Inheritance and genetic variation of shoot elongation before winter in oilseed rape (*Brassica napus* L.)

Mohammad Ghanbari

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Inheritance and genetic variation of shoot elongation before winter in oilseed rape (*Brassica napus* L.)

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Presented by

Mohammad Ghanbari

born in Zahedan, Iran

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D7

1. Name of supervisor: Dr. Christian Möllers

2. Name of co-supervisor: Prof. Dr. Konstantin Krutovsky

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Dedicated to

My mother’s soul in Paradise

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List of Abbreviations

ADFm acid detergent fibre of the defatted meal
ADLm acid detergent lignin of the defatted meal
AFLP amplified fragment length polymorphisms
AP1 APETALA1 gene
BOF begin of flowering
CBC cap-binding Complex
CBF c binding Factor
CCT CONSTANCE, CONSTANCE-like, TIMING of C
C18:1 oleic acid
C18:2 linoleic acid
C18:3 linolenic acid
DArT diversity arrays technology
DH doubled haploid
DHLE doubled haploid of L16 x Express617
DHSO doubled haploid of Sansibar × Oase
DOF duration of flowering
EFS early flowering in short days
EOF end of flowering
FLC FLOWERING LOCUS C
FRI FRIGIDA gene
FT FLOWERING LOCUS T
GC gas chromatography
GSL glucosinolates content
H^2 broad sense heritability
KASP KBioscience competitive Allele-Specific PCR
LT 50% lethality rate at 50%
LFY: LEAFY gene
MIM multiple interval mapping
NDFm neutral detergent fibre of the defatted meal
NIRS near-infrared reflectance spectroscopy
PH plant height
PodM protein content of the defatted meal
QTL quantitative trait locus
R^2 coefficient of determination
SD shoot diameter at root crown
SL shoot length from root crown to shoot apex
SNP single nucleotide polymorphisms
SSR simple sequence repeats

Chapter 1

General Introduction

Oilseed rape or rapeseed (*Brassica napus* L.) is the world's third-leading crop of vegetable oil (http://apps.fas.usda.gov). Oilseed rape cultivars are mainly cultivated for human nutrition and recently as animal feed and biodiesel production. Predominant oilseed rape genotypes that were released and commercialized in the last decades are double low cultivars. The term "double low" or "double zero" is commonly used to refer to oilseed rape with <2% erucic acid in the oil and <25 $\mu molg^{-1}$ glucosinolates in the seed (Bundessortenamt 2014). Oilseed rape is highly recognized for its nearly ideal fatty-acid profile that is, having low level of saturated fatty acids, high mono-unsaturated fatty acids and a good proportion of omega-3 and omega-6 polyunsaturated fatty acids (Schmidt and Bancroft 2011). *B. napus* is an amphidiploid species with 2n=38 (AACC) chromosomes derived from crosses between *B. rapa*, represented by AA genome, and *B. oleracea*, represented by CC genome. *B. napus* varieties are grown in different regions across the world, including Central and Western Europe, Canada, China and other parts of the world. This large adaptation has been achieved by spring and winter growth types, enabling genotypes to grow in diverse climates. The classification is based on vernalization requirement and not on the level of frost tolerance. However, winter types, also named biennial types, are generally assumed to have better winter survival which is correlated with cold acclimation and mechanisms preventing stem elongation before winter (Teutonico *et al*. 1993, Rapacz 1999). As any other crop, the yield potential of oil seed rape is also limited by environmental conditions such as biotic and abiotic factors hampering the successful production of this crop. Abiotic factors, like extreme low and high temperature are most significant constraints for the production of this crop in Central and Western Europe. Moreover in temperate regions, extreme low temperatures may occur in winter which may require enhanced stress tolerance of crop plants. For example, in 2012 following a rather normal winter, extreme low temperatures of up to −25 °C occurred in February in North Western Germany (https://www.wunderground.com, site visited March 3, 2016). Since at that time crops were not covered by snow, this caused severe frost damage in winter wheat, oilseed rape and other crops

(Christiane Möllers, personal communication). In addition, in January 2016 temperature dropped to -17 °C after a long warm period in December 2015, resulting in frost damage of initially formed buds in oilseed rape (own personal observation). Low temperatures have significantly limited plant reproduction and geographical distribution in North Europe and Canada. Therefore, plants have evolved variety of adaptive mechanisms to survive after exposure to freezing temperature and resume growth when threat of frost stress is passed (Fowler *et al.* 1981). Cold acclimation and vernalization requirement are both survival mechanisms adopted by winter type crops to cope with extreme freezing conditions during overwintering (Rapacz 2002a,b). In *Brassica napus* and other biennials plants, winter survival is a decisive attribute for favored overwintering that depends on the expression of many interacting traits (Kole *et al.* 2002). Plant breeders are striving for improving winter hardiness in the breeding material in order to secure yield stability in years with risk of frost damage. However, selection for improved winter hardiness is a difficult task, because firstly, efficient selection can only be performed in extreme winters, like e.g. in 2012, which in principle is not accessible and predictable in all regions. Secondly, winter hardiness is a complex trait, which consists of frost tolerance *per se*, disease resistance – especially under snow cover, flooding tolerance – after heavy rain falls on frozen soil, drought tolerance at long periods with frozen soil, and tolerance against soil movements due to rapidly changing temperatures. The reason for winterkill vary greatly from region to region and from year to year. It is discussed that vernalization genes and plant development stage are, also, interacting with winter hardiness, for instance, it is suggested that higher degree of vernalization leads to more winter hardy plants (Fowler *et al*. 1996a,b, Sãulescu and Braun 2001)**.**Vernalization is a complex physiological process that plays a determining role to accelerate floral transition in oilseed rape after enough exposure to nonfreezing low temperatures during rosette stage in the field sown conditions (Prásil *et al.* 2004). Although all winter oilseed rape varieties require vernalization, this requirement is not always correlated with winter hardiness. For instance, Teutonico *et al.* (1993), Rapacz and Chilmonik (2000) observed spring oilseed rape lines whose frost tolerance were the same as that of winter lines. Nonetheless, transition to the generative phase occurred earlier in spring types, which then at the bolting and flowering stage frequently suffer frost damage. As matter of fact, spring type plants have low capability to reduce and stop growth and shoot development when temperatures rise above 0 °C before and during winter (Rapacz 1999, Rapacz *et al.* 2001). Consequently, less assimilates are

accessible to increase concentration of cell constituents to prevent formation of of ice crystals in plant tissues under freezing conditions (Laroche *et al.* 1992, Fowler *et al.* 1996a). Cessation of growth during cold acclimation (Levitt 1972) and compact plant morphology have long been considered as one of the main requirements for overwintering (Rapacz 2002b). Too early elongation of the shoot before and during winter is particularly considered by oilseed rape breeders as a potential trait for selection of winter-hardy genotypes (Schulz 2007). Genotypes that show an enhanced shoot elongation before and during winter are very much prone to frost damage. However, so far, little is known about genetic variation and inheritance of shoot elongation before winter and its correlation to vernalization that is important mechanism for winter survival in oilseed rape. Furthermore, correlation between seed quality trait, that are economically important, and winter hardiness related traits are ambiguous in oilseed rape.

The present study aimed at performing field and greenhouse trials followed by statistical analysis and QTL mapping in two bi-parental doubled haploid (DH) populations of *Brassica napus* to shed light on genetic base and inheritance of shoot elongation before winter and its relation to vernalization requirement, flowering time and seed quality traits. Furthermore, a collection of 19 European winter oilseed rape breeding lines and open pollination (OP) cultivars were used to evaluate genetic variation of shoot elongation before winter and vernalization requirement in replicated field trials. The first DH population comprising 226 DH lines derived from a cross between the two winter oilseed rape cultivars ”Sansibar” and “Oase”, called DHSO population (Teh and Möllers 2016). The second DH population comprising 151 DH lines derived from a cross between the resynthesized line L16 and the inbred line 617 of the cultivar Express (Express617, Brandes 2016), called DHLE population. The two DH populations were selected from prior field tests regarding shoot length before winter and flowering time (Christian Möllers, personal communication). Furthermore, for the both populations genetic maps with sequence informative markers were available (Teh and Möllers 2016, Brandes 2016). The two DH populations and their parental lines were assessed in three different mega environments: (I) autumn sown environment, (II) spring sown environment, and (III) greenhouse environment with vernalized and non-vernalized plants. Multiple Interval Mapping (MIM, Kao *et a*l. 1999) was applied for QTL mapping based on genetic maps previously constructed for each of the two populations (Teh and Möllers 2016, Brandes 2016).

Therefore, the specific objectives of the present study were:

(1) To study genetic variation and inheritance of shoot elongation before winter in the 19 breeding lines and OP cultivars and two DH populations (DHLE & DHSO) in the autumn sown environment.

(2) To study genetic variation and inheritance of shoot elongation and tendency to form inflorescence in the 19 breeding lines and OP cultivars and two DH populations (DHLE & DHSO) in the spring sown environment.

(3) To study genetic variation and inheritance of shoot elongation in the two DH populations (DHLE & DHSO) in the greenhouse environment with vernalized and non-vernalized plants.

(4) To study correlation between studied traits in three different mega environments.

(5) To map and compare QTL identified for the studied traits in the two DH populations (DHLE & DHSO) in the three different mega environments.

(6) To identify candidate genes in genomic regions with major QTL for the traits of interest in the two DH populations (DHLE & DHSO).

Chapter 2

Literature review

Oilseed rape or rapeseed (*Brassica napus* L.) is a major oil crop in many parts of the world and is well adapted to cold, dry and moist growing conditions and is extensively cultivated in Europe, China, Canada and India (Downey and Rakow 1987). Today, oilseed rape is the world's third-leading source of vegetable oil and the second most important oilseed in the world after soybean (http://apps.fas.usda.gov). In 2014/15, oil production from oilseed rape amounted to 27.2 million tons, accounting for 15.5% of the world's vegetable oil supply (Figure 2.1). The main oilseed rape producing countries are China, Canada and India (Figure 2.2). In the EU-28 countries, 10.52 million tons of oilseed rape were produced in 2014/15, accounting for 38.6% of worldwide oilseed rape production. Cultivation acreage of oilseed rape is predicted to expand, particularly in European regions where demand is growing for renewable fuels such as biodiesel (Britz and Hertel 2011).

Oilseed rape varieties that meet the requirements of less than 2% erucic acid in the oil and less than 25 μmol/g glucosinolates in the seeds are called "canola" in Canada or ″double low″ (double zero) in Europe to distinguish them from traditional varieties that do not meet this standard (Bundessortenamt 2014). Depending on the fatty acid profile found in the seed of particular cultivars, oilseed rape is used for both edible and industrial purposes (Schmidt and Bancroft 2011). Typically, the fatty acid profile of edible modern oilseed rape ("00") oil contains 60% of oleic acid (18:1), 20% linoleic acid (18:2), 10% linoleic acid (18:3), 7% saturated fats and 1-2% erucic acid (Wittkop *et al.* 2009). For many years, grain yield has been the major breeding goal for oilseed rape researchers. However, since 1970 other breeding objectives have gained importance, such as early and simultaneous maturity, diseases resistance, oil and protein content, fatty acid composition, reduced fiber content, anti-nutritional factors and abiotic stresses tolerance

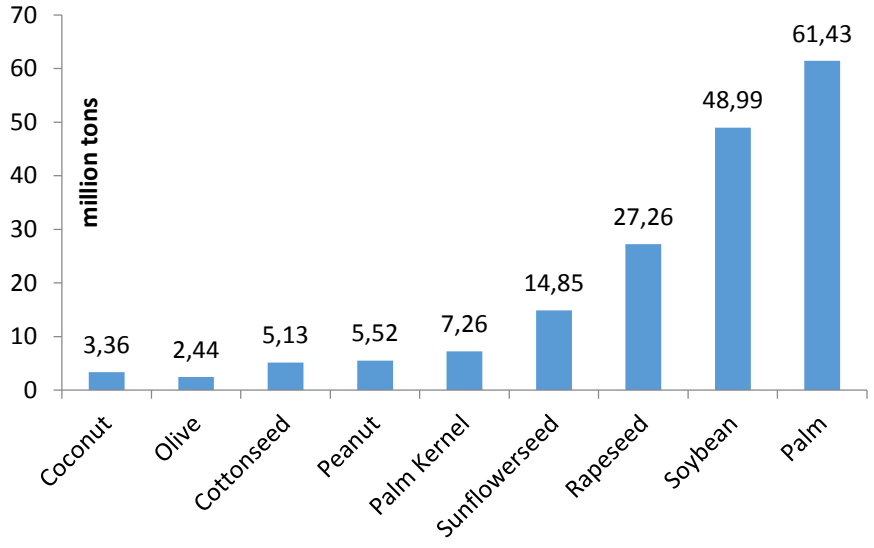

Figure 2.1: World Supply and distribution of major vegetable oils in 2014/2015 (in million tons). Source: USDA Foreign Agriculture Service; http://apps.fas.usda.gov/psdonline/circulars/oilseeds.pdf (site visited February 25, 2016)

