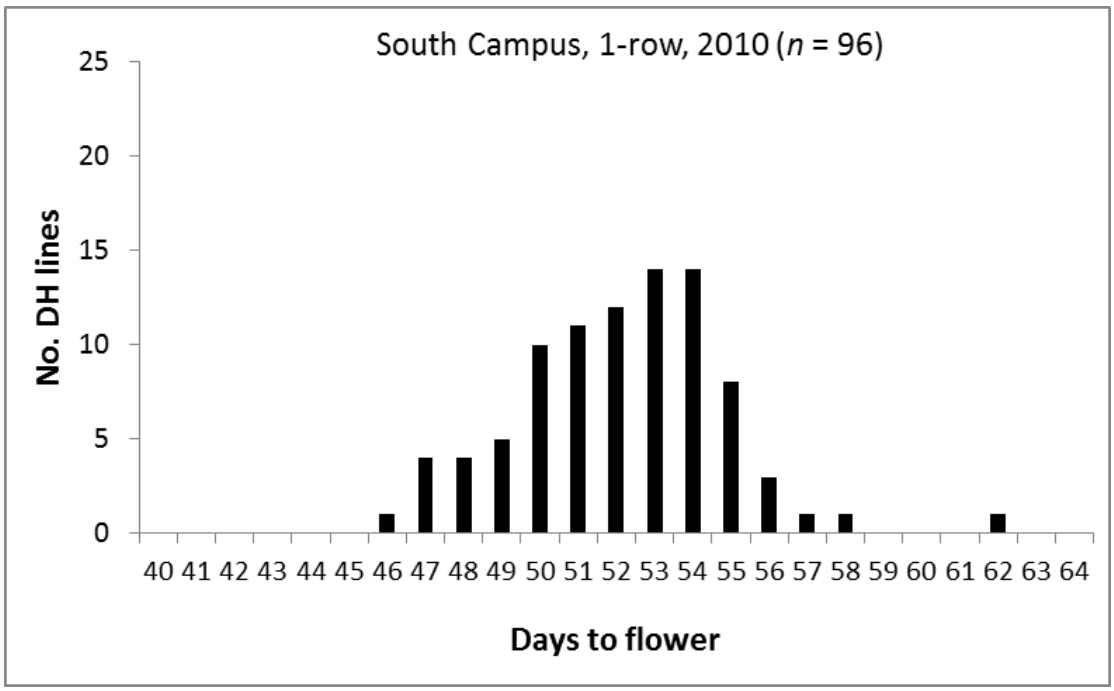

Figure 16a. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* in 1-row field trial at South Campus for days to flower. Experiment conducted in 2010.

Table 29a. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown in 1-row field trial at South Campus. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in 2010.

Population	No.	Mean ± S.D.	Range
DH lines	96	52.1 ± 2.7	46 - 62
Hi-Q	8	54.6 ± 0.7	54 - 56
RIL-144	2	46.6 ± 2.4	44 - 50
Peace	8	48.5 ± 0.7	46 - 51

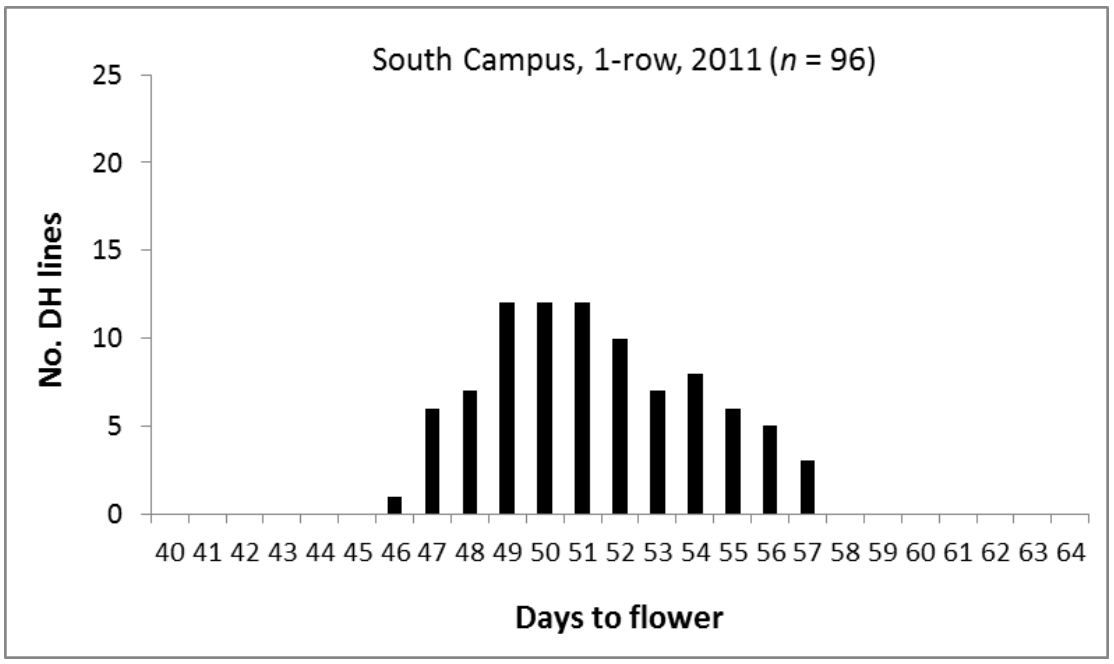

Figure 16b. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* in 1-row field trial at South Campus for days to flower. Experiment conducted in 2011.

Table 29b. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown in 1-row field trial at South Campus. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in 2011.

Population	No.	Mean ± S.D.	Range
DH lines	96	51.3 ± 2.7	46 - 57
Hi-Q	8	53.5 ± 0.9	52 - 55
RIL-144	2	47.0 ± 0.0	47 - 47
F_1 (Hi-Q × RIL-144)	2	50.5 ± 0.7	50 - 51
Peace	6	48.0 ± 2.0	46 - 53

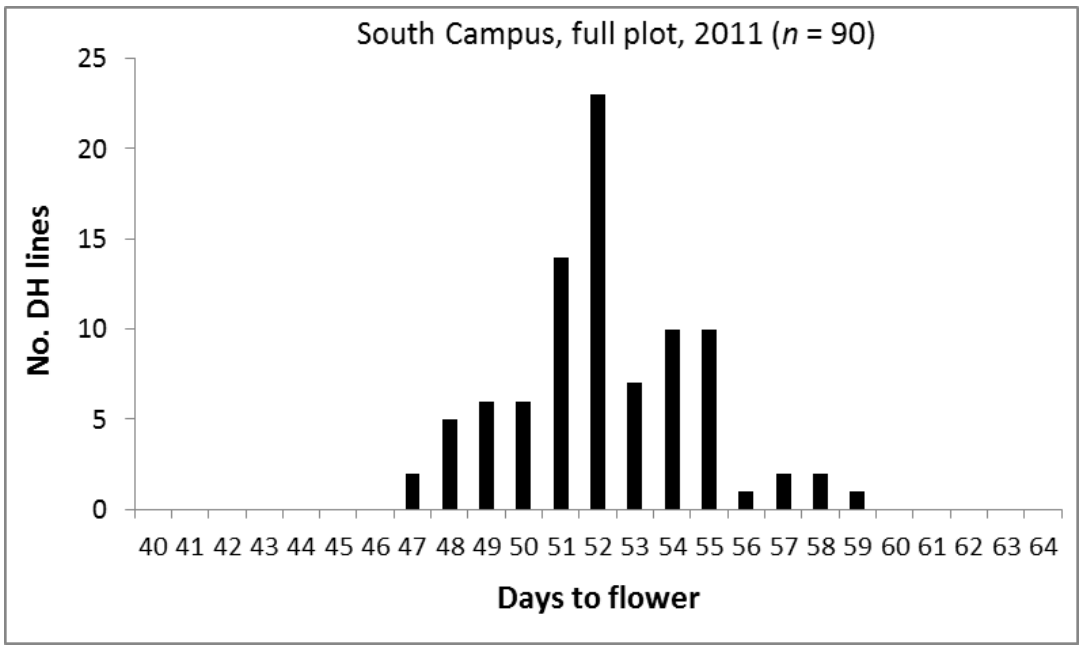

Figure 16c. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* in full plot field trial at South Campus for days to flower. Experiment conducted in 2011.

Table 29c. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown in full plot field trial at South Campus. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in 2011.

Population	No.	Mean ± S.D.	Range
DH lines	90	52.1 ± 2.7	47 - 59
Hi-Q	6	54.7 ± 1.0	53 - 56
RIL-144	2	47.0 ± 0.0	47 - 47
F_1 (Hi-Q × RIL-144)	2	50.5 ± 0.7	50 - 51
Peace	6	49.2 ± 2.0	47 - 52

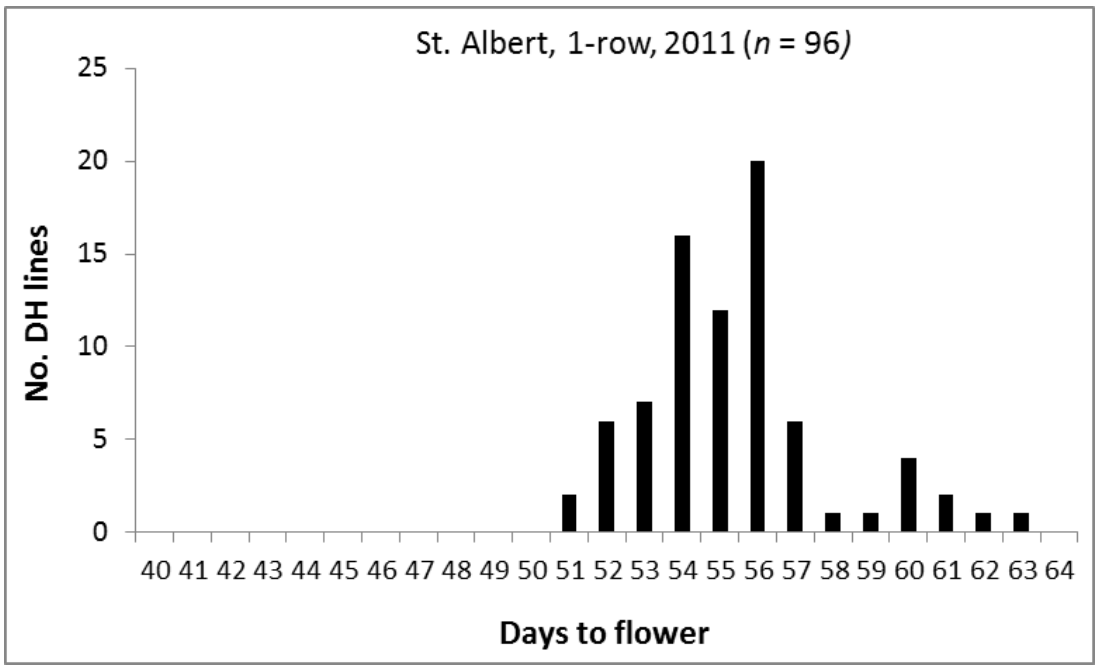

Figure 16d. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* in 1-row field trial at St. Albert for days to flower. Experiment conducted in 2011.

Table 29d. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown in 1-row field trial at St. Albert. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in 2011.

Population	No.	Mean ± S.D.	Range
DH lines	96	56.3 ± 2.1	51 - 63
Hi-Q	8	57.3 ± 1.3	56 - 59
RIL-144	2	53.0 ± 0.0	53 - 53
F_1 (Hi-Q × RIL-144)	1	55.0 ± 0.0	55 - 55
Peace	6	51.0 ± 1.6	49 - 53

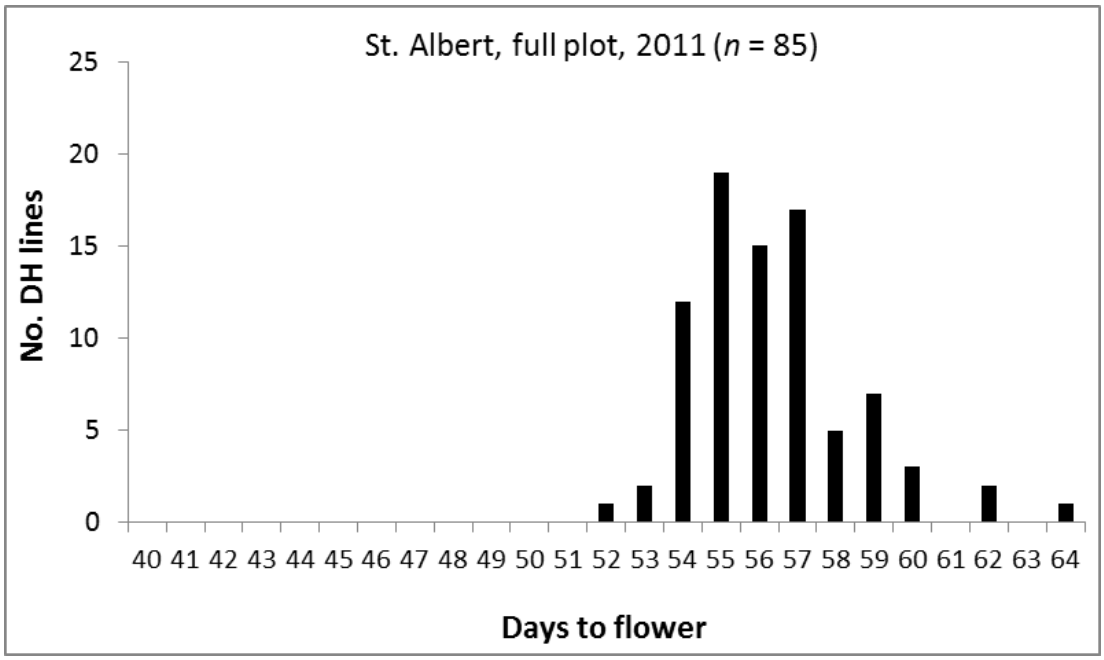

Figure 16e. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* in full plot field trial at St. Albert for days to flower. Experiment conducted in 2011.

Table 29e. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown in full plot field trial at St. Albert. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in 2011.

Population	No.	Mean ± S.D.	Range
DH lines	85	56.1 ± 2.4	52 - 64
Hi-Q	6	57.8 ± 1.3	57 - 60
RIL-144	2	54.0 ± 0.0	54 - 54
Peace	6	53.2 ± 1.0	52 - 54

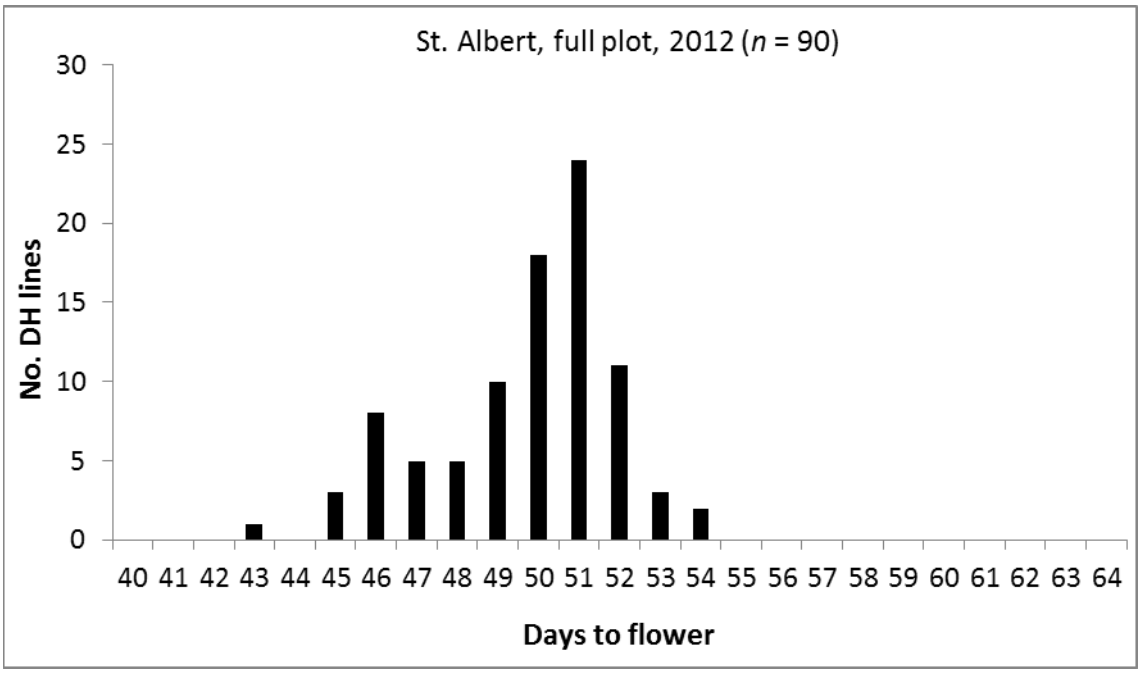

Figure 16f. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* in full plot field trial at St. Albert for days to flower. Experiment conducted in 2012.

Table 29f. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown in full plot field trial at St. Albert. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in 2012.

Population	No.	Mean ± S.D.	Range
DH lines	90	49.5 ± 2.2	43 - 54
Hi-Q	6	50.7 ± 2.3	45 - 53
RIL-144	2	43.0 ± 2.4	41 - 45
Peace	6	44.0 ± 1.4	43 - 45

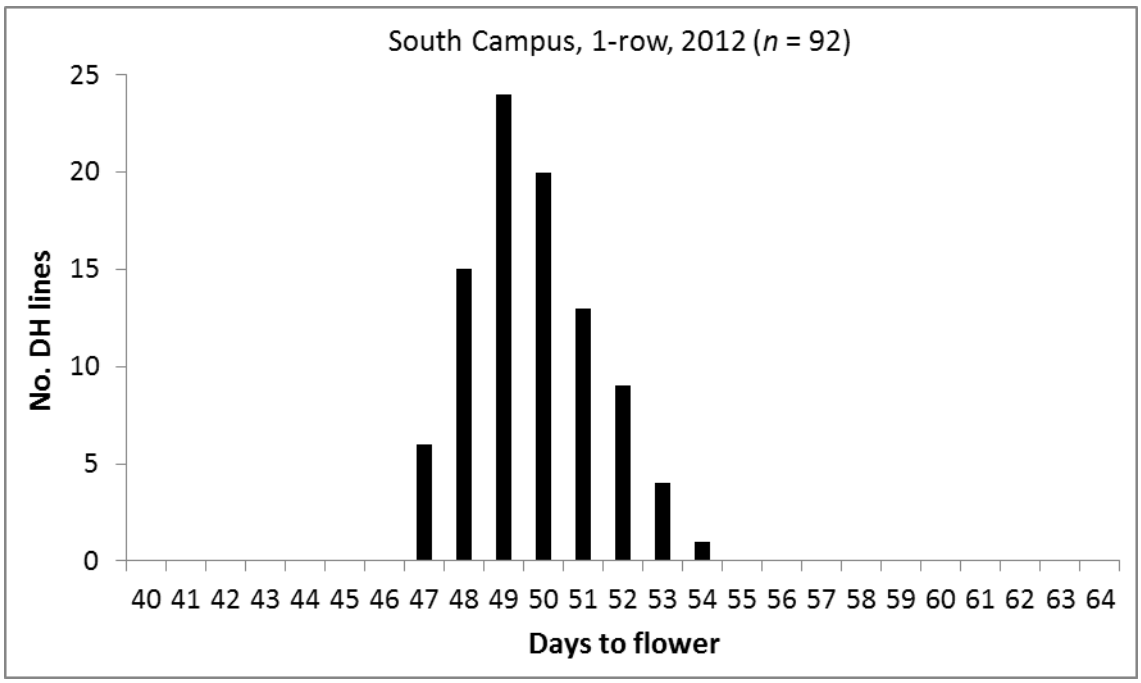

Figure 16g. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* in 1-row field trial at South Campus for days to flower. Experiment conducted in 2012.

Table 29g. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown in 1-row field trial at South Campus. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in 2012.

Population	No.	Mean ± S.D.	Range
DH lines	92	49.5 ± 2.7	47 - 54
Hi-Q	4	52.4 ± 0.5	52 - 53
RIL-144	2	47.0 ± 1.4	46 - 48
Peace	2	47.2 ± 2.2	44 - 49

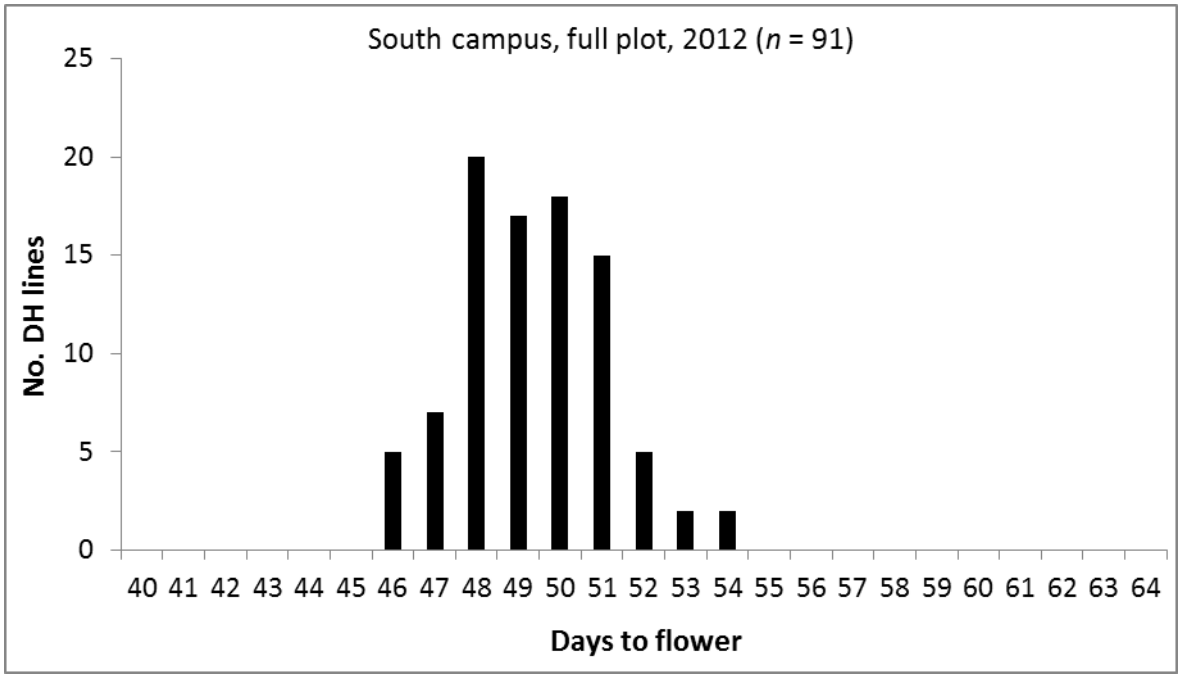

Figure 16h. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* in full plot field trial at South Campus for days to flower. Experiment conducted in 2012.