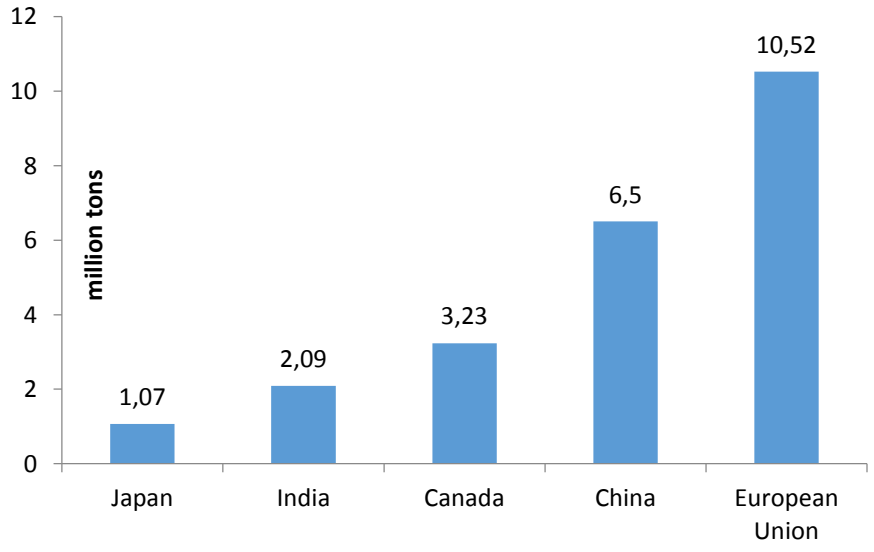

Figure 2.2: World supply and distribution of oilseed rape in 2014/2015 (in million tons). Source: USDA Foreign Agriculture Service; http://apps.fas.usda.gov/psdonline/circulars/oilseeds.pdf (site visited February 25, 2016)

Winter stress is one of the major common abiotic stresses that adversely influence plant growth and productivity in winter-type crops which are typically sown either in late summer or early fall (Chinnusamy *et al.* 2007). Plants overwinter as juvenile in rosette growth form and continue their development in the following spring (Kole *et al.* 2002). As other winter type crops, winter oilseed rape has to be winter hardy to endure unfavorable extreme low temperatures during overwintering, but the level of winter hardiness is not constant and dependent on both genotype and environment (Kole *et al.* 2002). The term winter or cold hardiness in a general sense is, the capability of a plant to withstand freezing temperatures. Collectively, the adaptive mechanisms associated with this ability are quite diverse; including cold acclimation to survive sub-zero temperatures and vernalization requirement to delay flowering (Gusta and Wisniewski 2013). It is well established, cold acclimation that typically occurs in autumn is an essential step to induce frost tolerance in acclimated plants and ideally, genotypes can maintain high level of winter hardiness over winter (Levitt 1980). The effect of cold acclimation can be understood when in the early fall, winter plants will not survive sub-freezing temperatures any better than spring plants (Rapacz and Chilmonik 2000, Waalen *et al.* 2014, Trischuk *et al.* 2014). However, cold acclimation is a complex multi-step process involving series of concrete physiological and biochemical changes and it is extremely influenced by environment and growing stage of the plant genotype (Rapacz and Janowiak 1998, Theocharis *et al.* 2012). It has been suggested that for cold acclimation to occur in winter cereals the plants must be in their vegetative state in order to respond to the environmental clues, however this has not been proven unequivocally (Andrews *et al.* 1960, Mahfoozi *et al.* 2001). The cold acclimation process results in the rapid accumulation of storage carbohydrates and high photosynthetic rate for the expression of freezing tolerance in different plants tissues (Levitt 1980, Livingston and Henson 1998, Rapacz *et al.* 2001). Recent studies of overwintering in cereals have shown that frost tolerance is strongly correlated with the capacity to increase photosynthesis and soluble carbohydrate pools during cold acclimation (Tognetti *et al.* 1990, Öquist *et al.* 1993). In field-grown oilseed rape cultivars some properties of photosynthetic apparatus, observed during winter, are correlated with growth rate and the progress of the cold acclimation (Rapacz 1998ab). It is shown that oilseed rape cultivars with higher photosynthetic electron transport rate maintained the cessation of elongation growth for a longer period, resulting in better preparation for overwintering (Rapacz and Chilmonik 2000). In addition, they found that less efficient photosynthetic electron

transport in autumn was observed in the spring cultivars in which the elongation of generative shoots was observed already during the first warm break in winter. The observation is in agreement with higher net assimilation rates and higher in situ CO, exchange rates in winter wheat cultivars than the respective cold-grown spring cultivars (Hurry *et al.* 1995). Such conditions may be described as conditions of elevated photosystem II (PSII) excitation pressure. Plants showed elevated PSII excitation pressure reflecting imbalances between energy supply and consumption when exposed to either high light intensity or low temperature (Rapacz 2002a,b). Both pre-hardening and growth under high PSII excitation pressure resulted in acclimation of the photosynthetic apparatus and the decrease in elongation growth rate, but it was not involved directly in the development of frost resistance (Rapacz 1998a,b).

From research point of view, frost stress is the main factor influencing winter survival in winter cereals and winter oilseed rape (Rapacz and Markowski 1999). Hence, artificial frost tests are exploited to select tolerant genotypes to frost in breeding projects. Although in some studies the correlation coefficients for frost tolerance between field and laboratory were between 0.77^{**} and 0.92^{**}, (Caradus and Christie 1980) the selected genotypes did not maintain winter hardiness in consecutive years, because freezing stress is assessed only under lab conditions while winter survival consists of others stresses such as disease, drought, etc., making selection of tolerant genotypes less efficient. In fact, controlled freezing tests obviously have an advantage over field-testing in which the evaluations are faster, give greater control of environmental conditions and provide the opportunity for application over time (Pomeroy and Fowler 1973), yet laboratory tests do not allow estimation of winter hardiness, which is a more relevant trait than freezing tolerance for winter survival. Also Rapacz *et al.* (2015) stated that cold acclimation under natural field conditions activates a greater array of freezing tolerance mechanisms than cold acclimation performed in under controlled environmental conditions in a laboratory. All the above barriers makes improvement of frost tolerance with less pace compared to breeding for biotic stresses with high correlation between field and controlled conditions. Also in the regions with harsh winters, spring cultivars of oilseed rape are alternative types for farmers to cultivate oilseeds.

In temperate regions, vernalization is the second important mechanism, after cold acclimation, for winter survival in winter crops (Trischuk *et al.* 2014). Vernalization prevents frost damage by prohibiting floral transition before winter and promoting transition to reproductive growth when optimum conditions are met (Zografos and Sung 2012). Vernalization enables a developmental

switch at the shoot apical meristem upon exposure to low temperatures making it competent to initiate floral apex. Vernalization is a quantitative response which is proportional to the duration of low temperature conditions, until a species or genotype specific requirement is reached (vernalization requirement), and further exposure to low temperature has no effect on accelerating the transition from vegetative to reproductive stage (Mahfoozi *et al*. 2001).

Several studies were undertaken to dissect genetic variation of vernalization requirement in winter crops. Early results on *B. napus* cultivars indicated that two recessive genes control vernalization (Thurling *et al*. 1979). In contrast, similar studies with *B. oleracea* indicated a polygenic inheritance with the annual growth habit being dominant (Kennard *et al.* 1994). QTL studies have shown that the vernalization requirement in *B. napus* is controlled by as many as three QTL (Ferreira *et al.* 1995). Very little is still known about the relationship between vernalization and frost tolerance. The relationship varies in different plant tissues and might have genetic association but the metabolic pathway is separated. Work on *Arabidopsis thaliana* suggested that completely separate pathways control freezing tolerance and vernalization (Chandler *et al.* 1996). In wheat, winter type varieties usually have a greater vernalization requirement and higher sensitivity to short days than spring type varieties, which enable them to survive in the vegetative phase over winter. Molecular markers have shown genes controlling vernalization in wheat are closely linked with frost tolerance genes (Galiba *et al*. 1995). It was indicated by Fowler *et al*. (2001) and Danyluk (2003) that genes that regulate vegetative/generative transition (*Vrn*, *Ppd*), also act to control genes affecting the expression of low temperature-induced genes associated with frost tolerance. Prásil *et al.* (2004) reported that genes controlling vernalization requirement act as master switch regulating genes of low temperature induced frost tolerance. They concluded that after saturation of vernalization requirement, winter wheat establishes only a low level of frost tolerance. It was also discussed that saturation of the vernalization requirement is main factor for the gradual loss in low-temperature tolerance observed in winter cereals after a long period at low temperatures (Fowler *et al.* 1996b). In such cases, vernalization genes may have a regulatory influence on low temperature gene expression in winter cereals since these genes were identified as key factors responsible for the duration of expression of low temperature-induced genes (Fowler *et al.* 1996a).

Despite the fact that some studies have reported a positive correlation between the degree of vernalization requirement and winter hardiness (Andersson and Olsson 1961, Kole *et al.* 2002, Hawkins *et al.* 2002, Casao *et al.* 2011), recently published results demonstrated that spring-type oilseed rape is, also, able to be winter hardy to a level comparable with winter-type, but only after cold pre-hardening which results in both the increase of photosynthetic activity and growth cessation during cold acclimation (Rapacz 1998a,b). The findings were confirmed with results obtained from field trials where some spring cultivars were characterized by frost tolerance similar to less tolerant winter cultivars (Teutonico *et al*. 1993). At the DNA level, Lorche *et al.* (1992) have found that low temperature induced amplification of rDNA and the differential methylation of EcoRI sites may possibly be related to the vernalization process but may not be related to the development of freezing tolerance. The above contradictory findings indicate that there is no simple relationship between frost tolerance and the degree of vernalization in plants.

Despite unknown genetic interaction between vernalization and winter hardiness, it is straightforward that due to lack of vernalization requirement, spring oilseed rape varieties are not able to cease plant growth in long warm-breaks before and during winter. Also winter varieties with low vernalization requirement may resume growth and break winter dormancy under such conditions and then are killed by freezing temperatures (Andersson and Olsson 1961). Therefore, in such situations vernalization requirements is highly correlated with frost damage. In Brassica species, further studies illustrated different linkage groups exist in both *B. napus* and *B. rapa* for capacity to attain freezing tolerance and vernalization (Ferreira *et al.* 1995, Teutonico *et al.* 1995). Teutonico *et al.* (1995) showed that freezing tolerance in the *Brassicaceae* might be controlled by number of genes in number of linkage groups throughout the genome. They also observed that regions linked to freezing tolerance in *B. rapa* did not appear to be linked with freezing tolerance in *B. napus***.**

During the last decades, double low cultivars of winter oilseed rape replaced traditional types in the cultivation acreage and they are subjected for enhancement oil and meal quality. Breeding for reduction in erucic acid and glucosinolates contents involved crossing with spring forms (Niewiadomski 1990), which may have influenced the correlation between winter hardiness and vernalization as well as the level of vernalization and frost tolerance in commercial oilseed rape cultivars. One major hazard for winter crops is when plants start bolting or flowering in a period during the winter when the temperature rises to spring-like level (Rapacz and Markowski 1999).

The situation is now more common, due to global warming phenomenon and serious loss in yield may occur if freezing temperatures follow. Therefore, understanding the genetic basis of the control of flowering time in *B. napus* is important for plant breeders in order to optimize crop performance in specific environments (Schmidt and Bancroft 2011). The time that a crop takes to flower is crucial and can affect seed yield, especially if one takes into account an ever-changing climate scenario. In winter type wheat, flowering time is induced by vernalization treatment. However, flowering is delayed during winter until favorable growth conditions in spring (Mahfoozi *et al.* 2001). Floral transition is profoundly affected in the process of vernalization (Chinnusamy *et al.* 2007). From physiology point of view, intermittent cold treatment delays flowering and induce *FLOWERIMG LOCUS C* (*FLC*) gene expression by approximately three folds (Seo *et al.* 2009). By contrast, prolonged expose to cold temperatures promotes flowering by suppressing the *FLC* gene (Sung and Amasino 2004, Kim *et al.* 2009).

In the model species *Arabidopsis thaliana*, four main floral transition pathways are vernalization, gibberellin, photoperiods and autonomous pathway, regulating the timing of flowering in response to different environmental and endogenous clues (Amasino 2010, Amasino and Michaels 2010). The key regulator of vernalization requirement is the MADS box transcription factor gene *FLOWERING LOCUS C* (*FLC*), which is a repressor of *FLOWERING LOCUS T* (*FT*) but is down-regulated by vernalization, thus enabling promotion of flowering by expression of *FT* in shoot apex (Amasino and Michaels 2010). Both *FLC* and its upstream regulator *FRIGIDA* (*FRI*) are major determinants of natural variation in flowering time (Irwin *et al.* 2012). Dominant alleles of *FRI* confer vernalization requirement causing plants to overwinter in vegetative stage, and several loss-of-function mutations are associated with early flowering (Johanson *et al*. 2000). *FRI* was suggested to up regulate *FLC* expression through interaction with the histone methyltransferase *EARLY FLOWERING IN SHORT DAYS* (*EFS*), which results in the modification of *FLC* chromatin (Ko *et al*. 2010, Irwin *et al.* 2012), and interaction with a nuclear cap-binding complex (*CBC*) with concomitant effects on *FLC* transcription and splicing (Geraldo *et al*. 2009). Photoperiod is another important ambient cue, affecting flowering time in oilseed rape. The key regulator in the photoperiodic pathway, which mediates the effect of day length on flowering time, is the *CCT* (*CONSTANS, CONSTANS-LIKE, TIMING OF CAB EXPRESSION* 1) domain transcription factor gene *CONSTANS* (*CO*) (Nelson *et al.* 2014). Under long-day conditions, *CO* protein accumulates at the end of the light phase and promotes

flowering by activating the expression of *FT* that is a central integrator of input signals from different pathways whose protein product is a major component of the mobile signal that moves to the shoot apical meristem and initiates flowering (Turck *et al*. 2008).

QTL mapping is intensively performed by researchers to find out the quantitative trait loci for flowering time in the natural or segregating populations. In *B. rapa* QTL linked to known function genes for flowering time (*VFR1, VFR2* and *VFR3 FR1, FR2* and *FR3*) were mapped in a segregating F2 population and a recombinant inbred line (RIL) population derived from a cross between an annual and a biennial oilseed type (Teutonico and Osborn 1994, Osborn *et al*. 1997), *VFR2* was estimated to have large effect and was suggested to be orthologue of *FLC* gene in *Arabidopsis*. A further study confirmed that *VFR2* locates at the *BrFLC1* locus, *FR1* at the position of *BrFLC2* and *FR2* at *BrFLC5* (Lou *et al.* 2007). The three *B. rapa* flowering time genes *BrFLC2, BrFLC3* and *BrFLC1* were assigned to linkage groups A02, A03 and A10, respectively (Kole *et al.* 2001). In addition, *VFR1* was mapped on A02 close to region syntenic to the *MAF* (MADS *Affecting Flowering*) region at the bottom of chromosome 5 in *Arabidopsis*.

Bolting time, as relevant trait for flowering time has also been analyzed under different conditions in a population derived from a cross between two heading Chinese cabbage (Zhang *et al.* 2006). 10 QTLs mapped to 6 linkage groups, however the linkage groups were not assigned to the chromosomes of reference genome in *B. oleracea*, therefore it was not possible to compare the mapped QTL to other flowering time QTL. Long *et al.* (2007) investigated flowering time in oilseed rape in 11 field environments and detected 5-18 QTL in each environment. When *Brassica napus* QTL were aligned with hundreds of flower transition genes in *Arabidopsis* by *in silico* mapping, 28% of the genes aligned with QTL regions, and 9% were consistent in interacting loci. The natural variation of the splicing site in *BrFLC1* was thought to contribute to flowering time in a study using 121 *B. rapa* accession (Yuan *et al*. 2009). Co-localization of the flowering-time QTL with flowering-related genes, including *brFLC1* and *brFLC2* in multiple segregating populations of *B. rapa* suggests that the function of *BrFLC* genes has changed due to genetic variation in vernalization requirement (Lou *et al*. 2007). Zou *et al*. (2012) identified nine *FLC* homologs in *Brassica napus* and found the coding sequences of all *BnFLC* were relatively conserved but the intron and promoter regions were more divergent.

Little is known about how flowering time is affected by frost stress, as main element of winter hardiness, and cold acclimation, which typically occurs before winter and it has been debating

whether there is simple correlation between them or complex models are involved. However, almost all researchers emphasized that cultivation time, shoot elongation before winter and shoot apex development are significantly correlated with frost damage in different field crops (Prásil *et al.* 2004, Velicka *et al.* 2005, Asghari *et al.* 2014). Under typical Western European growing conditions, elongation before winter to some extent is determined by sowing time in late summer or early autumn, therefore sowing date may play a decisive role in optimizing winter hardiness in winter type crops. Results of some investigations showed that successful over-wintering of oilseed rape was observed after they developed 6 to 8 leaves, a root collar diameter of 8 to 10 mm and a height of shoot not exceeding 30 mm in autumn (Cramer 1990). It is discussed that promotion of elongation growth leads to consumption of accumulated photosynthetic products, e.g. sugars and loss of frost tolerance (Levitt 1972). Rapacz *et al*. (2001) stated that decrease in frost tolerance observed in spring- type plants was associated with the beginning of elongation growth of petioles and epicotyl and expansion of leaf area. Also Waalen *et al.* (2014) showed that carbohydrate level and water content in the shoot apex of oilseed rape genotypes, during mid to late winter, are better predictors for winter hardiness than levels of theses parameters in leaves, especially sucrose, in the shoot apex meristem is good predictor of *LT50* and shoot regrowth after freezing stress over winter. Prásil *et al.* (2004) concluded that the initial growth of shoot apex was not associated with a loss in frost tolerance in wheat varieties; however, a much more advanced shoot length resulted in a decrease in the frost tolerance. Photoperiod is the second important factor after vernalization in winter type crop to resume growth during growing period. It is shown that vernalization requirement, solely, is not sufficient to induce reproductive transition in wheat (*Triticum aestivum* L.), but other ambient cues, such as the day length and temperature are, also, involved (Bergjord *et al.* 2009). In a research conducted on barley (*H. vulgare)* shoot apex development, flowering time and inflorescence development were accelerated when vernalized plants were grown in longer days (Sasani *et al.* 2009). As a result, onset of flowering was delayed in short days compared to plants grown in long days, after equivalent vernalization treatment. When considering the effect of photoperiod on shoot elongation before and after winter on *T. aestivum*. Prásil *et al.* (2014) found that vernalized plants followed by long days required less days to heading and shoot apex differentiation, while the length of the photoperiod (Long days vs. short days) during vernalization and cold acclimation did not influence the course of frost tolerance significantly. The results achieved by

Waalen *et al.* (2014), suggested that in winter oilseed, cells of the shoot apical meristem are initially differentiated by vernalization, then long day conditions induce the shoot apex to form flower primordial.

With respect to result of the QTL mapping for shoot development in winter crop a few studies have been conducted. Chen *et al.* (2010) reported that three major QTL were found to control variation in the developmental process of *T.aestivum*, and each of them was tightly linked with flowering genes, *VRN-A1*, *PPD-D1* and *VRN-D3* on chromosomes 5A, 2D and 7D respectively. Dechaine *et al.* (2014) characterized the genetic architecture of vegetative traits and begin of reproduction in different developmental stages in a recombinant inbred lines of *Brassica rapa* in the field and glasshouse. They detected 10 QTLs in different environments for the vegetative and generative traits during life cycle in a bi-parental population of *B. rapa*. The results showed differential expression of QTL for stem length over developmental time, indicting a three-way interaction between QTL, environment and ontogeny stage. So far, no scientific attempt was reported to study genetic variation and QTL mapping on shoot elongation before winter in oilseed rape. The present study aimed to shed light on genetic basis and inheritance of shoot elongation before winter and its correlation with vernalization requirement, flowering time and seed quality trait in a set of 19 winter oilseed rape breeding lines and open pollination (OP) cultivars and two bi-parental DH populations of *Brassica napus* in three mega environments.

Chapter 3

Genetic variation of shoot elongation before winter and its correlation with vernalization requirement in winter oilseed rape cultivars (*Brassica napus* L.)

3.1 Abstract

Complexity of winter hardiness and low efficiency of laboratory test has propelled breeding strategies toward the contributing traits for improved winter hardiness. Shoot elongation before winter is a decisive feature that contributes to the winter hardiness of oilseed rape. The present study was carried out to investigate genetic variation of shoot elongation before winter and its correlation with vernalization requirement in 19 European breeding lines and open pollination (OP) cultivars of winter oilseed rape. All 19 genotypes were tested in field experiments in two different mega environments: autumn sown and spring sown. Shoot length from root neck (crown) to shoot apex and shoot diameter at the root neck were measured around three months after sowing date in respective environments. Large phenotypic variation with significant genotypic variance was found for shoot length in the autumn sown and spring sown environment which were named shoot length before winter and shoot length in the spring environment, respectively. Broad sense heritability was quite high (h^2=97%) for shoot length in the spring sown environment, while medium heritability (h^2=62%) was observed for shoot length before winter. Spearman's rank correlation gave rather medium positive correlation (r_s=0.48*) between shoot length before winter and shoot length in the spring sown environment. Scatter plot of Spearmen's rank correlation distributed the genotypes in two clusters; first cluster consisted of genotypes with short shoot length in the autumn sown and spring sown environment, including R53 and L16, Mohican, Lorenz and Sollux, Zenith, Apex and Akela. Second cluster consisted of cultivars which were short before winter but long in the spring sown environment including Montego, Tenor, Adriana, Sansibar, Oase, Express 617, SGEDH13, SGDH14, King 10 and hybrid cultivar Visby. Gaoyou was the only cultivar representing the longest shoot length in the both environments. Rather low vernalization requirement was found in the majority of genotypes which increases risk of frost damage in the regions with long warm periods before and during winter. Shoot length before winter differed between the genotypes, however no serious frost damage was observed during implementation of this study at the four locations. Two resynthesized lines R53 and L16 with short shoot length in the two environment, were recognized from the traditional oilseed genotypes. Whereas no substantial differentiation was found between double low and double high quality genotypes among traditional oilseed genotypes.

3.2 Introduction

Oilseed rape (*Brassica napus* L.) is a major oilseed in many parts of the world with well adaptation to cold, dry and moist growing conditions and is extensively cultivated in Northern Europe, China, Canada and India (Downey and Rakow 1987). Despite large adaptation, winter canola survival is a limiting factor to the success of the crop in the regions with extreme freezing temperatures. Therefore in the countries with Northern latitudes such as Canada and Norway spring types of oilseed rape are mainly grown on farmlands. However, in temperate regions such as Central and Northern Europe winter stress is one of the main common abiotic stresses that adversely affect plant growth and productivity in winter type crops which are typically sown either in late summer or early fall (Chinnusamy *et al.* 2007). Many variables affect winter hardiness and the inability to control these variables severely restricts the usefulness of natural environments in the evaluation of cold hardiness of potential genotypes in breeding programs (Gusta *et al.* 1977). Moreover, winter hardiness is not a stable attribute in oilseed rape varieties making less efficiency of selection for enhanced winter hardiness. In temperate regions, vernalization is the second important mechanism for winter survival in winter crops (Trischuk *et al.* 2014). Vernalization prevents frost damage by repressing floral transition before winter and promoting transition to reproductive growth when optimum conditions are met (Zografos and Sung 2012). Some studies have reported a positive correlation between the degree of vernalization requirement and winter hardiness in winter type crops, suggesting that selection of genotypes with higher vernalization requirement leads to more winter hardy plants in breeding programs (Andersson and Olsson 1961, Kole *et al.* 2002, Hawkins *et al.* 2002, Casao *et al.* 2011). However, recently published results demonstrated that spring type oilseed rape is able to be winter hardy to a level comparable with winter type, but only after cold hardening which results in both the increase of photosynthetic activity and growth cessation during cold acclimation (Rapacz 1998a,b). These observations were confirmed with results obtained from field trials in which some spring oilseed varieties were characterized by frost tolerance similar to less tolerant winter cultivars (Teutonico *et al*. 1993). However, winter survival frequency in spring type plants, which typically lack vernalization requirement, is usually very low compared to winter cultivars, because of frost damage (Teutonico *et al.* 1993, Fowler *et al.* 1979). One of the possible reasons of low frost tolerance might be due to the limited capability to prevent shoot

elongation during winter when temperature rises only slightly above 0 °C during overwintering (Laroche *et al.* 1992, Murelli *et al.* 1995, Fowler *et al*. 1996b). Consequently, less energy is available for processes associated with cold acclimation (Fowler *et al.* 1996a, Stanca *et al.* 2003). Further studies suggested that spring type plants cannot recover high photosynthetic activity after shift from warm to cold acclimating temperatures, while the high photosynthetic rate during cold acclimation is a prerequisite for the expression of freezing tolerance in frost tolerant plants as it provides carbohydrates for cold acclimation (Hurry *et al.* 1995 and Rapacz 1999).

One major hazard for winter crops is when plants start bolting or flowering in long warm periods during the winter when the temperature rises to spring like levels. The situation is now more common, due to climate changes and global warming phenomenon that may result in serious yield losses if freezing temperature follows. Therefore, investigating the genetic basis of vernalization requirement is important for understanding variation in flowering time and winter survival in *B. napus* (Schmidt and Bancroft 2011). During the last 25 to 30 years, double low cultivars of winter oilseed rape replaced traditional oilseed rape types in cultivation. Reduction in erucic acid and glucosinolates involved crossing with spring forms (Finlayson *et al.* 1973, Niewiadomski 1990), which may have influenced the correlation between winter hardiness and vernalization as well as the level of vernalization and frost tolerance of currently cultivated oilseed rape. It is discussed that intensive selection on double zero quality cultivars led to lower vernalization requirement, earlier shoot elongation and flowering and at least in some areas with long warm periods in winter this could be a cause of serious winter damage (Rapacz and Markowski 1999). Therefore, the present study aimed to study genetic variation for the elongation of shoot before winter and its correlation with vernalization requirement in 19 winter oilseed genotypes including breeding line and OP cultivars with different genetic and quality background.

3.3 Materials and Methods

3.3.1 Plant material

The seed material consisted of 19 European breeding lines and open pollination (OP) cultivars of winter oilseed rape with different quality background, ranging from double zero (0,0) to double high (+,+) quality (Table 3.1). All cultivars are inbred lines except Visby that is a hybrid cultivar. The line SGDH14 is a doubled haploid line that was developed based on F1-microspore culture technic from a cross between the old German cultivar Sollux and the Chinese cultivar Gaoyou (Zhao *et al.* 2005). Furthermore, SGEDH13 is a doubled haploid line derived of a cross between line SGDH14 and the cultivar Express. The seed material was obtained from breeding companies and the Department of Crop Sciences at Göttingen University.

Table 3.1: Name and features of the 19 breeding lines and cultivars of winter oilseed rape

Genotype name	Seed quality	Genetic background
Adriana	00	Line cultivar
Akela	00	Line cultivar
Apex	00	Line cultivar
Express 617	00	Line cultivar, inbred line
Gaoyou	++	Chinese Line cultivar
King10	00	Line cultivar
L16	0+	Resynthesized line
Lorenz	00	Line cultivar
Mohican	00	Line cultivar
Montego	00	Line cultivar
Oase	00	Line cultivar
R53	++	Resynthesized line
Sansibar	00	Line cultivar
SGDH14	++	DH line
SGEDH13	++	DH line
Sollux	++	Line cultivar
Tenor	00	Line cultivar
Visby	00	Hybrid cultivar
Zenith	00	Line cultivar

00 less than 2% erucic acid in the oil and less than 25 $\mu molg^{-1}$ glucosinolates in the seed
0+ less than 2% erucic acid in the oil and more than 25 $\mu molg^{-1}$ glucosinolates in the seed
++ more than 2% erucic acid in the oil and more than 25 $\mu molg^{-1}$ glucosinolates in the seed

3.3.2 Field sown experiments

All 19 breeding lines and cultivars, named 19 genotypes in this study, were phenotypically tested in field sown experiments in the two different environments: autumn sown and spring sown. Since the two environments diffed, they were named the two mega environments.