Table 29h. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown in full plot field trial at South Campus. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in 2012.

Population	No.	Mean ± S.D.	Range
DH lines	91	48.9 ± 2.9	46 - 54
Hi-Q	6	49.5 ± 2.4	45 - 53
RIL-144	2	43.0 ± 2.8	41 - 45
Peace	6	45.8 ± 2.7	41 - 48

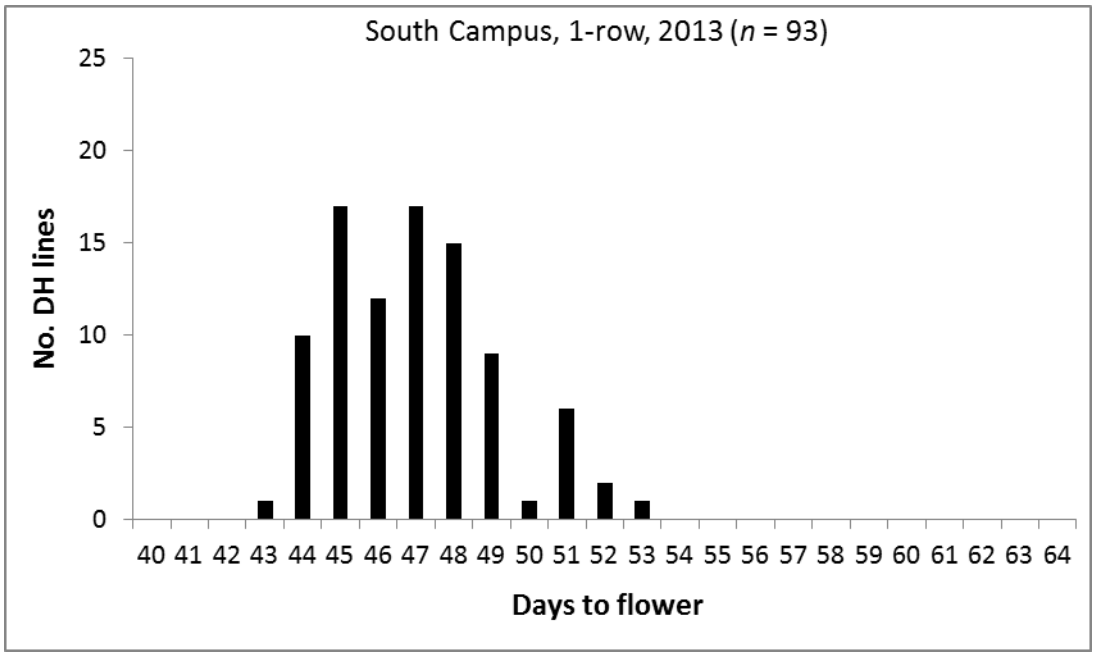

Figure 16i. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* in 1-row field trial at South Campus for days to flower. Experiment conducted in 2013.

Table 29i. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown in 1-row field trial at South Campus. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in 2013.

Population	No.	Mean ± S.D.	Range
DH lines	93	46.8 ± 2.1	43 - 53
Hi-Q	3	49.0 ± 0.0	49 - 49
RIL-144	3	43.0 ± 0.0	43 - 43
Peace	3	43.0 ± 0.0	43 - 43

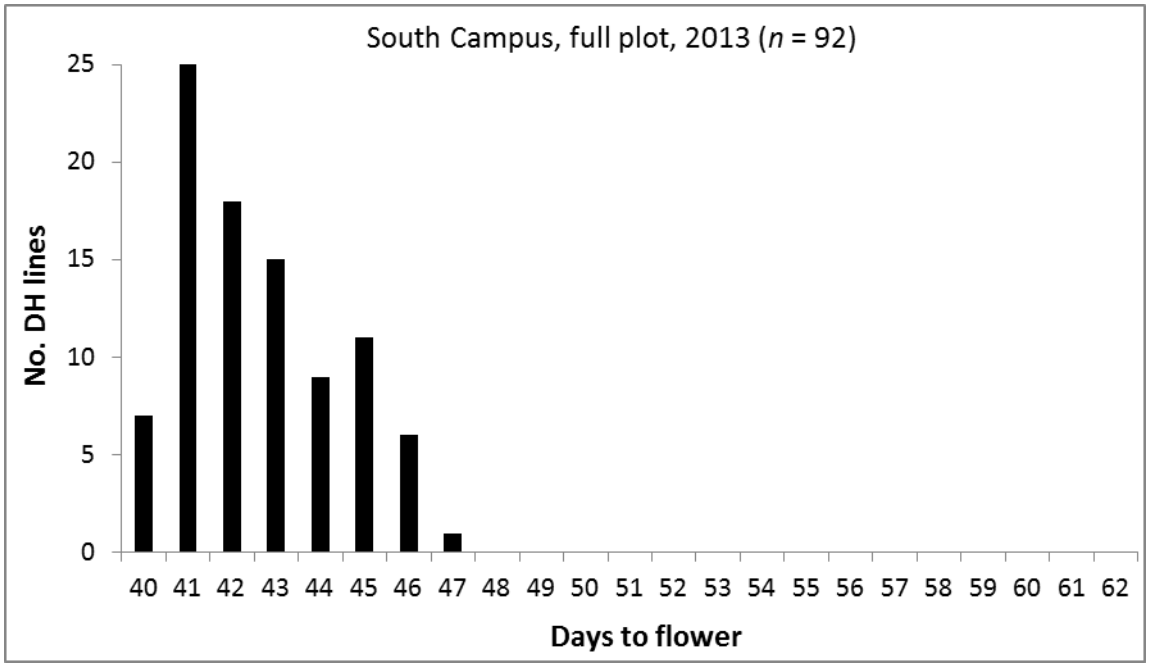

Figure 16j. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* in full plot field trial at South Campus for days to flower. Experiment conducted in 2013.

Table 29j. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown in full plot field trial at South Campus. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in 2013.

Population	No.	Mean ± S.D.	Range
DH lines	92	42.1 ± 1.8	40 - 47
Hi-Q	3	43.3 ± 1.8	41 - 45
RIL-144	3	39.7 ± 0.6	39 - 40
Peace	5	39.3 ± 0.5	39 - 40

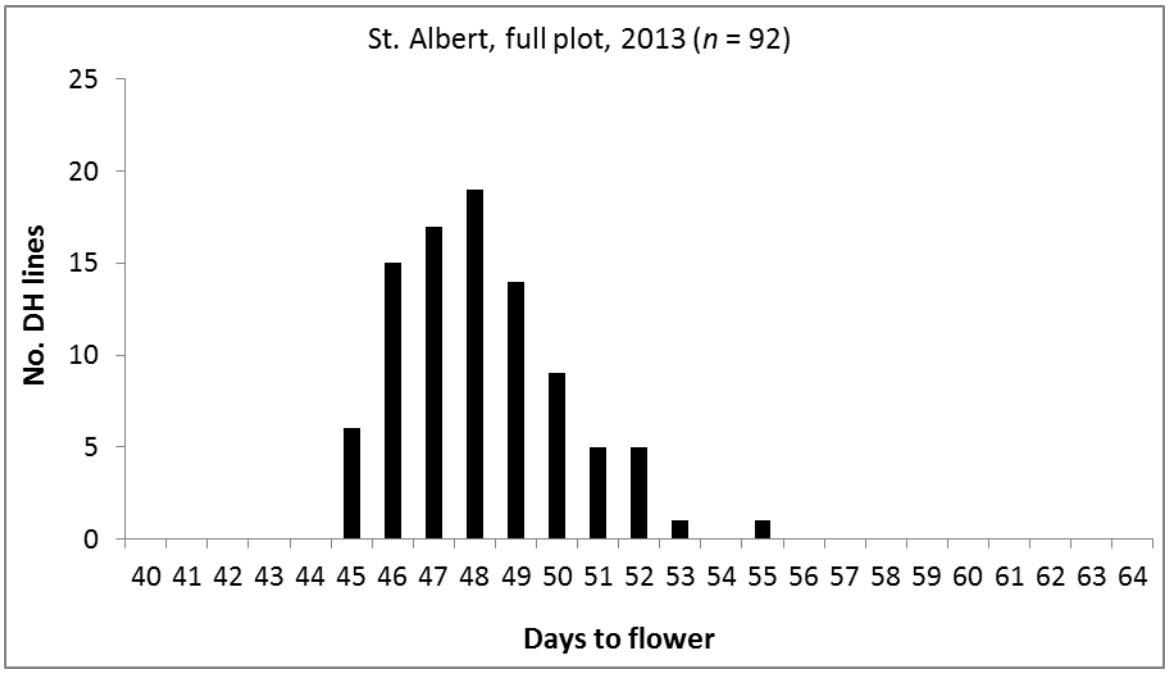

Figure 16k. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* in full plot field trial at St. Albert for days to flower. Experiment conducted in 2013.

Table 29k. Descriptive statistics for days to flower of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown in full plot field trial at St. Albert. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in 2013.

Population	No.	Mean ± S.D.	Range
DH lines	92	47.8 ± 2.0	45 - 55
Hi-Q	3	53.0 ± 1.6	51 - 55
RIL-144	3	45.3 ± 1.5	44 - 47
Peace	6	44.5 ± 1.9	44 - 45

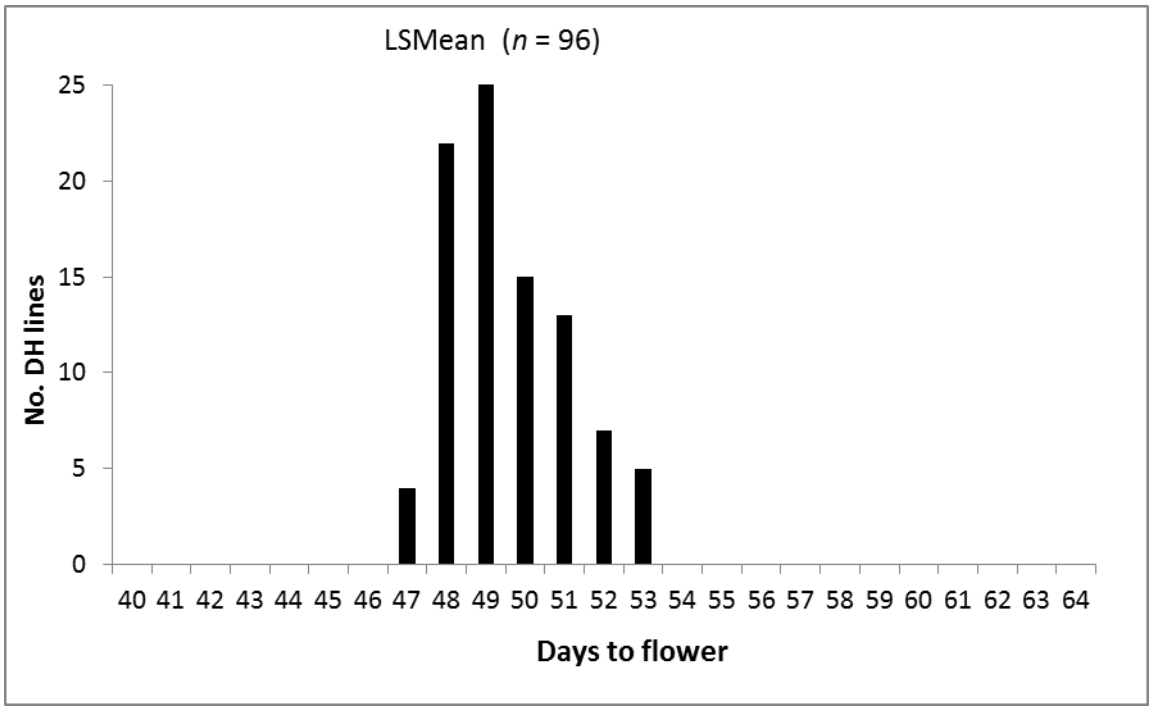

Figure 16l. Frequency distribution of the LS means of DH lines of Hi-Q × RIL-144 cross of *Brassica napus* for days to flower in field trials conducted in 2010 to 2013 at St. Albert and South Campus locations.

Table 29l. Descriptive statistics based on LS means of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* for days to flower in field trials conducted in 2010 to 2013 at St. Albert and South Campus locations.

Population	No.	Mean ± S.D.	Range
DH lines	96	49.1 ± 1.6	47 - 53
Hi-Q	61	50.3 ± 1.4	47 - 52
RIL-144	25	44.1 ± 1.2	40 - 48
Peace	60	46.8 ± 0.8	43 - 50

Table 30. Spearman's correlation coefficients for days to flower among the field trials conducted in 2010 to 2013 with the DH lines of Hi-Q × RIL-144 cross of *Brassica napus*.

	SA2011R	SC2011F	SC2010R	SC2011R	SA2012F	SC2012R	SC2012F	SC2013R	SC2013F	SA2013F
SA2011F	0.515[a]	0.684	0.68	0.661	0.288	0.533	0.616	0.671	0.546	0.593
	<.0001[b]	<.0001	<.0001	<.0001	0.008	<.0001	<.0001	<.0001	<.0001	<.0001
	69[c]	82	82	79	81	80	81	80	82	82
SA2011R		0.545	0.473	0.618	0.238	0.562	0.631	0.623	0.626	0.541
		<.0001	<.0001	<.0001	0.040	<.0001	<.0001	<.0001	<.0001	<.0001
		74	74	75	73	76	73	76	77	77
SC2011F			0.646	0.707	0.365	0.384	0.593	0.731	0.710	0.621
			<.0001	<.0001	0.001	0.001	<.0001	<.0001	<.0001	<.0001
			87	84	86	85	86	85	87	87
SC2010R				0.652	0.325	0.439	0.595	0.641	0.604	0.545
				<.0001	0.002	<.0001	<.0001	<.0001	<.0001	<.0001
				84	86	85	86	85	87	87
SC2011R					0.28	0.391	0.585	0.708	0.681	0.613
					0.009	0.001	<.0001	<.0001	<.0001	<.0001
					83	86	83	85	87	87
SA2012F						0.118	0.621	0.294	0.245	0.26
						0.279	<.0001	0.006	0.022	0.014
						84	86	84	86	86
SC2012R							0.315	0.439	0.378	0.402
							0.003	<.0001	0.001	<.0001
							84	86	88	88
SC2012F								0.663	0.634	0.625
								<.0001	<.0001	<.0001
								84	86	86
SC2013R									0.757	0.607
									<.0001	<.0001
									88	88
SC2013F										0.656
										<.0001
										90

[a]Spearman correlation coffcient, [b]probablty of the correlation coffcient ($p \leq 0001$ is highly significan correlation), [c]degree of freedom

SA2011F = Days to flower in full plot field trail at St. Albert in 2011; SA2011R = Days to flower in 1-row field trail at St. Albert in 2011; SC2011F = Days to flower in full plot field trail at South Campus in 2011, SC2010R = Days to flower in 1-row field trail at South Campus in 2010; SC2011R = Days to flower in 1-row field trail at South Campus in 2011; SA2012F = Days to flower in full plot field trail at St. Albert in 2012; SC2012R = Days to flower in 1-row field trail at South Campus in 2012, SC2012F: Days to flower in full plot field trail at South Campus in 2012; SC2013R = Days to flower in 1-row field trail at South Campus in 2013; SC2013F = Days to flower in full plot field trail at South Campus in 2013; SA2013F = Days to flower in full plot field trail at St. Albert in 2013.

3.6. Field Evaluation of the DH Lines for Seed Yield

Seven trials seeded in 2011, 2012 and 2013 at three locations in Alberta (Table 3). Seed yield data of the two trials seeded in 2012 at South Campus and St. Albert locations, and the trials seeded at South Campus in 2013 could not be used due to damage caused by hail and root maggot, respectively. In the four trials, the parent Hi-Q produced on an average 2,516 kg/ha; while the early flowering line RIL-144 produced significantly lower seed yield (1,915 kg/ha) than Hi-Q. Average seed yield of the DH population was 2,151 kg/ha, which is also lower than Hi-Q (Tables 31e).

The DH population showed continuous variation for seed yield (Fig. 17a to 17d) as could be expected for a quantitative trait. Correlation between the experiments for seed yield was non-significant in most cases (Table 32) except the trials in Killam and St. Albert in 2013. This suggests that performance of the DH lines was not consistent across the environments – high genotype × environment interaction is evident for seed yield. The two experiemnts seeded in Killam and St. Albert in 2013 showed sisgificant correlation, therefore LS means for seed yield was calculated based on these two data set. Few DH lines produced seed yield as high as Hi-Q (Fig. 17a to 17e).

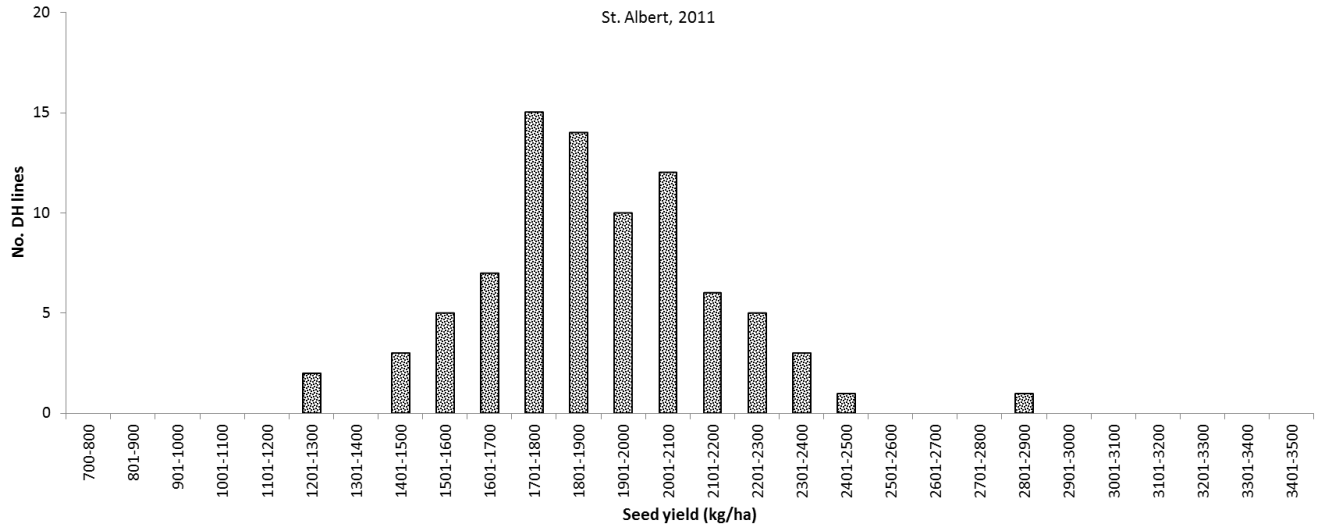

Figure 17a. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* for seed yield in full plot field trial at St. Albert. Experiment conducted in 2011.

Table 31a. Descriptive statistics for seed yield (kg/ha) of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown in full plot field trial at St. Albert. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in 2011.

Population	No.	Mean ± S.D.	Range
DH lines	85	1890.0 ± 262	1212 - 2825
Hi-Q	6	2459.6 ± 251	2020 - 2671
RIL-144	2	2023.9 ± 293	1516 - 2509
Peace	6	2147.6 ± 327	1817 - 2609

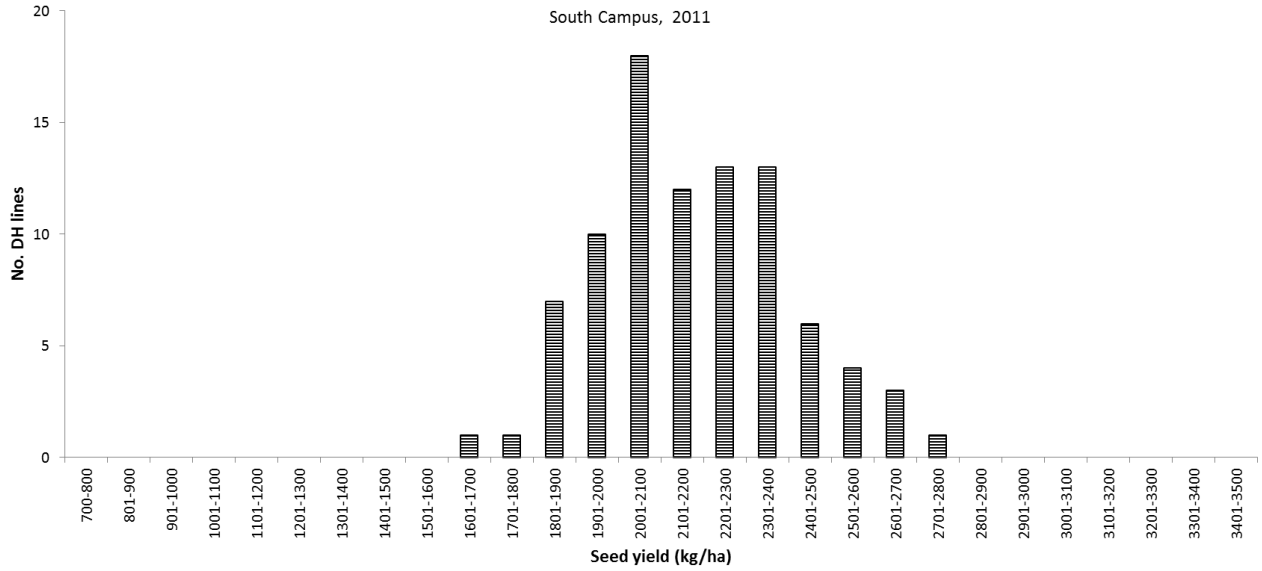

Figure 17b. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* for seed yield in full plot field trial at South Campus. Experiment conducted in 2011.