3.3.2.1 Autumn sown environment

The seed material of 19 genotypes were sown in North-Western Germany at four locations with two replicates during growing seasons 2014/15 and no replicate in 2015/16. In 2014/15 the locations were Peine (Limagrain GmbH), sown on 21 August, and Einbeck (KWS Saat SE), sown on 4 September. In 2015/16 the locations were Peine (Limagrain GmbH), sown on 20 August, and Göttingen-Reinshof, sown on 28 August. Hundred seeds from each line were sown in small field-plots with double rows in Peine and Göttingen with 2 m length, 0.5 m space between plots and plant-to-plant distance in the row was 10 cm. In Einbeck, seeds were sown as one row with 3 m length and 0.8 m space between the rows and plant-to-plant distance in the row was 6 cm. All agronomic practices, such as fertilizer, herbicide and insecticides were applied at each location according to common practices. Three to four months after sowing date, five representative plants were harvested by cutting the stem below the root neck (crown). Harvesting time in 2014/15 for Peine and Einbeck were 8 and 24 December 2014 and in 2015/16 for Peine and Göttingen were 30 November 2015 and 11 January 2016, respectively. Shoot length from root neck (crown) to shoot apex and shoot diameter at the root neck were measured using a slide gauge and a metering rule and they were called in this study shoot length before winter and shoot diameter before winter.

3.3.2.2 Spring sown environment

The seed material of 19 genotypes were sown with two replicates at four location in 2013 and 2014 and with no replicate at two locations in 2015. Four locations were Göttingen-Reinshof, sown on 19 and 30 April 2013, 20 March 2014 and 24 March 2015 (no replicate). Two locations were in Einbeck (KWS Saat SE), sown on 4 April 2014 and 10 April 2015 (no replicate). All

plants were grown by sowing 100 seeds in 2 m long double rows and 0.5 m space between plots with 10 cm distance between plants in the row in Göttingen and 3 m single row and 8 cm space in Einbeck. Three to four months after sowing date (Göttingen 18.07.2013, 7.08.2013, 02.07.2014, 26.06.2016; Einbeck 06.07.2014, 13.07.2015), seven representative plants were harvested by cutting the stem below the root neck (crown). Shoot length from root neck to shoot apex and shoot diameter, at the root neck, were measured using metering rule and slide gauge. Percentage of tendency to form inflorescence was scored as 100% for visible buds or flower and 0% for lack of buds in each of the seven plants.

3.3.3 Statistical analysis

Analysis of variance and descriptive statistics were done by PLABSTAT (Utz 2011) and R (i386 3.0.3). Analysis of variance was done for the autumn sown and spring sown environment, separately. In each mega environment, location and year were defined as experiment, meaning four experiments in the autumn sown environment and six experiments in the spring sown environment. Due to unbalance replicates at different locations, genotype's mean over replicates were used for ANOVA. Linear mixed-effect model (R package {nlme} version 3.1-125) was applied to test significant difference for the studied traits. Therefore, experiment and sample plants were random factor versus, genotypes were fixed factor. The statistic model used for ANOVA is shown as follow:

$$X_{ijk} = \mu + g_i + e_j + g_i e_j + p_k{:}g_i e_j$$

where X_{ijk} is phenotypic observation of genotype i in experiment j and plant k, μ is overall mean, g_i and e_j are effects of genotype i and experiment j, respectively, $g_i e_j$ is residual error to test g_i and e_j and $p_k{:}g_i e_j$ is sampling residual error to test $g_i e_j$ effect. Broad sense heritability (h^2) of genotypes mean over experiments was calculated by following equations suggested by Hill et al. (1998)

$$h^2 = \sigma^2_G / (\sigma^2_G + \sigma^2_{GE}/E + \sigma^2 p{:}ge/EP)$$

where σ^2_G and σ^2_{GE} are genetic and genetic x experiment variance components, respectively. *E* is number of experiments and *P* is number of sample plants. Least significant difference (LSD) was utilized for mean comparisons at $P<0.05$ for shoot length before winter and shoot length in the spring sown environment. Mean values of the genotypes across the experiments were used to calculate Spearman's rank correlation coefficients between the traits that was done by R software (i386 3.0.3).

3.4 Results

3.4.1 Phenotypic analysis

The analysis of variance indicated significant genotypic variance components for the all studied traits in the two mega environments (Table 3.2). Significant variance components were observed for experiment and genotype x experiment interaction (G x E) that was bigger than genotypic variance for shoot length before winter and shoot diameter in the spring sown environment, that caused medium heritability for respective traits, while for shoot length and the appearance of visible buds in the spring sown environment high heritability was found in the 19 oilseed rape genotypes.

Large phenotypic variability was found among the plant material for the measured traits in the two mega environments (Table 3.3). Shoot diameter before winter ranged from 7 to 12 mm, while it ranged from 18 to 26 mm in the spring sown environment. Shoot length before winter ranged from 23 to 66 mm, in contrast shoot length ranged from 187 to 1296 mm in the spring sown environment. In the two environments, the Chinese cultivar Gaoyou showed the maximum value for shoot length. Tendency to from inflorescence, shown by percentage of buds, was observed for almost all genotypes, nonetheless Akela was the only genotype with no visible buds during implementation of the study. Results of mean comparisons (LSD) demonstrated that no significant difference was found between genotypes with less than 11.7 mm difference for shoot length in the autumn sown environment and less than 203.4 mm difference in the spring sown environment (Figure 5.1.1 and Figure 5.1.2). For instance, the two cultivars Sansibar and Oase showed significant difference for shoot length in the autumn sown environment, while no significant difference was observed for shoot length in the spring sown environment.

Table 3.2: Variance components and heritability of the traits in 19 oilseed cultivars in the two mega-environments

Environment	Trait	Variance components			Heritability (%)
		Genotype (G)	Experiment (E)	G x E	
Autumn sown	Shoot length[a]	100.97**	128.9**	166.43**	62
	Shoot diameter[a]	0.88**	0.49**	1.91**	69
Spring sown	Shoot length[a]	152235**	31595*	23070**	97
	Shoot diameter[a]	4.75**	7.54**	7.54**	73
	Buds[b]	0.99*	0.09**	0.9**	87

*and** denote significance at P<0.05 and P<0.01, respectively.
[a] and [b] denote millimeter (mm) and percentage (%), respectively.

Table 3.3: Descriptive statistics of the traits in 19 cultivars of winter oilseed rape in the two mega environments

Genotype*	Autumn sown		Spring sown		
	Shoot diameter[a]	Shoot length[a]	Shoot diameter[a]	Shoot length[a]	Buds[b]
	LSD5%=2.1	LSD5%=11.7	LSD5%=3.7	LSD5%=203.4	LSD5%=34
L16	12	23	24	259	39
R53	10	24	25	200	32
Apex	9	25	26	668	60
Adriana	11	26	22	1186	82
Mohican	11	26	25	298	27
Tenor	8	26	19	1009	100
Lorenz	11	27	**26**[c]	334	32
Montego	10	28	20	1017	**100**[c]
Sansibar	11	29	26	844	76
Sollux	11	29	21	**187**[d]	14
King10	10	33	23	990	80
SGEDH13	**12**[c]	35	23	1204	86
SGDH14	12.	38	23	1089	**100**[c]
Express617	10	39	25	991	80
Oase	11	43	21	733	80
Akela	9	48	20	232	**0**[d]
Visby	10	48	21	1216	98
Zenith	**7**[d]	**23**[d]	23	579	26
Gaoyou	9	**66**[c]	**18**[d]	**1296**[c]	**100**[c]

LSD 5%: least significant difference at level of P< 0.05
[a] and [b] denote millimeter (mm) and percentage (%) respectively
[c] and [d] denote maximum and minimum mean for each trait, respectively
* genotypes are ascending sorted for shoot length in the autumn sown

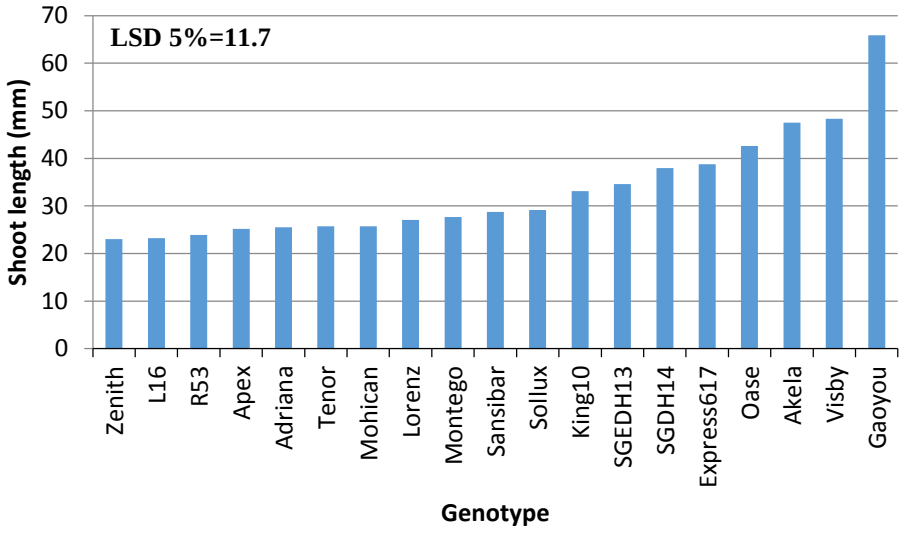

Figure 3.1.1: Mean comparison of 19 genotypes of winter oilseed rape for shoot length in the autumn sown environment.

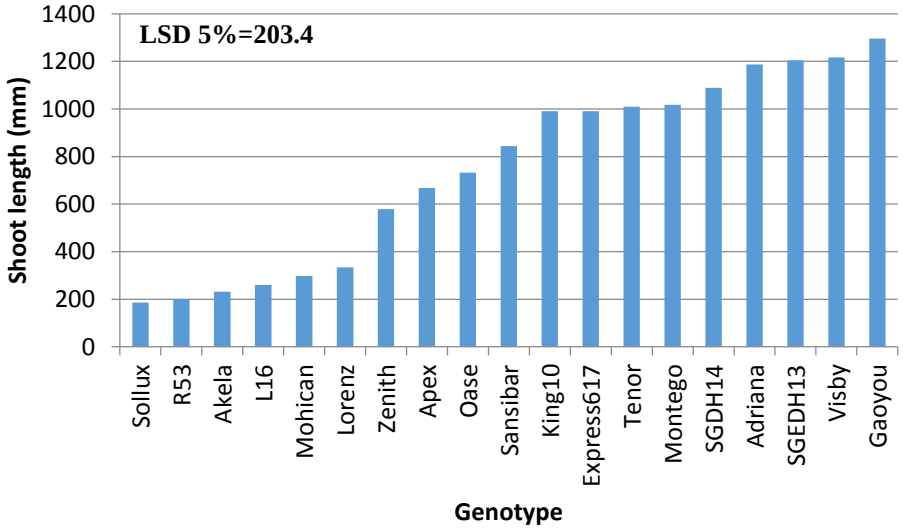

Figure 3.1.2: Mean comparison of 19 genotypes of winter oilseed rape for shoot length in the spring environment.

3.4.2 Correlation Analysis

Spearman's rank correlation analysis illustrated low to high phenotypic correlation ranging from 0.1 to 0.88 between the studied traits in 19 genotypes (Table 3.4). No significant correlation was found between shoot length and shoot diameter in the autumn sown environment, whereas a negative significant correlation (r_s=-0.42**) was found between them in the spring sown environment. Furthermore, no significant correlation was found between the shoot diameters in two mega-environments. Highly positive correlation was found between shoot length and visible buds in the spring sown environment (r_s=0.88**), showing that shoot elongation was significantly followed by tendency to form inflorescence in the spring sown environment. Spearman correlation revealed a positive correlation (r_s= 0.48*) between shoot length before winter and shoot length in the spring sown environment. Likewise, shoot length before winter had a rather low positive correlation with buds in the spring environment (r_s= 0.45*). Scatter plot of spearman's rank correlation depicted moderate positive correlation (r_s= 0.48*) between shoot length before winter and shoot length in the spring sown environment (Figure 3.2). The two resynthesized lines L16 and R53 and three cultivars Mohican, Lorenz and Sollux had the shortest shoot length at both mega environments. In contrast, the two cultivars Gaoyou and Visby showed the longest shoot length at both mega environments. The cultivar Oase had a relatively long shoot before winter and a moderate shoot length in the spring sown environment. Cultivar SGDH14 was found in the middle between its parents Sollux and Gaoyou. However, SGEDH13 was at the same position as its parents Express and SGDH14.

Table 3.4: Spearman's rank correlation of the traits in 19 cultivars of winter oilseed in two mega environments

Environment	Trait	Autumn sown	Spring sown		
		Shoot length	Shoot diameter	Shoot length	Shoot diameter
Autumn sown	Shoot diameter	0.15			
Spring sown	Shoot length	0.48*	0.1		
	Shoot diameter	-0.52*	0.15	-0.42*	
	Buds	0.45*	0.03	0.88**	-0.44*

*and** denote significance at P<0.05 and P<0.01, respectively

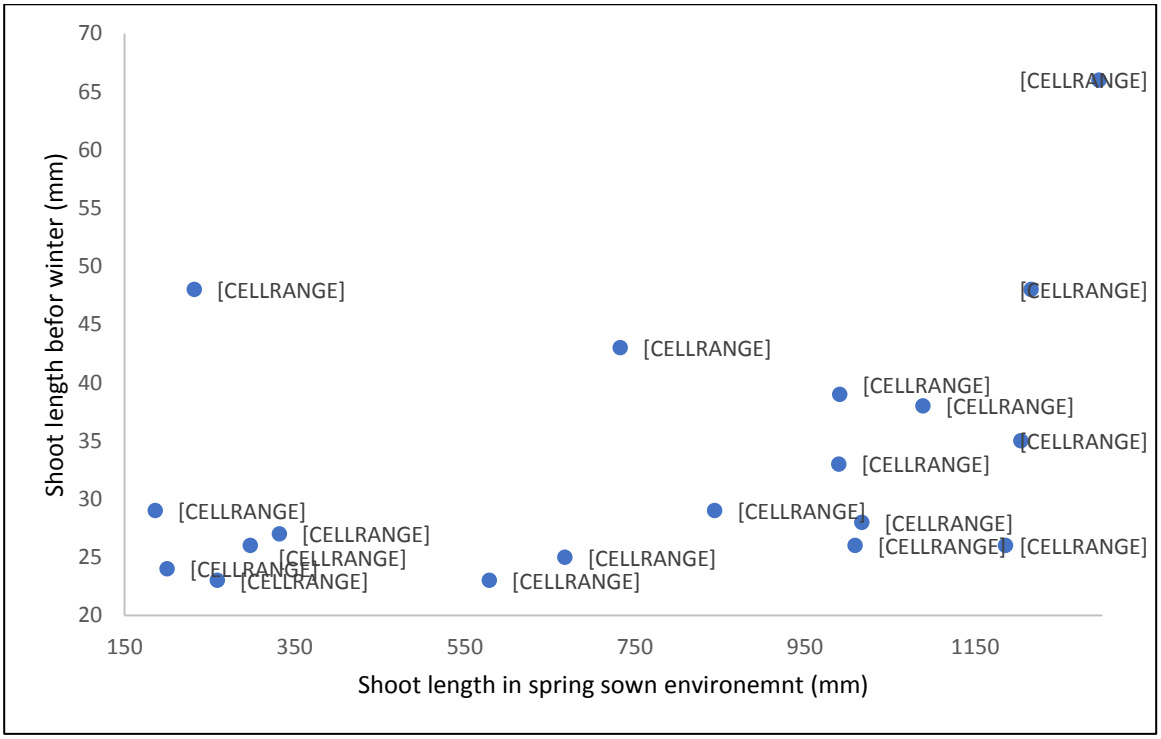

Figure 3.2: Scatter plot of 19 winter oilseed rape genotypes between shoot length before winter and shoot length in the spring sown environment

3.5 Discussion

3.5.1 Phenotypic analysis

Significant genotypic difference for shoot length before winter in 19 winter oilseed rape genotypes indicates large genetic variation in the current genepool of winter oilseed rape. This variation was described earlier by Schulz (2007) in a set of 15 winter oilseed rape cultivars. He stated that with respect to frost tolerance of winter oilseed rape, the too early elongation of the epicotyl before and during winter is considered a particularly relevant trait. Low heritability for shoot length and shoot diameter before winter corresponds to a phenomenon in plant development process, called plant plasticity. Plasticity often involves altering gene expression and plant physiology in response to environmental cues (El-Soda *et al.* 2014). As a result of phenotypic plasticity, the magnitude of genetic variation between plants in the initial steps of plant development i.e. at the rosette stage is environment-specific, whereas later in development and in adult plants it is more stable across environments (Dechaine *et al.* 2014). Large genetic variation along with high heritability found for vernalization requirement, determined by shoot length and tendency to from inflorescence in the spring sown environment, are frequently reported in long researches (Rapacz *et al*. 2001, Prásil *et al.* 2004, Waalen *et al.* 2014). High heritability shows stable genetic variability in different environments that is a desire feature to increase selection efficiency for traits of interest in plant breeding programs. Therefore, shoot length and the appearance of visible buds in the spring sown experiment can be exploited for selection of genotypes with low or high vernalization requirement. Gaoyou, SGDH14 and Montego showed 100% of visible buds, while Akela and Sollux had the lowest percentage of visible buds in the spring sown environment. As a result, using of segregating populations derived from cross between the above contrasting genotypes could increase the detection power of QTL mapping. Novickiene *et al.* (2010) compared growth parameters of three winter oil seed varieties during hardening period in autumn in growing seasons 2007 and 2008. They observed significant differences for leaf number, root diameter, leaf weight and root weight showing

different acclimation response among cultivars. Additionally they concluded that apical bud and root crown diameter are important for winter hardiness.

3.5.2 Correlation analysis

With respect to the processes involved in winter kill and winter hardiness, it can be noticed that dynamics of freezing tolerance are characterized by three stages in wheat; a hardening period, a maintenance period, and a dehardening period (Prásil *et al.* 2014) and each stage is influenced by vernalization, photoperiod, developmental and other environmental factors (Sãulescu and Braun 2001). Genetic relationship between vernalization requirement and winter hardiness has been studied in different crops. However, no explicit correlation was found between them (Fowler *et al*. 2001, Danyluk 2003, Waalen *et al*. 2014). In oilseed rape opposite results are reported, Andersson and Olsson (1961) pointed out that higher vernalization caused more winter-hardy plants, while Markowski and Rapacz (1994) denied it and they reported a line with high vernalization requirement and low frost tolerance. In this study, moderate correlation was found between shoot length before winter and shoot length in the spring sown environment. The medium correlation suggested genetic association between two traits, however the magnitude of correlation was not high enough to conclude stable correlation in winter oilseed rape that might be the reason for no simple correlation reported by other studies (Rapacz and Markowski 1999, Prásil *et al*. 2004, Waalen *et al.* 2014). Also it can be speculated that independent regulator genes are involved for both traits as different generic pathways are shown between frost tolerance and vernalization requirement (Ferreira *et al.* 1995, Teutonico *et al.* 1995). Scatter plot classified eighteen genotypes into two groups; first group comprised of genotypes with short shoot length in the two environments; including R53 and L16, Mohican, Lorenz and Sollux, Zenith, Apex and Akela. Second group consisted of cultivars with short length before winter and enhanced shoot length in the spring environment including Montego, Tenor, Adriana, Sansibar, Oase, Express617, SGEDH13, SGDH14, King10 and hybrid Visby. Gaoyou was the only genotype, which was long in both mega environment. Gaoyou is a Chinese semi-winter inbred line with (++) quality developed by Zhejiang Agriculture University (Zhao *et al*. 2005), thus, spring alleles in Gaoyou increased shoot length when temperature arose above 0 °C during winter. Two resynthesized lines L16 and R53 showed same and strong vernalization requirement and the

minimum shoot length before winter, while traditional line and cultivars showed large variation for shoot length. Girke *et al*. (2011) observed large differences in the genetic distance of resynthesized lines to the winter oilseed rape genepool. The large difference can be used for integrating desired genes lost during domestication or intensive selection for improvement of oil quality traits in oilseed rape genepool. Genetic variation found among cultivars with double zero quality for shoot length in the two environments (see Table 3.1) can support the hypothesis that intensive selection has changed the relation between vernalization requirement and frost tolerance in modern oilseed cultivars. Rapacz and Markowski (1999) compared European winter oilseed rape cultivars cultivated in the 1970s and in the mid-1990. They reported that significant correlation between vernalization requirement and frost tolerance in old oilseed rape cultivars have been decreased due to improved winter hardiness in modern cultivars during last 20 years. Low vernalization recorded in this study for majority of lines and cultivars is a hazard in the current climates condition. In which global warming has caused long warm periods during winter especially in Northern hemisphere in the last decades. The warm breaks during before or during winter promotes shoot development and tendency to form inflorescence in cultivars with low vernalization that caused reduction in plant capacity to withstand following freezing temperature. Using QTL mapping on population derived from cross between genotypes with different shoot length before winter and spring sown environment gives more genetic variability to dissect genetic relationship between vernalization requirement and shoot length before winter. Therefore in the chapters 4 and 5, two doubled haploid populations L16 x Express617 (DHLE) and Sansibar x Oase (DHSO) whose parental lines showed significant difference for shoot length before winter are used to study inheritance of shoot length before winter and its association with vernalization requirement in winter oilseed rape.

Chapter 4

Genetic analysis and inheritance of shoot elongation before winter and its relation with other traits in the doubled haploid population L16 x Express617 (*Brassica napus* L.)

4.1 Abstract

Shoot elongation before winter is considered as a critical component of the complex trait winter hardiness in winter oilseed rape. Thereby genotypes with an enhanced shoot length before winter are very much prone to frost damage. The present work has been conducted to study the inheritance of shoot elongation in a DH population derived from a cross between the resynthesized line L16 and the winter rapeseed cultivar Express617 in three mega environments: (a) autumn sown (b) spring sown and (c) greenhouse with zero, four and eight weeks vernalization treatments. Shoot length and shoot diameter were measured around three months after sowing in each environment. Furthermore, begin of flowering, plant height at end of flowering and seed quality traits were measured in the autumn sown environment. Large and significant genotypic variance components were found for all the traits in the three environments. Shoot length in the autumn sown environment, also named shoot length before winter, was significantly positive correlated with shoot length in the three greenhouse treatments. However, no significant correlation was observed between shoot length before winter and shoot length in the spring sown environment. Shoot length before winter was, also, not correlated with begin of flowering neither in the autumn sown nor in the greenhouse environment. Six main QTL for shoot length before winter contributed 49.2% to the phenotypic variance. For shoot length in the spring sown environment a major QTL with R^2=35.5% was localized on linkage group C09 which along with two QTL on A02 and A07 explained 68% of the observed variance. No collocation of QTL were found between shoot length before winter and shoot length in the spring sown and greenhouse environment with zero and 8 weeks vernalization treatment. On linkage group A02 a hotspot comprised QTL for begin of flowering and plant height in the autumn sown environment, shoot length and visible buds in the spring sown environment, shoot length and shoot diameter in 4 weeks vernalized conditions in the greenhouse environment. An identified candidate gene for the hotspot on A02 is a copy of the *FT* gene. Identification of candidate gene in the vicinity of the biggest QTL for shoot length in the spring sown environment indicated a paralogue of gene *FLC* on linkage group C09. QTL analysis in the present study also revealed that linkage groups A07 and C09 consisted of genomic regions influencing both seed quality traits and the studied traits in three different environments.

4.2 Introduction

The seed oil of *B. napus*, in comparison with other oilseeds, contains low levels of saturated fatty acids, a high percentage of oleic acid and an optimal ratio of polyunsaturated fatty acids for human nutrition (Schmidt and Bancroft 2011). Moreover, after oil extraction the remaining meal is a valuable feedstuff for animal feeding and a potential protein source for human consumption. The favourable composition of amino acids in the meal, including comparatively high contents of the essential sulphuric amino acids methionine and cysteine has placed rapeseed meal as substitute for soybean meal (Downey 1990). All above benefits along with growing demand for biodiesel production has dramatically increased cultivation acreage of oilseed rape cultivars with in the last decades. However, the genetic base of oilseed rape (*Brassica napus*) is quite narrow due to its limited geographic range and intensive breeding (Girke *et al.* 2012). Resynthesized lines could be employed to give more chance to find desired alleles or to increase the genetic distance for optimal utilization of heterosis (Girke *et al*. 2012). Becker *et al.* (1995) investigated genetic distances between resynthesized lines and *B. napus* varieties with RFLP and allozyme markers and stated that resynthesized lines might be a valuable source for broadening the genetic base of the present breeding material of *B. napus*.

Intensive selection for improvement oil quality in oilseed rape cultivars is believed to have decreased vernalization requirement in double zero cultivars while frost tolerance has improved in modern cultivars (Rapacz and Markowski 1999). Low vernalization increases risk of frost damage in some areas with long warm periods during winter, because plants may break growth cessation and then are killed by subsequent freezing temperatures (Andersson and Olsson 1961).Under typical Western European growing conditions, shoot elongation before winter to some extent is determined by sowing time in late summer or early autumn, therefore sowing date may play a decisive role in optimizing winter hardiness in winter type crops (Crosatti *et al.* 2008). Results of some investigations showed that successful overwintering of oilseed rape was observed after they developed 6 to 8 leaves, a root collar diameter of 8 to 10 mm and a height of shoot not exceeding 30 mm in autumn (Cramer 1990). Rapacz *et al*. (2001) stated that decrease in frost tolerance observed in spring type plants was associated with the beginning of elongation growth of petioles and epicotyls and also expansion of leaf area. Promotion of stem elongation

leads to consumption of accumulated photosynthetic products, e.g. sugars and loss of frost tolerance (Levitt 1972). Also Waalen *et al.* (2014) showed that carbohydrate level and water content in the shoot apex of oilseed rape genotypes, during mid to late winter, are better predictors for winter hardiness than levels of theses parameters in leaves. Furthermore, their results highlighted that the potential of water content and carbohydrate content, especially sucrose, in the shoot apex meristem is good predictor of *LT50* and shoot regrowth after freezing stress over winter. Prásil *et al.* (2004) concluded that the initial growth of shoot apex was not associated with a loss in frost tolerance in wheat varieties, however, a much more advanced shoot length resulted in a decrease in tolerance. From above literatures, vernalization and cessation of shoot elongation before winter are considered by breeder as survival mechanisms for optimal overwintering in winter type crops. However, correlation between them are not considered properly. Regarding the QTL mapping for genes regulating shoot development in winter type crops few studies have been conducted. Chen *et al.* (2010) reported that three major QTL were found to control variation in the developmental process of *T. aestivum*, and each of them was tightly linked with flowering genes, *VRN-A1*, *PPD-D1* and *VRN-D3* on chromosomes 5A, 2D and 7D respectively. Dechaine *et al.* (2014) detected QTL x environment interaction for the vegetative traits and onset of reproduction over ontogeny in a bi-parental population of *Brassica rapa*.