Table 31b. Descriptive statistics for seed yield (kg/ha) of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown in full plot field trial at South Campus. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in 2011.

Population	No.	Mean ± S.D.	Range
DH lines	90	2178.2 ± 223	1697 - 2711
Hi-Q	6	2440.2 ± 257	2147 - 2728
RIL-144	2	2158.2 ± 282	1833 - 2483
Peace	6	2413.4 ± 320	1932 - 2807

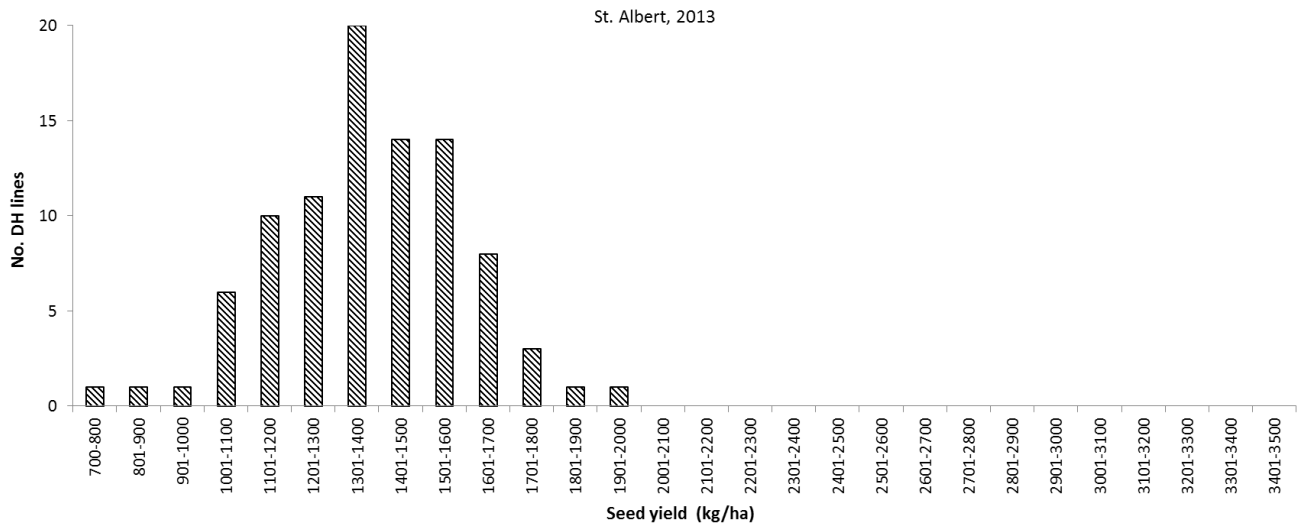

Figure 17c. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* for seed yield in full plot field trial at St. Albert. Experiment conducted in 2013.

Table 31c. Descriptive statistics for seed yield (kg/ha) of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown in full plot field trial at St. Albert. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in May 2013.

Population	No.	Mean ± S.D.	Range
DH lines	92	1281.3 ± 208.3	759 - 1973
Hi-Q	3	1543.8 ± 161.1	1435 - 1729
RIL-144	3	1054.7 ± 77.9	986 - 1139
Peace	6	1389.4 ± 148.8	1226 - 1585

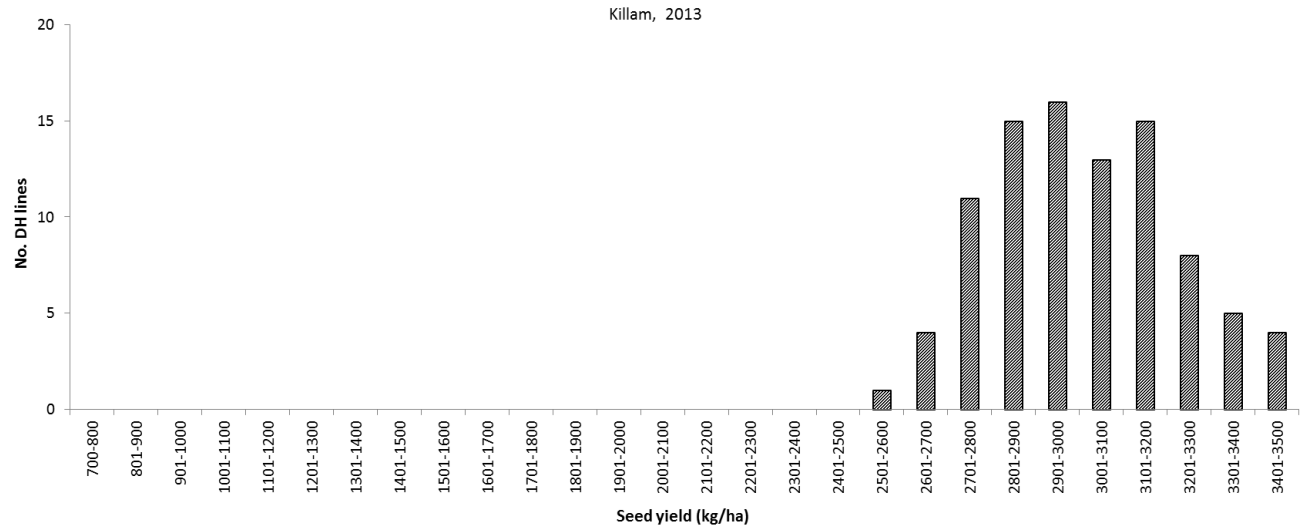

Figure 17d. Frequency distribution of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* for seed yield in full plot field trial at Killam. Experiment conducted in 2013.

Table 31d. Descriptive statistics for seed yield (kg/ha) of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown in full plot field trial at Killam. The early flowering *B. napus* cv. Peace is included as check. Experiment conducted in 2013.

Population	No.	Mean ± S.D.	Range
DH lines	92	3004.7 ± 202.2	2582 - 3480
Hi-Q	3	3488.1 ± 128.7	3345 - 3595
RIL-144	2	2775.5 ± 94.9	2673 - 2861
Peace	6	3076.5 ± 228.9	2762 - 3345

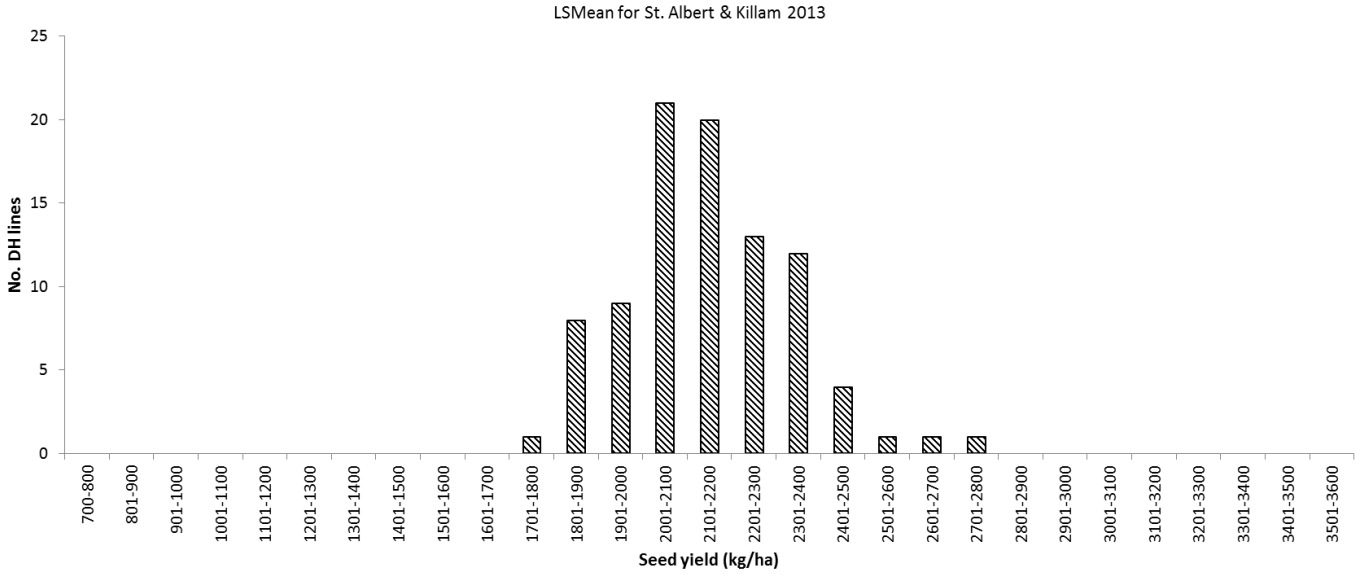

Figure 17e. Frequency distribution of the LS means of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown in Killam and St. Albert for seed yield. Experiments conducted in 2013.

Table 31e. Descriptive statistics based on LS means of the parents and DH lines of Hi-Q × RIL-144 cross of *Brassica napus* grown in Killam and St. Albert for seed yield. The early flowering *B. napus* cv. Peace is included as check. Experiments conducted in 2013.

Population	No.	Mean ± S.D.	Range
DH lines	91	2150.9 ± 205	1781 - 2773
Hi-Q	6	2516.0 ± 145	1735 - 3595
RIL-144	5	1915.1 ± 86	986 - 2861
Peace	12	2233.0 ± 189	1626 - 3345

Table 32. Spearman's correlation coefficients among field trial for seed yield of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus*

	SA2013F	SA2011F	SA2011F
Killam2013F	0.469*** (88)[a]	0.0923 (86)	0.125 (81)
SA2013F		0.172 (85)	0.174 (81)
SA2011F			0.182 (82)

*** $p<0.001$; [a]In brackets, *df*

3.7. Correlation Between Seed Yield, and Flowering Time and Dry Matter

Among the four yield trials, seed yield data from St. Albert 2011 showed significant positive correlation with days to flower data from the field trials, as well as flowering data from the growth chamber experiments conducted under 14, 16 and 18 h photoperiod. This suggests that, at this location in 2011, the late flowering DH lines produced greater seed yield. However, seed yield data from the other three trials, South Campus 2011, St. Albert 2013 and Killam 2013, did not show any significant correlation with days to flower (Table 33).

As found with days to flower, the leaf, root and total dry matter also showed significant positive correlation with seed yield data from St. Albert 2011. However, the coefficients of correlations were stronger with root and total dry matter than with leaf dry matter. Seed yield in South Campus 2011, St. Albert 2013 and Killam 2013 did not show significant correlation with these physiological traits; however, in all cases, the values were positive (Table 34) and stronger with root and total dry matter.

Correlation between seed yield, and days to flower and dry matter suggests that the early flowering DH lines in general produced lower dry matter and seed yield. However, the non-significant correlation observed in most cases suggests that earliness in canola can be improved to some extent through the use of the early flowering lines developed in this project without significant yield loss.

Table 33. Spearman's correlation coefficients between seed yield and days to flower of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus*.