Modern oilseed rape possess less than 2% amount of anti-nutritional fatty acid erucic acid (C22:1) in oil. Oil from low erucic-acid oilseed rape has a desirable fatty acid composition with oleic acid replacing erucic acid as the main component. Typically, the fatty acid profile of edible modern oilseed rape ("00") oil contains 60% of oleic acid (18:1), 20% linoleic acid (18:2), 10% linoleic acid (18:3), 7% saturated fats and 1-2% erucic acid (Wittkop *et al.* 2009). The development of cultivars combining high oleic acid and low linolenic acid is a breeding goal to provides a higher oxidative stability at high cooking temperatures, is related to mutations in the *Fatty Acid Desaturase* genes *FAD2 and FAD3*, which control the enzymes involved in desaturation of oleic (C18:1) to linoleic acid (C18:2) and linoleic to linolenic acid (C18:3), respectively (Wittkop *et al.* 2009, Abbadi and Leckband 2011). Modern oilseed cultivars are characterized not only for the favourable composition of fatty acid but also for an increase in the oil content. However, complex genetic structure of oil content in *B. napus* is only poorly understood due to complex polyploidy level in the genome. One approach to address this

complexity is QTL mapping, which is used to identify significant genomic regions associated with quantitative traits on a molecular linkage map. The QTL associated with oil content in oilseed rape (*Brassica napus* L.) have been identified using different populations and different mapping methods (Zhao *et al.* 2005, Teh and Möllers 2016). The number of QTL involved in oil content reported ranged from 1 to 18. Moreover, it was revealed that a single QTL could explain from 1.2 to 15.7% of the phenotypic variance that collectively with other QTL could explain up to 51% of the total phenotypic variance for oil content (Ecke *et al.* 1995) or up to 80% of oil content variation based on additive effects of QTL and additive × additive epistatic loci (Zhao *et al.* 2005). Additional investigations are essential to achieve a deeper knowledge of this trait in order to further improve oil content in high-yielding cultivars. In this context, the identification and utilization of genes contributing to oil content via comparative QTL mapping in different genetic backgrounds could help to identify gene loci with a key influence on this complex trait in oilseed rape (Chen *et al.* 2010). In a first such comparative study, Delourme *et al.* (2006) identified orthologous genomic regions involved in oil content in different genetic backgrounds and identified novel alleles in individual lines. Although genetic studies for seed quality traits and developmental traits are extensively conducted in different population of *B. napus*, little is known regarding the correlation between developmental processes and economic traits. The present study was carried out to investigate the inheritance and genetic basis of shoot elongation before winter and its relation to vernalization requirement, flowering time and seed quality traits in a DH population constructed from a cross between the resynthesized line L16 and the winter rapeseed cultivars Express617. The two parents differed in their vernalization requirement and flowering time. L16 is late flowering whereas Express617 is comparatively early flowering (Brandes 2016, and personal communication). In addition genetic variation of 19 different winter oilseed rape genotypes represented significant difference between L16 and Express617 for shoot length before winter and vernalization requirement (See chapter 3). It is anticipated that by the use of the DHLE population, higher number of major and minor QTL could be detected.

4.3 Material and Methods

4.3.1 Plant material

A doubled haploid bi-parental population, consisting of 151 inbred lines, derived from a cross between the resynthesized line L16 and the winter rapeseed line Express617 was used for a series of field and greenhouse trials. The resynthesized line "L16" is an interspecific hybrid between a Chinese broccoli (*B. oleracea* convar. botrytis var. alboglabra) as a mother and a Chinese cabbage (*B. rapa* ssp. pekinensis) as a father. L16 has high glucosinolate (~50μmolg^{-1}) content in the seed and less than 2% of erucic acid in the oil (Brandes 2016). This may indicate that L16 is not a true resynthesized line but rather a semi-synthesized line derived from a cross with a low erucic acid line or cultivar. On the other hand, Express617 is characterized as double low quality in Europe or "canola" in Canada, meaning less than 2% erucic acid in the oil and less than 25 μmolg^{-1} glucosinolates in the seed. The two parents were selected from their contrasting characteristics. L16 is late flowering with short shoot length before winter, whereas Express617 is comparatively early flowering with enhanced shoot length before winter (Brandes 2016; personal communication). Furthermore, results of spring sown experiments of 19 winter oilseed breeding lines and cultivars indicated that L16 has significant higher vernalization requirement than Express617 (See Table 3.3, Chapter 3). The doubled haploid population was developed through microspore culture using F1 plants of the cross between L16 x Express617 as microspore donors, and was named as DHLE population (Doubled Haploid of L16 and Express617; Brandes 2016).

4.3.2 Field and greenhouse experiments

The DHLE population and the parental lines were phenotypically characterized in field and greenhouse experiments. The field experiments were performed in two different mega environments. Those included the normal sowing experiments at end of August/beginning of September and spring sowing experiments at end of March/ beginning of April. Since the greenhouse, autumn and spring sown experiments represented very different environments; they were called mega environments.

4.3.2.1 Autumn sown environment

The DHLE population and its parental inbred lines were sown at four locations in observation field-plots during growing seasons 2014/15 and 2015/16. In 2014/15 the locations were Peine (Limagrain GmbH), sown on 21 August, and Einbeck (KWS Saat SE), sown on 4 September. In 2015/16 the locations were Peine, sown on 20 August, and Göttingen-Reinshof, sown on 28 August. All locations were in North Western Germany. Hundred seeds from each line were sown in small field-plots with double rows in Peine and Göttingen with 2 m length, 50 cm space between plots and plant-to-plant distance in the row was 10 cm. In Einbeck seeds were sown as one row with 3 m length and 80 cm space between plots and plant-to-plant distance in the row was 6 cm. All agronomic practices, such as fertilizer, herbicide and insecticide were applied at each location according to common practices. Three to four months after sowing, five representative plants were selected and harvested by cutting the stem below the root neck (crown). Harvesting time in 2014 for Peine and Einbeck were 8 and 24 December and in 2015 for Peine and Reinshof were 30 November 2015 and 11 January 2016. Shoot length before winter from root neck to shoot apex and shoot diameter before winter at the root neck were measured using a slide gauge and a metering rule. Begin of flowering from first of January and plant height at end of flowering were recorded in the following spring at four locations. In addition, data for begin of flowering and plant height at end of flowering was kindly provided by Haiko Brandes for the two autumn sown experiments Reinshof, sown on 3 September 2012 and Peine sown on 1 September 2012 (Brandes 2016).

4.3.2.2 Spring sown environment

The DHLE population and its parental inbred lines were sown at four locations in North Western Germany. Two locations were in Göttingen-Reinshof, sown on 20 March 2014 and 24 March 2015 and two locations were in Einbeck (KWS Saat SE), sown on 4 April 2014 and 10 April 2015.

All plants were grown by sowing 100 seeds using 2 m double rows and 50 cm space between plots and 10 cm between plants in Göttingen and 3 m single row with 80 cm between plots and plant-to-plant distance in the row was 6 cm in Einbeck. Three to four months after sowing, seven

representative plants were selected and harvested by cutting the stem below the root neck (crown). Harvesting time for Göttingen and Einbeck in 2014 were 3 and 7 July and in 2015 were 26 June and 13 July, respectively. Shoot length from root neck to shoot apex and shoot diameter, at the root neck, were measured using metering rule and slide gauge. Percentage of tendency to form inflorescence was scored as 100% for presence of visible buds or flower and 0% for lack of buds for each of the seven plants.

4.3.2.3 Greenhouse environment

The greenhouse experiments were conducted during December 2014 to July 2015 in Göttingen. The DHLE population and its parental lines were sown in 96-multipot trays, in compost soil. Four seeds, with one seed in each slot, were sown for each genotype. The greenhouse experiments were performed with three different vernalization treatments: 0, 4 and 8 weeks chilling treatment. For 8 weeks vernalization treatment, the two independent experiments were sown with one week interval. The first experiment was sown on 12 December 2014 and the second experiment was sown on 19 December 2014. The plants of two experiments were allowed to grow to the four leaf stage and thereafter they were transferred to a vernalization chamber, set to 4 °C and 8 hours light, on 12 and 19 January 2015, respectively. The plants were taken out from the vernalization chamber after 8 weeks (12 and 19 March) and they were further cultivated in the greenhouse at around 20 °C, then they were harvested on 12 and 19 May 2015.

For the 4 weeks and 0 week vernalization treatments, the two experiments were sown by providing 1 week gap between the sowing dates, i.e. on 12 and 19 February 2015, respectively. In case of the 4 weeks vernalization treatment, the plants were allowed to grow until four leaf stage. Thereafter, plants were transferred to the vernalization chamber on 9 and 17 March 2015 for the two experiments, respectively. After around 4 weeks of vernalization, plants were taken out from the vernalization chamber on 7 and 14 April 2015 and they were further cultivated in the same greenhouse as mentioned above, then they were harvested on 7 and 14 June 2015. The plants without vernalization were sown on 12 and 19 February 2015 and kept constantly in the greenhouse without vernalization treatment and they were harvested on 12 and 19May 2015. Through this staggered arrangement, it was tried to grow all plants after vernalization treatment at the same time in the greenhouse that was possible for 8 weeks vernalized and non-vernalized

plants (12 and 19 May). However, due to late sowing time, 4 weeks vernalized plants were harvested around 4 weeks later than other plants (7 and 14 June). Harvesting time was scheduled three month after sowing time in 0 week vernalized plants and two months following the transfer to the greenhouse in 4 and 8 weeks vernalized plants. In the all treatments, four plants per genotype were harvested by cutting the stem below the root neck (crown). Shoot length from root neck (crown) to shoot apex and shoot diameter at the root neck were measured using metering rule and slide gauge. Moreover, days to begin of flowering from sowing date was scored in the 8 weeks vernalized plants.

4.3.3 Seed quality traits

The DHLE population and the parental inbred lines were sown end of August /beginning of September at four locations in North Western Germany. Two locations were Göttingen-Reinshof, during growing seasons 2012/13 and 2013/14 and two locations were Einbeck (KWS Saat SE) and Peine (Limagrain GmbH) during growing season 2014/2015. Sowing condition and plots size are already mentioned in 4.3.2.1. At harvesting time, seeds of ten open pollinated plants from each line were harvested and bulked for analyses. Oil content, protein content, glucosinolates, were measured by near-infrared reflectance spectroscopy (NIRS) using commercial calibration raps2012.eqa provided by VDLUFA Qualitätssicherung NIRS GmbH (Teichstr. 65, D-34130 Kassel, Germany). Furthermore, neutral detergent fibre of defatted meal (NDFm), acid detergent fibre of defatted meal (ADFm) and acid detergent lignin of defatted meal content (ADLm) were measured by NIRS using calibration provided by Dimov *et al.* (2012). All the above traits are expressed on a seed basis at 91% dry matter content. Protein content of the defatted meal (PodM) was calculated as:

$$\%\text{Protein of the defatted meal (PodM)} = [\%\ \text{Seed protein} / (100 - \%\text{Oil})] * 100$$

Fatty acid composition was initially measured by NIRS, then mean of genotypes across four experiments were used to select 25 genotypes with maximum content of oleic acid (C18:1) and 25 genotypes with minimum content of oleic acid (C18:2). Then seeds from the 50 genotypes were collected from each of four experiments (200 samples) and the fatty acid composition were

analyzed by Gas Chromatography (GC) using method adapted from Thies (1971). Approximately 200 mg of seed, 1 ml of Na-methylate-methanol (0.5 mol L^{-1}), and one stainless steel rod (1.1 cm length; 0.4 cm diameter) were added in a propylene tube. The seeds were then homogenized using a custom-built vertical homogenizer (Institute of Applied Plant Nutrition, Georg-August-University Göttingen) for 3 min. Following incubation for 20 min at room temperature, 300 µl iso-octane and 100 µl 5% NaHSO4 in water were added, briefly vortexed, and centrifuged for 3 min at 4000 rpm. About 200 µl of the upper phase was pipetted into a GC vial and 3 µl was injected into a gas chromatograph (Thermo Trace GC Ultra), equipped with auto sampler, split injector (split ratio 70:1), flame ionization detector (320 °C), and capillary FFAP phase (0.25 mm × 25 m; Macherey & Nagel). Hydrogen (carrier gas) pressure was set at 100 kPa. Oven temperature was set at 210 ◦C. Total analytical time was 6 min. Data from NIRS and GC was used to develop a new NIRS calibration equation to estimate fatty acid composition in the DHLE population in each field experiment separately. The fatty acids content reported in this study include oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3), expressed as percentage of total fatty acids in mature seeds. Thousand seed weight was measured from weight conversation of 500 seeds at all four locations.

4.3.4 Linkage map

Linkage map was constructed by Brandes (2016) using MAPMAKER/EXP 3.0 (Lincoln et al., 1992) (Table 4.1). The resulting linkage map for the DHLE population has 4003 SNP markers mapped to 19 linkage groups, representing 19 chromosomes in *B. napus*, and covered 2050.3 cM with a mean interval distance of 0.5 cM between markers (Brandes 2016). The map has an average density of 2.02 markers per cM with distribution of markers varied 0.2 to 3.8 marker/cM across the linkage groups. The A genome comprised more markers (2252) as compared to the C genome (1751), with a mean interval distance between markers of 2.3 cM in the A genome and 1.6 cM in the C genome. The number of markers mapped in an individual linkage group ranged from 52 (A08) to 389 (A03). Also pairwise recombination and LOD scores indicated that markers were well allocated to linkage groups based on LOD and recombination frequency (Figure 4.1). For QTL mapping, a subset of 788 markers were manually selected by Brandes (2016) (Figure 4.2 and Figure 4.3) from the high-fidelity markers on the basis that the distance

between adjacent markers was about 5 - 10 cM. The term framework map was used to refer to the map used for QTL mapping.