	DTF18h-18C	DTF16h-20C	DTF14h-18C	DTF10h-18C	DTF16h-18/8C	DTF16h-20/16C	DTF-Fld
Yield-SC 2011	0.121 (84)	0.036 (84)	0.103 (84)	0.030 (84)	0.133 (84)	-0.107 (85)	0.088 (85)
Yield-SA 2011	0.267* (80)	0.433*** (80)	0.330** (80)	0.156 (80)	0.401*** (80)	0.282** (81)	0.332** (81)
Yield-SA 2013	0.108 (85)	0.108 (85)	-0.020 (85)	0.131 (85)	0.001 (85)	-0.043 (86)	-0.025 (86)
Yield-KL 2013	0.081 (86)	0.068 (86)	-0.013 (86)	0.046 (86)	0.092 (86)	-0.048 (87)	-0.036 (87)

* $p<0.05$, ** $p<0.01$, *** $p<0.001$; In brackets, *df*

Definition of variables:
Yield-SC2011: Seed yield/ha in South Campus in 2011 full plot field trial
Yield-SA2011: Seed yield/ha in St. Albert in 2011 full plot field trial
Yield-SA2013: Seed yield/ha in St. Albert in 2013 full plot field trial
Yield-KL2013: Seed yield/ha in Killam in 2013 full plot field trial
DTF18h-18C: Least square means of days to flowering under 18 h photoperiod and 18 °C constant temperature in growth chamber
DTF16h-20C: Least square means of days to flowering under 16 h photoperiod and 20 °C constant temperature in growth chamber
DTF14h-18C: Least square means of days to flowering under 14 h photoperiod and18 °C constant temperature in growth chamber
DTF10h-18C: Least square means of days to flowering under 10 h photoperiod and 18 °C constant temperature in growth chamber
DTF16h-18/8C: Least square means of days to flowering under 16 h photoperiod and 18/8 °C (day/night) temperature in growth chamber
DTF16h-20/16C: Least square means of days to flowering under 16 h photoperiod and 20/16 °C (day/night) temperature in growth chamber
DTF-Fld: Least square means of days to flowering from 11 station year (Table 2) field trials conducted in Alberta in 2010 to 2013

Table 34. Spearman's correlation coefficients between seed yield, and leaf, root and total dry matter of the DH lines of Hi-Q × RIL-144 cross of *Brassica napus*.

	Leaf-DM	Root-DM	Total-DM
Yield-SC 2011	0.147 (84)	0.125 (84)	0.144 (84)
Yield-SA 2011	0.275* (80)	0.418*** (80)	0.427*** (80)
Yield-SA 2013	0.137 (85)	0.165 (85)	0.187 (85)
Yield-KL 2013	0.079 (86)	0.118 (86)	0.176 (86)

* $p<0.05$, *** $p<0.001$; [a]In brackets, *df*

Definition of variables:

Yield-SC2011: Seed yield/ha in South Campus in 2011 full plot field trial

Yield-SA2011: Seed yield/ha in St. Albert in 2011 full plot field trial

Yield-SA2013: Seed yield/ha in St. Albert in 2013 full plot field trial

Yield-KL2013: Seed yield/ha in Killam in 2013 full plot field trial

Leaf-DM: Least square means of leaf dry matter of the DH lines grown under 16 h photoperiod and 18/8 ºC (day/night) temperature in growth chambers

Root-DM: Least square means of root dry matter of the DH lines grown under 16 h photoperiod and 18/8 ºC (day/night) temperature in growth chambers

Total-DM: Least square means of total (leaf + stem + root) dry matter of the DH lines grown under 16 h photoperiod and 18/8 ºC (day/night) temperature in growth chambers

3.8. Molecular Mapping of the Flowering Gene(s) and Dry matter

The parent RIL-144 was developed from an interspecific cross of Hi-Q ($A^nA^nC^nC^n$) × *B. oleracea* (C^oC^o) through self-pollination of $A^nC^nC^o$ hybrid plants. Assuming no allosyndesis between the A and C genome chromosomes, the A genome of the RIL-144, theoretically, should be exactly the same as the *B. napus* parent Hi-Q, while its nine C genome chromosomes expected to carry alleles from both *B. oleracea* and Hi-Q. In this regard, the 10 A genome chromosomes in the DH lines, which developed from Hi-Q × RIL-144 cross, should be exactly the same as the 10 A genome chromosomes of the *B. napus* parent Hi-Q or RIL-144; while the DH lines should differ only for the C genome alleles. Therefore, a genetic linkage map was constructed only for the nine C genome chromosomes and used for QTL mapping.

3.8.1. Screening of molecular markers

A total of 783 SSR markers from the nine C genome linkage groups (LGs) were screened for polymorphism between Hi-Q and RIL-144. Of the tested markers, 86 (11%) were polymorphic (Table 35). This low polymorphism was due to close relationship between the two parents, as discussed above.

Of the 66 SSR primer pairs designed based on the flowering time gene sequence obtained from NCBI and TAIR data bases, 52 generated PCR products; however, only two showed polymorphism between the parents. These two primer pairs were BnFRI4 (based on *B. napus FRI* gene) and BF4 (based on *A. thaliana FT* gene). Of the remaining 14 flowering time gene based markers published by different authors or obtained from other researcher, all produced PCR products, however only one (BnC2.FTb, Wang et al. 2009) was polymorphic between the parents. Thus, a total of three flowering time gene based markers were included in the construction of the genetic linkage map; these markers amplified four loci.

We also evaluated 939 SSR markers from the A genome for polymorphism between Hi-Q and RIL-144 to detect if any change in the A genome occurred during the development of the RIL-144 from the Hi-Q × *B. oleracea* cross.

Table 35. Number simple sequence repeat (SSR) markers tested for polymorphism between the parental lines, Hi-Q and RIL-144, for construction of a genetic linkage map.

LG	Polymorphic	Monomorphic	Missing amplification	Total
A1	11	43	12	66
A2	18	59	9	86
A3	9	94	18	121
A4	8	46	5	59
A5	13	49	5	67
A6	15	75	21	111
A7	10	50	4	64
A8	10	95	25	130
A9	20	135	24	179
A10	6	44	6	56
Total A genome	120	690	129	939
C1	6	109	31	146
C2	7	42	18	67
C3	10	25	2	37
C4	10	38	12	60
C5	14	29	13	56
C6	7	37	14	58
C7	9	65	49	123
C8	4	24	12	40
C9	9	36	24	69
Flowering genes & others	3	63	14	80
LG not known	7	36	4	47
Total C genome	86	504	193	783
Total A and C genomes	**206**	**1,194**	**322**	**1,722**

3.8.2. Construction of linkage map

A total of 63 SSR, which includes the three flowering time gene based markers, and two AFLP markers were used. These markers detected 83 loci which were used to construct the C genome linkage map. The linkage map covered 1,193 cM of the nine C genome chromosomes (Table 36, Fig. 18). Genome coverage of our C genome linkage map is very similar to the recently published *B. napus* maps (e.g. Luo et al. 2014). The three SSR markers designed based on flowering time genes mapped to the linkage group C2.

Table 36. Number marker loci mapped on the 19 linkage groups of *Brassica napus* and genome size coverage.

LG	No. loci	Length in cM
C1	7	162.5
C2	10	111.2
C3	8	126.5
C4	11	119.8
C5	12	227.4
C6	10	163.0
C7	5	77.1
C8	13	125.2
C9	7	80.4
Total	**83**	**1,193.1**

As discussed above (section 3.8), theoretically, the A genome of the DH population would be the same as Hi-Q or RIL-144. However, polymorphism between Hi-Q and RIL-144 for some of the A genome markers was found, and these constituted few small linkage groups (data not presented). Whether this is the result of allosysnesis between the A and C genome chromosomes and/or any other changes in the genome that occurred during the development of RIL-144 from the Hi-Q × *B. oleracea* interspecific cross need to be resolved. High homoeology between the A and C genome chromosomes (Sharpe et al. 1995, Parkin et al. 2003, Panjabi et al. 2008) and occurrence of allosyndetic pairing between A and C chromosomes (Attia and Röbbelen 1986, Attia et al. 1987, Mason et al. 2010) as well as introgression of genes from one of these genome to the other (Rahman 2001) has been reported previously.

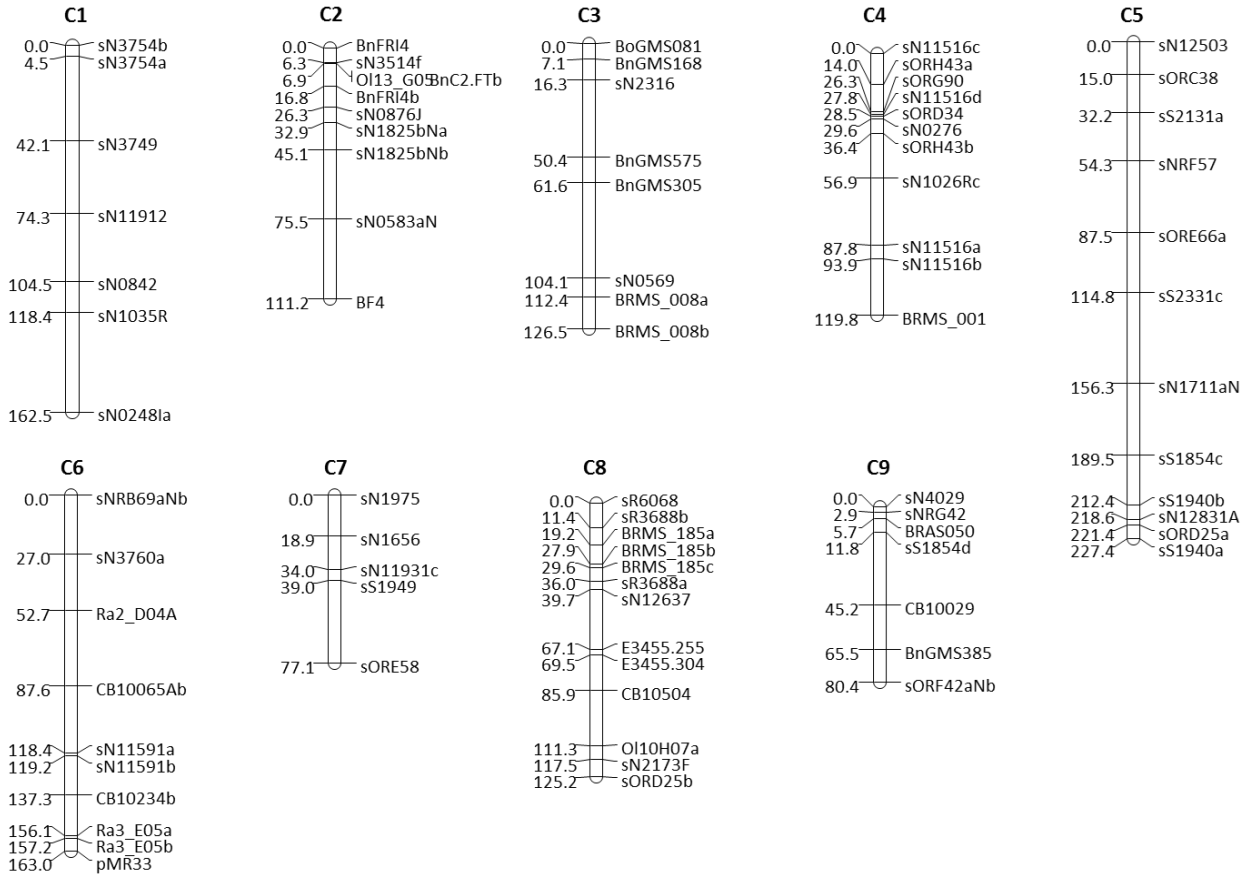

Figure 18. A genetic linkage map of the *Brassica* C genome constructed based on a doubled haploid (DH) population derived from Hi-Q × RIL-144 cross of *B. napus*.