Table 4.1: Marker distribution, size and marker density between markers of each linkage group in the linkage map of the DHLE population (Brandes 2016)

Linkage group	No. markers *per* linkage group	Size (cM)	Marker density (cM^{-1})
A01	187	83	2.25
A02	108	95.5	1.13
A03	389	138.8	2.80
A04	303	78.9	3.84
A05	139	116	1.20
A06	355	120.4	2.95
A07	312	92.4	3.38
A08	52	62.8	0.83
A09	176	112.4	1.57
A10	231	69.5	3.32
C01	207	108.7	1.91
C02	176	131.6	1.34
C03	286	196.1	1.46
C04	199	105.4	1.89
C05	160	121.6	1.32
C06	138	91.2	1.51
C07	209	108	1.94
C08	199	83.1	2.39
C09	177	134.8	1.31
A genome	2252	969.8	2.33
C genome	1751	1080.5	1.67
Whole genome	4003	2050.3	2.02

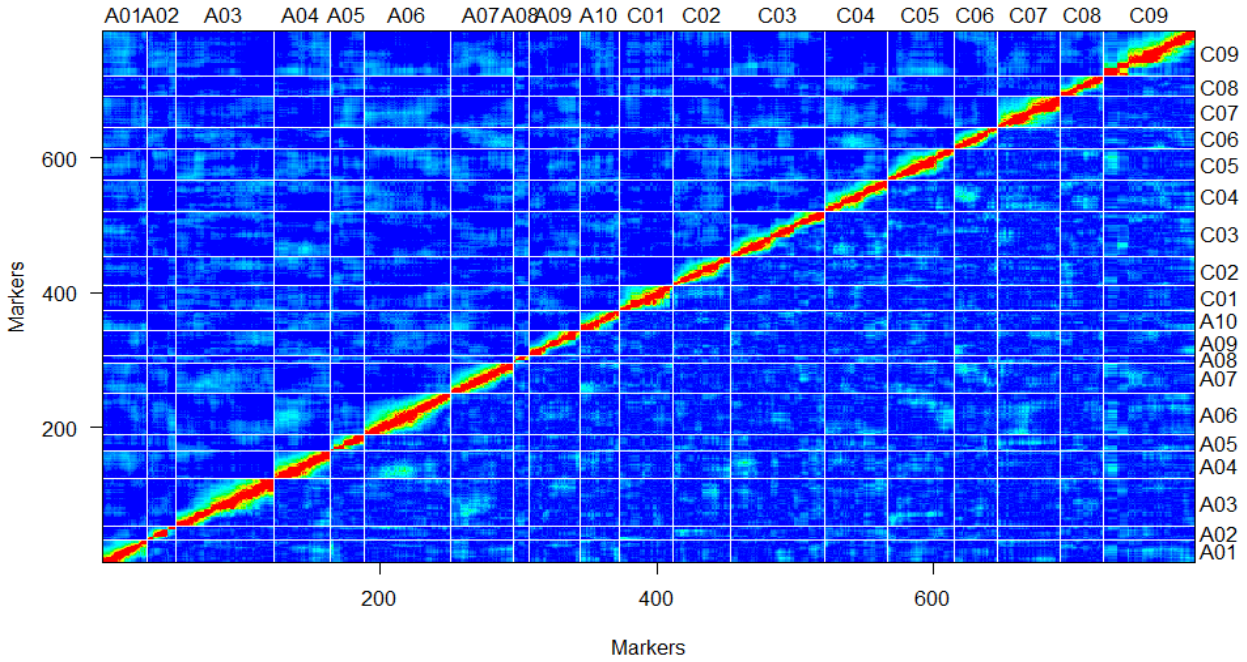

Figure 4.1: Estimated recombination fractions (upper left) and LOD scores (Lower right) for all pairs of markers in the DHLE population. Dark red indicates pairs of markers that appear to be tightly linked (very low recombination), dark blue indicates pairs that are not completely linked (very high recombination). Green points (combination of red and blue points) indicated marker's pairs ranging from very high recombination to very low recombination.

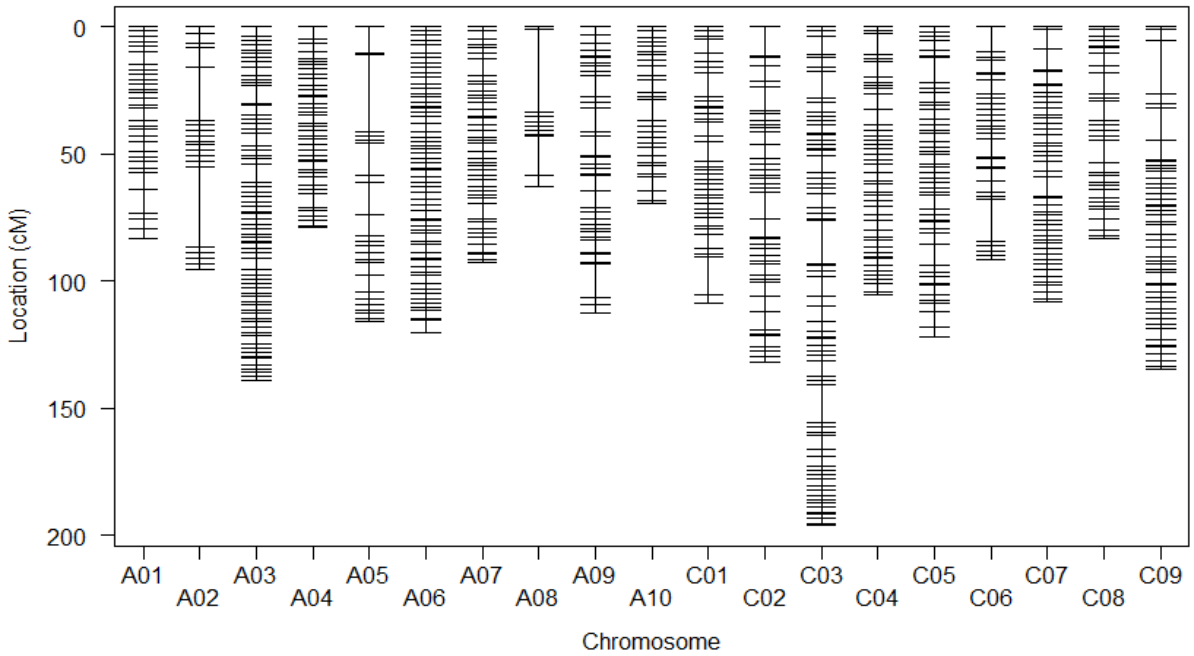

Figure 4.2: Genetic framework map of the DHLE population, with approximately 5 to 10 cM marker spacing

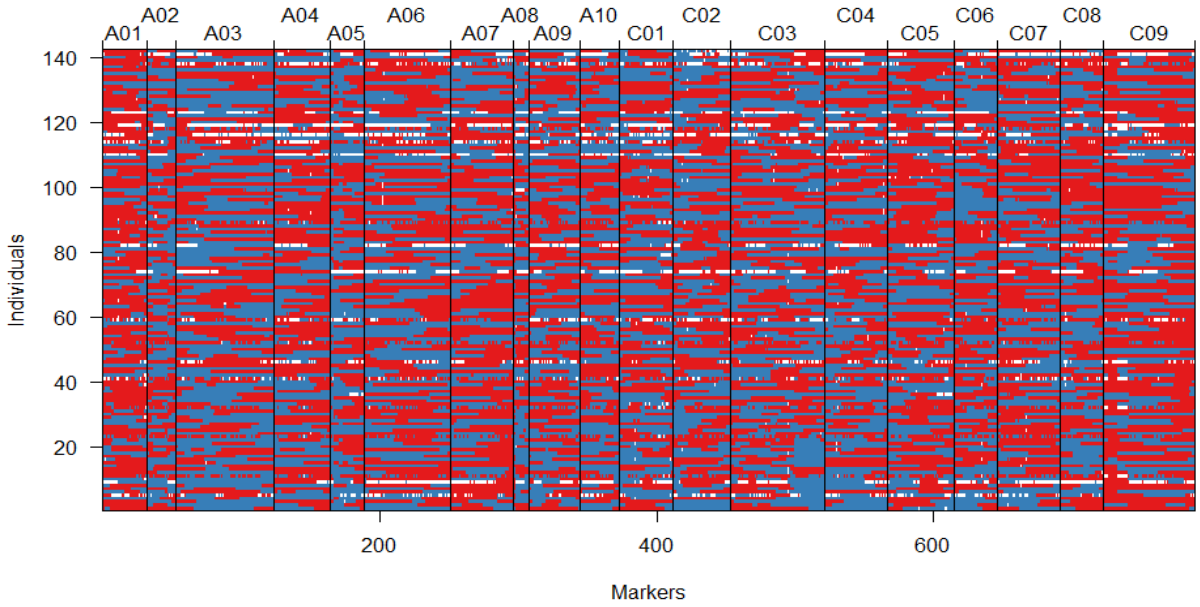

Figure 4.3: Genotype data for the DHLE population. Blue and red pixels correspond to alleles coming from L16 and Express617, respectively. White pixels indicate missing genotype data.

4.3.5 Statistical analysis

Analysis of variance and descriptive statistic were done by PLABSTAT (Utz 2011) and R (i386 3.0.3). Analysis of variance was separately done for each mega environment in which, location and year were treated as experiment factor in each mega environment. Linear mixed-effect model (R version 3.1-125, package {nlme}) was applied to test significant difference for the traits, hence experiment and plant sample (sub-sample) were defined as random factor and genotype was fixed factor. The statistic model used for ANOVA is shown below.

$$X_{ijk} = \mu + g_i + e_j + g_i e_j + p_k{:}g_i e_j$$

where X_{ijk} is phenotypic observation of genotype i in experiment j and plant k, μ is a general mean, g_i and e_j are effects of genotype i and experiment j, $g_i e_j$ is residual error to test main effect of genotype i and experiment j and $p_k{:}g_i e_j$ is sampling residual error to test $g_i e_j$ effect. Broad sense heritability (h^2) of genotypes mean over experiments were calculated as follow:

$$h^2 = \sigma^2_G / (\sigma^2_G + \sigma^2_{GE}/E + \sigma^2_{p:ge}/EP)$$

where σ^2_G and σ^2_{GE} are genotype and residual error variance components, respectively, E is the number of experiments and P is the number of measured plants. The following ANOVA model was used in variance analysis for seed quality traits and thousand kernel weight.

$$X_{ij} = \mu + g_i + e_j + g_i e_j$$

where X_{ij} is observation of genotype *i* in experiment *j*, μ is general mean, *gi* and *ej* are effects of genotype *i* and experiment *j*, $g_i e_j$ is residual error of model. Broad sense heritability (h^2) of mean values over experiments was calculated as follow:

$$h^2 = \sigma^2_G / (\sigma^2_G + \sigma^2_{GE}/_E)$$

where σ^2_G and σ^2_{GE} are genotype and residual error variance components, respectively, E is number of experiments. Genotypes mean across the experiments were used to calculate Spearman's rank correlation coefficients between the traits which was done by R software (i386 3.0.3).

4.3.6 QTL mapping and identification of candidate genes

QTL mapping was implemented using the software WinQTL Cartographer software (ver. 2.5) (Wang *et al.* 2012). QTL were initially detected with composite interval mapping (CIM) using stepwise regression model 6 (Silva *et al*. 2012) for each trait, the LOD significance threshold (α=0.05) was estimated by 1000 permutation tests. Five markers as default model for CIM were allowed as co-factor in forward and backward regression method in the analysis. If this was not done, other QTL would inflate the residual sum-of-squares, and reduce the power to detect a putative QTL in the region of interest. CIM tests were performed, at 1 cM steps with a 10 cM window size, to prevent any background markers within 10 cM of a putative QTL from being included in the final results. Peaks were treated as separate QTL when the distance is more than 10 cM and the minimum LOD value exceeds one between any two adjacent peaks. Subsequently, multiple interval mapping (MIM) (Kao *et al.* 1999, Silva *et al.* 2012) was applied to refine the QTL position, the QTL effect in detected QTL and also to search for more QTL, and to investigate epistatic effects among the detected QTL. The MIM model was built upon a priori

model from CIM analysis and progressively refined using the BIC-M2 = 2ln(n) criterion. QTL positions that did not remain significant when fitted with others were then dropped from the model. QTL effects and their percentage of phenotypic variance explained by individual and all the QTL were estimated with the final model fitted in MIM. A one-LOD drop from the peak position was used as a confidence interval for each QTL.

To identify candidate genes in hotspot of genetic map, physical position of interval flanking markers (SNP) for was identified by performing a BLAST of marker's sequences against sequence reference genome of *Brassica napus.* (http://www.brassicadb.org/brad/). The position of the best hit was recorded only when the marker sequence from particular linkage group fell onto the same corresponding chromosome in *Brassica napus*. We used reference sequence genomes of *Brassica rapa* (http://www.brassicadb.org/brad/) and *Brassica oleracea* (http://www.ocri-genomics.org/bolbase/) to search for syntenic genes in *B. napus*. Then, the physical position of the syntenic genes were determined in the *Brassica napus* determined by BLAST search of genes sequence against reference sequence genome of *Brassica napus*.