3.8.3. QTL mapping

Flowering time:

A total of 11 QTL were detected through CIM method; five of these QTL detected on the chromosomes C1 (two QTL), C2, C4 and C9 with LOD score of >3, and six on the chromosomes C2, C5 (three QTL) and C8 (two QTL) with LOD score <3 (Table 37, Fig. 19). Among these, the QTL on C1, located between the flanking markers sN11912 and sN0842, was detected in field trials and in all six growth chamber experiments conducted under different photoperiod. This QTL was detected with LOD score of >3 in most cases, and explained 16-37% of the total phenotypic variance. Additive effect of this QTL was one to two days in most cases except the 10 h experiment where additive effect of this QTL was 6.6 days. Single marker analysis also showed strong association (p = 0.001) of the marker sN0842 from this QTL region with days to flower under all conditions (Table 38). The DH population used in this study was developed from a spring × spring *B. napus* cross; detection of this QTL in all experiments including the experiments conducted at different photoperiod with constant temperature of ≈ 18 °C indicate that this genomic region may carry gene(s) affecting flowering time through the autonomous pathway of flowering.

Among the six flowering time experiments conducted in growth chamber, the 10 h photoperiod experiment can be considered a short-day condition. Under this condition, a QTL on C9 at confidence interval of 2.9 – 5.7 cM and flanked by the markers sNRG42 and BRAS050 was detected through CIM method (Table 37, Fig. 19). Strong association (p <0.001) of the marker BRAS050 from this QTL region with days to flower detected through single marker analysis also substantiate this finding (Table 38). This QTL exerted an additive effect of 4.0 days at 10 h photoperiod and explained 15% of the total phenotypic variance (Table 37). The fact that this QTL was not detected in any other growth chamber experiments through CIM method suggests that this genomic region may carry gene(s) affecting flowering through the photoperiod pathway of flowering.

A QTL on the linkage group C2, flanked by the markers sN1825bNa and sN1825bNb, was detected in two 16 h photoperiod experiments with 20 °C constant or 20/16 °C (day/night) temperature (Table 37, Fig. 19). This QTL explained about 10% of the total phenotypic variance;

however, this QTL could not be detected in the other four growth chamber experiments as well as in field trial through CIM method.

Seven QTL on the linkage groups C1, C4, C5 (three QTL) and C8 (two QTL) were identified where the allele from the early flowering line RIL-144 delayed flowering in the DH population (Table 37). This is not surprising given that some of the DH lines flowered later than the late-flowering parent Hi-Q. The fact that the RIL-144 carry allele causing lateness of flowering in combination with allele(s) from Hi-Q suggests that days to flower in RIL-144 is under complex genetic control.

Digenic epistasis analysis for additive × additive gene and epistasis × environment interaction was conducted using QTLNetwork 2.0 (http://ibi.zju.edu.cn/software/qtlnetwork/) (Yang et al. 2008) at each cM interval. The two QTL detected at 10 h photoperiod on the linkage groups C1 and C9 showed an epistatic interaction with additive effect of 6.8 ± 2.4 days (p = 0.005) (Table S4). However, no epistatic interaction between the QTL could be detected under other three photoperiod conditions.

Based on days to flower data from field trials, digenic epistasis analysis for additive × additive gene and epistasis × environment interaction was also conducted. No digenic epistasis or epistasis × environment interaction could be identified in this study.

Leaf dry matter:

Three QTL on the linkage groups C1, C4 and C5 was detected with LOD score of >2 (Table 39, Fig. 19). Among these, the QTL on C1, flanked by the markers sN11912 and sN0842, was detected with LOD score of 3.5. Single marker analysis also showed strong association of the SSR marker sN0842 from this QTL region with leaf dry matter (Table 40). Additive effect of this QTL was 0.3 g, and the QTL explained 18.7% of the total phenotypic. This QTL resided in the same genomic region where a major flowering time QTL was detected (Table 37, Fig. 19). For this QTL, the allele from Hi-Q increased leaf dry matter as well as the number of days required to flower. In addition to this, a QTL on C4, flanked by the marker sN11516a and sN11516b, was detected with LOD score of 2.7. However, single marker analysis detected this QTL with p = 0.01 and explained only 9.0% of the total phenotypic variance.

Root dry matter:

Two QTL on the linkage groups C1 and C2 were detected where the QTL on C1 was detected with LOD score of 4.9 (Table 39, Fig. 19). This C1 QTL explained 32.5% of the total phenotypic variance with an additive effect of 0.6 g, i.e. the effect of this QTL on root dry matter was almost double as compared to its effect on leaf dry matter. Single marker analysis also detected this QTL with $p = 0.001$ (Table 40). The QTL on C2 was detected with LOD score of 2.4 and explained about 10% of the total phenotypic variance; additive effect of this QTL was about one-sixth of the QTL detected on C1. Single marker analysis also showed minor effect of this QTL on root dry matter (Table 40).

Total dry matter:

Two QTL on the linkage groups C1 and C9 were detected with LOD scores of 6.3 and 2.6, respectively (Table 39, Fig. 19). These two QTL resided in the same genomic regions where the two major flowering time QTL were detected in this study. The QTL on C1 was also detected for leaf and root dry matter. This QTL explained 32.5% of the total phenotypic variance and exerted an additive effect of 0.6 g on total dry matter. Single marker analysis also showed major effect of this QTL on total dry matter (Table 40). The effect of the QTL on C9 on total dry matter was about one-third when compared with the effect of the C1 QTL (Table 39). Single marker analysis also suggested minor effect of this QTL on total dry matter (Table 40).

Among the QTL detected for leaf, root and total dry matter, the QTL on C1 can be consider as major one affecting all three traits. This QTL, as well as other QTL located in the same genomic region where flowering time QTL were detected (Fig. 19). Co-localization of the QTL affecting days to flower and plant dry matter indicates that the same genomic region may carry genes affecting flowering time and bio mass production, or the gene(s) controlling one of these traits exert pleiotrophic effect on the other trait. Further study would be needed to unveil this.

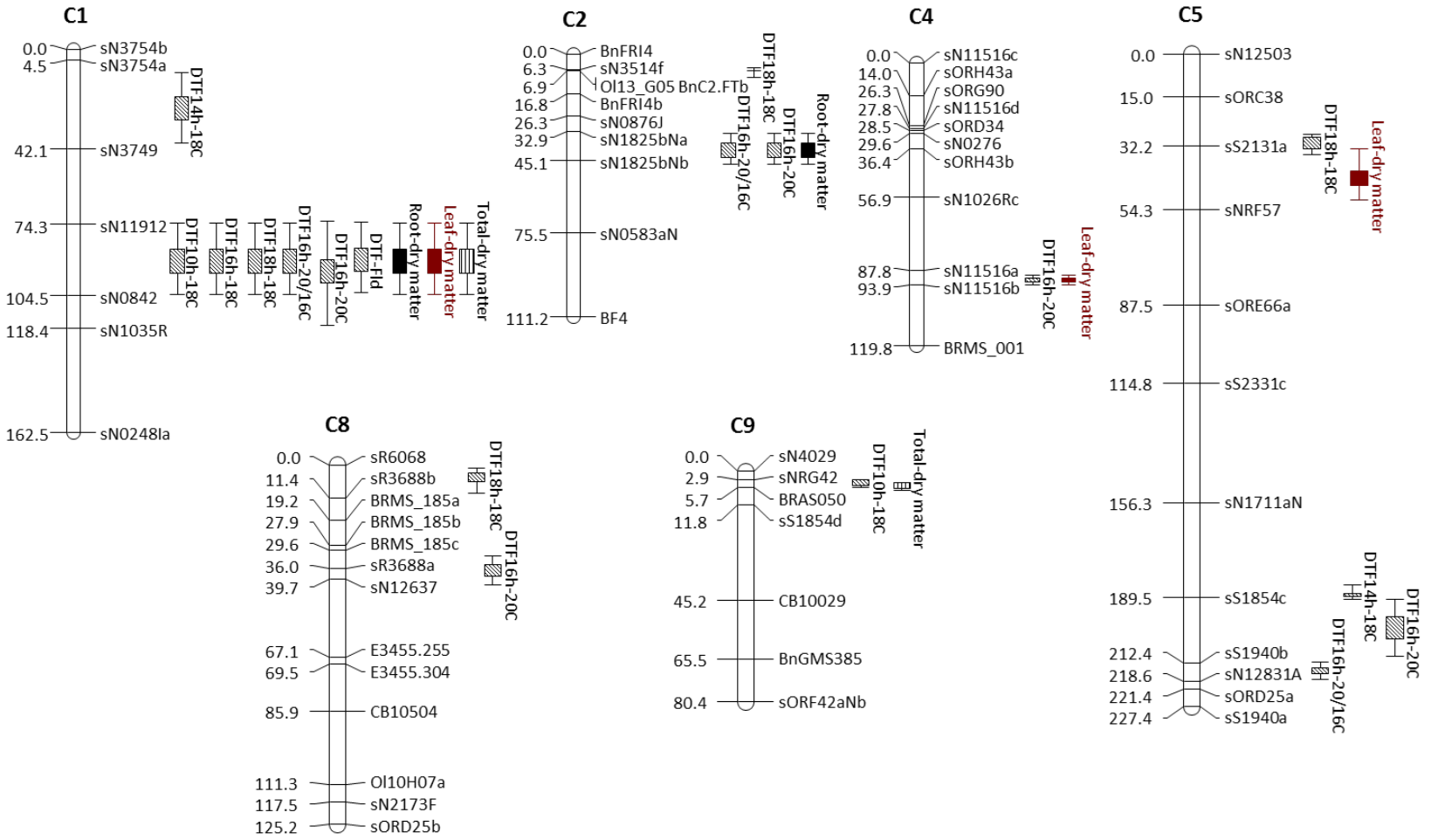

Figure 19. QTL mapping of days to flower under different growth conditions, and leaf, root and total dry matter in spring *Brassica napus* based on a genetic linkage map constructed by the use of a doubled haploid (DH) population derived from Hi-Q × RIL-144 cross.

Table 37. QTL mapping of days to flower in the doubled haploid (DH) population of Hi-Q × RIL-144 cross of *Brassica napus* through composite interval mapping (CIM) method.

Traits/Linkage group	Marker interval	Confidence Interval (cM)	R^2 (%)[a]	Additive effect[b]	LOD Score
DTF10h-18 °C					
C1	sN11912 - sN0842	74.3 - 104.5	32.2	6.6	5.9
C9	sNRG42 - BRAS050	2.9 - 5.7	14.7	4.0	3.3
DTF14h-18 °C					
C1	sN3754a - sN3749	4.5 - 42.1	10.8	-0.6	3.1
C1	sN11912 - sN0842	74.3 - 104.5	16.2	0.9	2.5
C2	BnC2.FTb	6.9	12.1	0.7	2.2
C5	sS1854c	189.5	09.3	-1.1	2.1
DTF16h-18/8 °C					
C1	sN11912 - sN0842	74.3 - 104.5	19.9	1.9	4.5
DTF18h-18 °C					
C1	sN11912-sN0842	74.3-104.5	32.53	1.29	3.5
C5	sS2131a	32.2 - 32.2	14.6	0.9	2.4
C8	sR6068 - sR3688b	0 - 11.4	14.1	-1.1	2.4
DTF16h-20/16 °C					
C1	sN11912-sN0842	74.3-104.5	36.68	1.86	4.8
C2	sN1825bNa - sN1825bNb	32.9 - 45.1	10.8	0.8	3.2
C5	sS1940b - sN12831A	212.4 - 218.6	9.6	-1.7	2.1
DTF16h-20 °C					
C1	sN11912-sN1035R	74.3-118.4	29.30	2.3	4.2
C2	sN1825bNa - sN1825bNb	32.9 - 45.1	9.2	1.3	2.1
C4	sN11516b	93.9 - 93.9	13.5	-4.7	4.1
C5	sS1854c - sS1940b	189.5 - 212.4	17.7	-3.4	2.3
C8	BRMS_185c - sR3688a	29.6 - 36	11.4	-4.3	2.7
DTF-Field					
C1	sN11912 - sN0842	74.3 - 104.5	19.5	0.8	3.7

[a]percentage of variation explained by each QTL.[b]Additive effect (number days) is the effect of substitution of allele from one parent by the other; negative values indicate that the alleles from RIL144, positive values indicate hat the alleles from Hi-Q

Table 38. QTL mapping of days to flower in the doubled haploid (DH) population of Hi-Q × RIL-144 cross of *Brassica napus* through single marker (SM) analysis method.

Traits/Linkage group	Marker ID	Position	R^2(%)[a]	p^b
DTF10h-18 °C				
C1	sN0842	104.5	29.9	0.001
C2	Ol13_G05	6.9	17.1	0.001
C2	BnC2.FTb	6.9	5.7	0.01
C9	BRAS050	5.7	5.7	0.001
DTF14h-18 °C				
C1	sN0842	104.5	26.1	0.001
C2	Ol13_G05	6.9	16.3	0.001
C2	BnC2.FTb	6.9	5.9	0.01
DTF16h-18/8 °C				
C1	sN0842	104.5	37.9	0.001
C1	sN1035R	118.4	2.2	0.01
C1	sN0248Ia	162.5	4.5	0.05
C2	Ol13_G05	6.9	13.7	0.001
C2	BnC2.FTb	6.9	4.6	0.05
C5	sNRF57	54.3	3.2	0.05
C9	sN4029	0	3.9	0.05
C9	sNRG42	2.9	1.9	0.05
DTF18h-18 °C				
C1	sN0842	104.5	12.1	0.001
C2	Ol13_G05	6.9	5.9	0.05
C8	sR3688b	11.4	7.8	0.001
C9	sNRG42	2.9	0.8	0.05
C9	BRAS050	5.7	4.1	0.01
DTF16h-20/16 °C				
C1	sN0842	104.5	24.3	0.001
C2	Ol13_G05	6.9	23.6	0.001
C2	BnC2.FTb	6.9	64.7	0.001
C2	sN1825bNb	45.1	8.7	0.01
C9	BRAS050	5.7	4.9	0.05
DTF16h-20 °C				
C1	sN0842	104.5	26.9	0.001
C2	Ol13_G05	6.9	35.8	0.001
C2	BnC2.FTb	6.9	13.1	0.001
C2	sN1825bNb	45.1	4.07	0.05
C9	BRAS050	5.7	6.9	0.01
DTF-Field				
C1	sN0842	104.5	34.8	0.001
C2	Ol13_G05	6.9	21.3	0.001
C2	BnC2.FTb	6.9	3.0	0.001
C2	sN1825bNb	45.1	5.3	0.01

[a]percent phenotypic variation explained by QTL. [b]significant at 5%, 1%, 0.1% and 0.01% levels (F statistic).

Table 39. QTL mapping of leaf, root and total dry matter in the doubled haploid (DH) population of Hi-Q × RIL-144 cross of *Brassica napus* through composite interval mapping (CIM) method.

Traits/Linkage group	Marker interval	Confidence Interval (cM)	R^2 (%)[a]	Additive effect[b]	LOD Score
Leaf dry matter					
C1	sN11912 - sN0842	74.3 - 104.5	18.7	0.3	3.5
C4	sN11516a - sN11516b	93.9 - 93.9	38.5	0.5	2.7
C5	sS2131a - sNRF57	32.2 - 54.3	9.6	-0.2	2.1
Root dry matter					
C1	sN11912 - sN0842	74.3 - 104.5	32.5	0.6	4.9
C2	sN1825bNa - sN1825bNb	32.9 - 45.1	9.9	0.1	2.4
Total dry matter					
C1	sN11912 - sN0842	74.3 - 104.5	32.5	0.6	6.3
C9	sNRG42 - BRAS050	2.9 - 5.7	7.27	0.2	2.6

[a]percentage of variation explained by each QTL.[b]Additive effect (in gram) is the effect of substitution of allele from one parent by the other; negative values indicate that the alleles from RIL144, positive values indicate hat the alleles from Hi-Q

Table 40. QTL mapping of leaf, root and total dry matter in the doubled haploid (DH) population of Hi-Q × RIL-144 cross of *Brassica napus* through single marker (SM) analysis method.

Traits/Linkage group	Marker ID	Postion	R^2(%)[a]	p^b
Leaf dry matter				
C1	sN0842	104.5	15.1	0.001
C4	BRMS_001	119.8	9.0	0.01
C9	BRAS050	5.7	2.9	0.01
Root dry matter				
C1	sN0842	104.5	31.1	0.001
C2	Ol13_G05	6.9	22.6	0.001
C2	BnC2.FTb	6.9	6.01	0.001
C2	sN1825bNb	45.1	8.1	0.001
C9	BRAS050	5.7	5.0	0.01
Total dry matter				
C1	sN0842	104.5	20.8	0.001
C2	Ol13_G05	6.9	12.6	0.001
C2	BnC2.FTb	6.9	2.8	0.01
C4	BRMS_001	119.8	7.7	0.01
C9	BRAS050	5.7	9.0	0.001

[a]percent phenotypic variation explained by QTL. [b]significant at 5%, 1%, 0.1% and 0.01% levels (F statistic).

4. Summary and Conclusions

All the traits investigated in this research, viz. days to flower under different photoperiod and temperature conditions, and leaf, root and total dry matter, are quantitative in nature. QTL mapping identified two major loci on the chromosomes C1 and C9 involved in the control of flowering variation in this DH population. The C1 QTL might carry the gene(s) controlling flowering time variation through the autonomous pathway of flowering, while the C9 QTL may carry the gene(s) that affects flowering through the photoperiod pathway. The QTL markers identified in this study can be used in breeding for earliness of flowering.

A total of five QTL affecting leaf, root and total dry matter were identified of which the QTL on C1 was detected for all traits. This, as well as other QTL detected for leaf, root and total dry matter co-located in the genomic region where flowering time QTL was detected.

Days to flower and dry matter data in most cases showed non-significant correlation with seed yield; however, the coefficients of correlations were positive in most cases. This suggests that, earliness in canola can be improved to some extent without significant yield loss; however, selection for extreme early flowering type will cost seed yield.

5. Implications for Alberta's Agriculture

The genomic region and molecular markers identified in this research can be used by canola breeders in marker-assisted selection for earliness of flowering for the development early flowering/maturing canola cultivars. Furthermore, fine mapping of these genomic regions can be done to identify and sequence the genes involved in the control of flowering time through the photoperiod and autonomous pathway of flowering. This will enable to develop gene-based markers as well as identify the allelic variations present within the genes.

Knowledge gained from this research, such as the correlations between seed yield and days to flower and plant dry matter, can be used by canola breeders to develop a knowledge-based strategy for the improvement of earliness in *B. napus* canola without sacrificing significant yield loss. Furthermore, the mapping population and the genetic linkage map constructed in this research can be used for mapping of other traits with focus on the C genome of *B. napus*. The

germplasm developed in this research can be used in breeding for the development of hybrid parent lines with diversified C genome.

6. Targets Achieved Compared to Those Contemplated

All the three objectives laid out in this research project as short-term goals, such us (i) understanding the genetic control of flowering and maturity in the early flowering *B. napus* line, (ii) mapping of the flowering time gene(s) and identify molecular markers for earliness of flowering for use in marker assisted breeding, and (iii) study the effect of the early flowering gene(s) introgressed from *B oleracea* on physiology of the plant, are broadly met in this project. However, we did not record maturity data as days to flower correlates well with days to maturity (Mahmood et al, 2007, Miller 2001) and flowering data from greenhouse also agree with field data (Cruz et al. 2007).

7. Future Research

Molecular markers for the flowering time genes identified in this research can be used to develop near isogenic lines (NILs). This will enable investigating the impact of the specific flowering time allele on physiology of the plant and seed yield. The mapping population and the genetic linkage map constructed in this research project can be used for mapping of other traits, such as oil content, of the Brassica C genome. The high yielding DH lines, which flowers earlier than Hi-Q, can be used in breeding to improve both days to flower and seed yield simultaneously. Furthermore, the genomic regions identified in this research affecting days to flower and plant dry matter can be further dissected through fine mapping, cloning and sequencing of the genes to develop gene-based markers.

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Table S1. Analysis of variance for days to flower of the 94 doubled haploid lines derived from Hi-Q × RIL-144 cross of *Brassica napus* tested under four photoperiod conditions.

Effect	Num. DF	F-value	*P*
Genotype	93	2.37	<0.0001
Photoperiod	3	2447.3	<0.0001
Genotype (Photoperiod[1])	279	1.31	0.0054

[1]Photoperiods: 10 h, 14h, 16h and 18h

Table S2. Least squares mean differences for days to flower of the DH lines of Hi-Q × RIL-144 of *Brassica napus* grown in growth chamber under different photoperiods.

Photoperiod I	Photoperiod II	LS mean difference	Standard error	*t*-value	*P*	Adjusted *P* (Dunnett's test)
10h	14h	42.9	0.6	75.34	<.0001	<.0001
10h	18h	41.8	0.6	73.4	<.0001	<.0001
14h	18h	-1.1	0.5	-2.09	0.0374	0.1006

Table S3. Analysis of variance for days to flower of the 94 doubled haploid lines derived from Hi-Q × RIL-144 cross of *Brassica napus* tested in field in 1-row and full-plot trials.

a) 1-row trials:

Effect	Num. DF	F-value	*P*
Genotype	91	1.31	0.0277
Environment	3	80.02	<0.0001
Genotype × Environment	273	0.31	1.0000

b) Full-plot trials

Effect	Num. DF	F-value	*P*
Genotype	91	1.39	0.0107
Environment	1	45.99	<0.0001
Genotype × Environment	91	0.45	1.0000

Table S4. Epistasis effect of QTL on days to flower under 10 h photoperiod in the DH lines derived from Hi-Q × RIL-144 cross of *Brassica napus*.

QTL on LG	Marker interval	Position (cM)	Range (cM)	QTL on LG	Marker interval	Position (cM)	Range (cM)	Additive effect (d)	SE	*P*-value
C1	sN0842-sN1035R	110.4	104.5-118.4	C9	BRAS050-sS1854d	9.6	5.9-14.6	6.8	2.4	0.005