4.4 Results

4.4.1 Phenotypic analysis

For the DH population L16 x Express617 (DHLE), highly significant genotypic variance components were found for all traits in the three mega environments: autumn sown, spring sown and greenhouse (Table 4.2.1). The variance components of the experiment and genotype x experiment were significant for most traits in the three mega environments. Estimation of broad-sense heritability classified traits in two groups. First group comprised traits with high heritability ranging from 73 to 94% including shoot length in the spring sown and greenhouse environment, plant height at end of flowering (EOF) and begin of flowering in the autumn sown environment and begin of flowering in the greenhouse with eight weeks vernalization treatment. Shoot length before winter and shoot diameter in the three mega environments were placed in the second group in which heritability ranged from 11 to 61%, indicating high influence of genotype x environment interaction for the respective traits. Tendency to form influence in the spring sown environment, measured by the percentage of visible buds, showed high heritability (h^2=80%). In the autumn sown environment, large phenotypic variation with normal or near-normal distributions were found for shoot length, shoot diameter, begin of flowering and plant height at EOF (Fig. 4.4.1). Furthermore, transgressive segregation was observed with extreme values at both ends of the distributions exceeding the mean values of both parents. Parental differences for shoot length and begin of flowering were more pronounced than for other traits in the autumn sown environment (Table 4.3.1). Express617 with 61 mm shoot length and around five earlier days of begin of flowering differed from L16 with 34 mm shoot length. In the spring sown environment, parent L16 with 218 mm shoot length was significantly shorter than Express617 with 855 mm shoot length. In addition, large phenotypic variation was observed for shoot length that ranged from 87 mm to 1255 mm, resulting in a bimodal frequency distribution of the DH population (Table 4.3.1 and Figure. 4.4.1). Bimodal frequency segregation suggested involvement of a major gene in inheritance of the trait. Also, comparison of frequency distribution of shoot length and the percentage of visible buds indicated that shoot elongation caused tendency to form buds in which, genotypes shorter than L16 showed lower tendency to

form inflorescence, whereas genotypes longer than Express617 showed almost 100% buds formation. For shoot diameter in the spring sown environment, no significant difference was found between parents L16 and Express617, however transgressive segregation and phenotypic variation ranged from 17 mm to 29 was found across spring sown field experiments.

Table 4.2.1: Variance components and heritability of the DHLE population in the three mega environments

Environment	Trait	Variance components (σ^2)			Heritability (%)
		Genotype (G)	Experiment(E)	G x E	
Autumn Sown	Shoot length[a]	35.9**	468**	151.9**	51
	Shoot diameter[a]	0.2**	9.01**	0.9**	29
	BOF	4.1**	46.15**	4.14	84
	Plant height at EOF[a]	61.4**	228**	46.7	73
Spring Sown	Shoot length[a]	132083*	53745**	34581	94
	Shoot diameter[a]	10.93**	1.03*	10.25**	22
	Buds[b]	0.12**	0.01**	0.08**	80
Greenhouse 0 week	Shoot length[a]	53.4**	5.9**	20.7**	77
	Shoot diameter[a]	0.05**	0.04**	0.05**	43
Greenhouse 4 weeks	Shoot length4[a]	7129**	5.8**	3009**	77
	Shoot diameter[a]	0.88**	0.04	9.02*	11
Greenhouse 8 weeks	Shoot length[a]	1670**	5.52	2153**	73
	Shoot diameter[a]	0.11**	0.07	0.14**	61
	BOF	69**	0.15	186**	80

*and** denote significance at P<0.05 and P<0.01, respectively
[a] and [b] denote millimeter (mm) and percentage (%) , respectively
BOF: begin of flowering (from first of January)
EOF: end of flowering (from first of January)

Table 4.3.1: Descriptive statistics of the parents and the DHLE population (n=151) in the autumn and spring sown environment

Environment	Trait	Parents		Doubled haploid population (n=151)				
		L16	Express617					
		Mean		Min	Max	Mean	*F*-value	LSD 5%
Autumn sown	Shoot length[a]	34	61	21	71	42	1.87**	18
	Shoot diameter[a]	10	9	8	14	11	1.41**	2.4
	BOF(days)	118	113	112	124.1	119	6.02**	2.5
	Plant height at EOF[c]	1591	1492	1343	1871	1612	3.63**	135
Spring sown	Shoot length[a]	218	855	87	1255	659	15.5**	264.8
	Shoot diameter[a]	23	26	17	29	23	1.2*	5.2
	Buds[b]	25	100	0	100	61	6.2**	4.2

*and** denote significance at P<0.05 and P<0.01, respectively
[a] and [b] denote millimeter (mm), percentage (%), respectively
BOF: begin of flowering (from first of January)
EOF: end of flowering

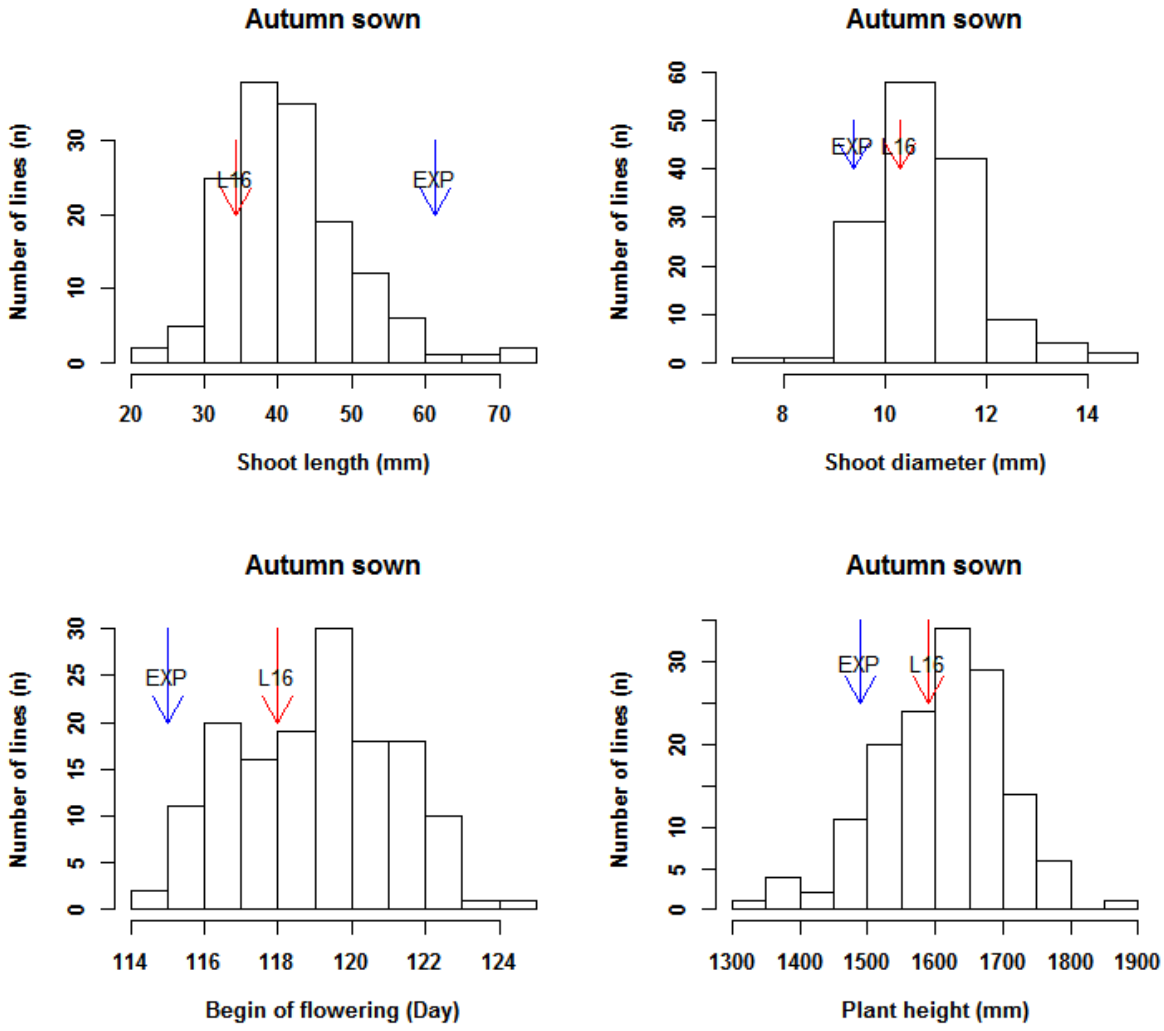

Figure 4.4.1: Frequency distribution of shoot length, shoot diameter, begin of flowering, plant height in the DHLE population in the autumn sown environment. Arrows indicate the parents mean across the field experiments. (*continued on the next page*)

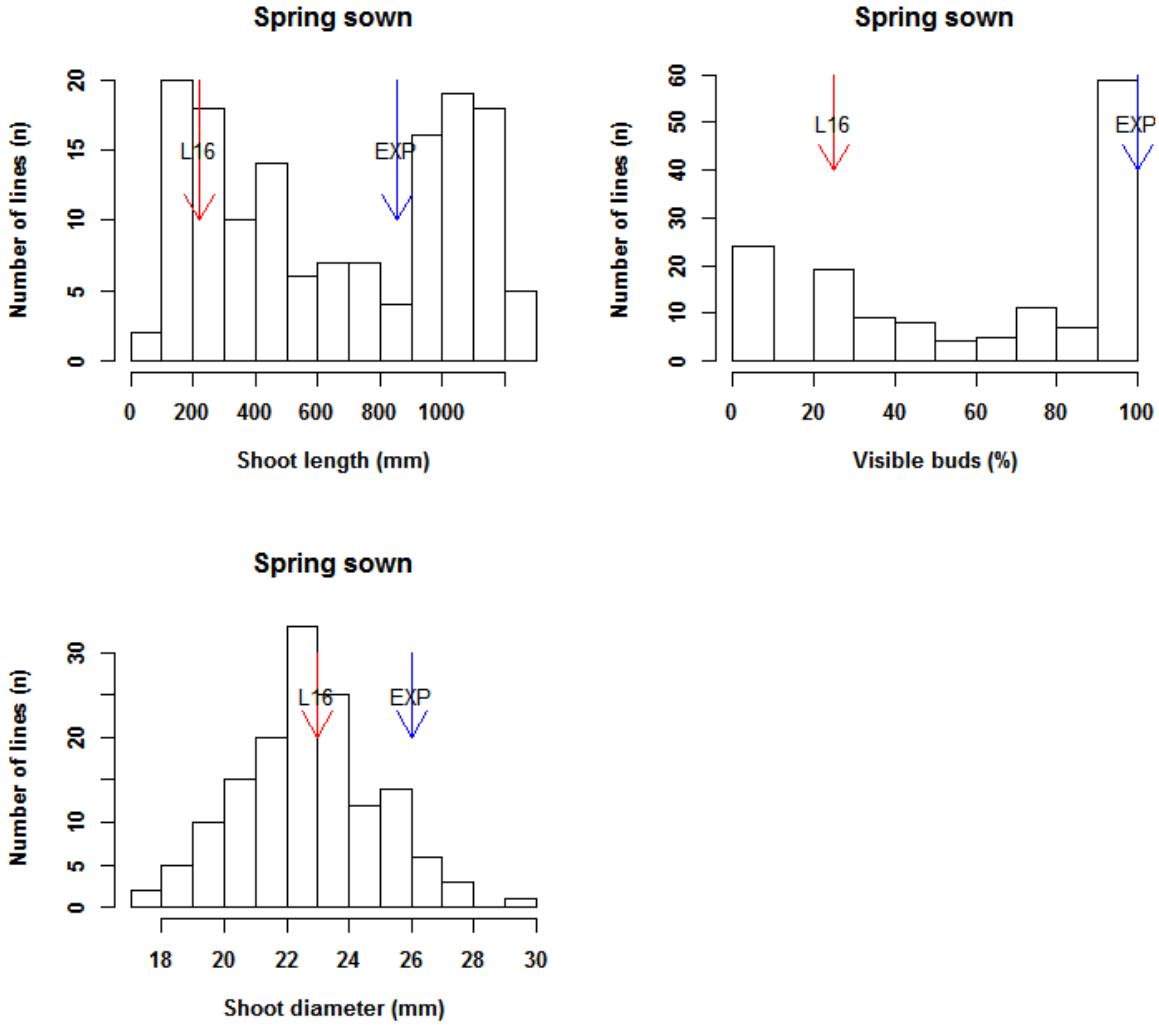

Figure 4.4.1: (*continued from the previous page)* Frequency distribution of shoot length, shoot diameter and visible buds in the DHLE population in the spring sown environment. Arrows indicate the parents mean across the field experiments.

In the greenhouse environment, in which genotypes after zero (non-vernalized), four and eight weeks of vernalization were tested; large phenotypic variation with normal or near normal frequency distribution was found for shoot length which ranged from 15 mm to 71 mm for non-vernalized plants and ranged from 270 mm to 518 mm for 8 weeks vernalized plants (Table 4.3.2, Figure 4.4.2). A skewed frequency distribution with maximum difference between the parents was found for shoot length in 4weeks vernalized plants, demonstrating large variability in vernalization requirement between the DH lines. Significant difference for shoot length was found between the parental lines. L16 had shorter shoot length and thinner stem diameter than Express617 in all the greenhouse treatments. Population mean for shoot length in non vernalization treatment was 33 mm that was roughly 10 folds smaller than shoot length (370) in eight weeks vernalized plants. Begin of flowering was, also, observed in plants with eight weeks vernalization treatment which ranged from 120 to 160 days, implying that vernalization requirement was completed in the entire DHLE population, while begin of flowering was partially observed in plants with 4 weeks vernalization treatment (data not shown).

Table 4.3.2: Descriptive statistics of the parents and the DHLE population (n=151) in greenhouse environment

Experiment	Trait	Parents		Doubled haploid population (n=151)				
		L16	Express617					
		Mean		Min	Max	Mean	*F*-value	LSD 5%
Greenhouse 0 weeks	Shoot length[a]	21	48	15	71	33	4.32**	11.21
	Shoot diameter[a]	4	5	3	6	4	1.75**	0.78
Greenhouse 4 weeks	Shoot length[a]	32	425	28	400	126	4.25**	131
	Shoot diameter[a]	4.4	4.5	4	26	6	1.12	1.1
Greenhouse 8 weeks	Shoot length[a]	332	459	270	518	370	3.66**	69.5
	Shoot diameter[a]	4.1	4.3	3	8	4	2.50**	2.5
	BOF (days)	150	141	120	160	139	6.20**	10.1

*and** denote significance at P<0.05 and P<0.01, respectively.
[a] denotes millimeter (mm)
BOF: begin of flowering (from sowing time)

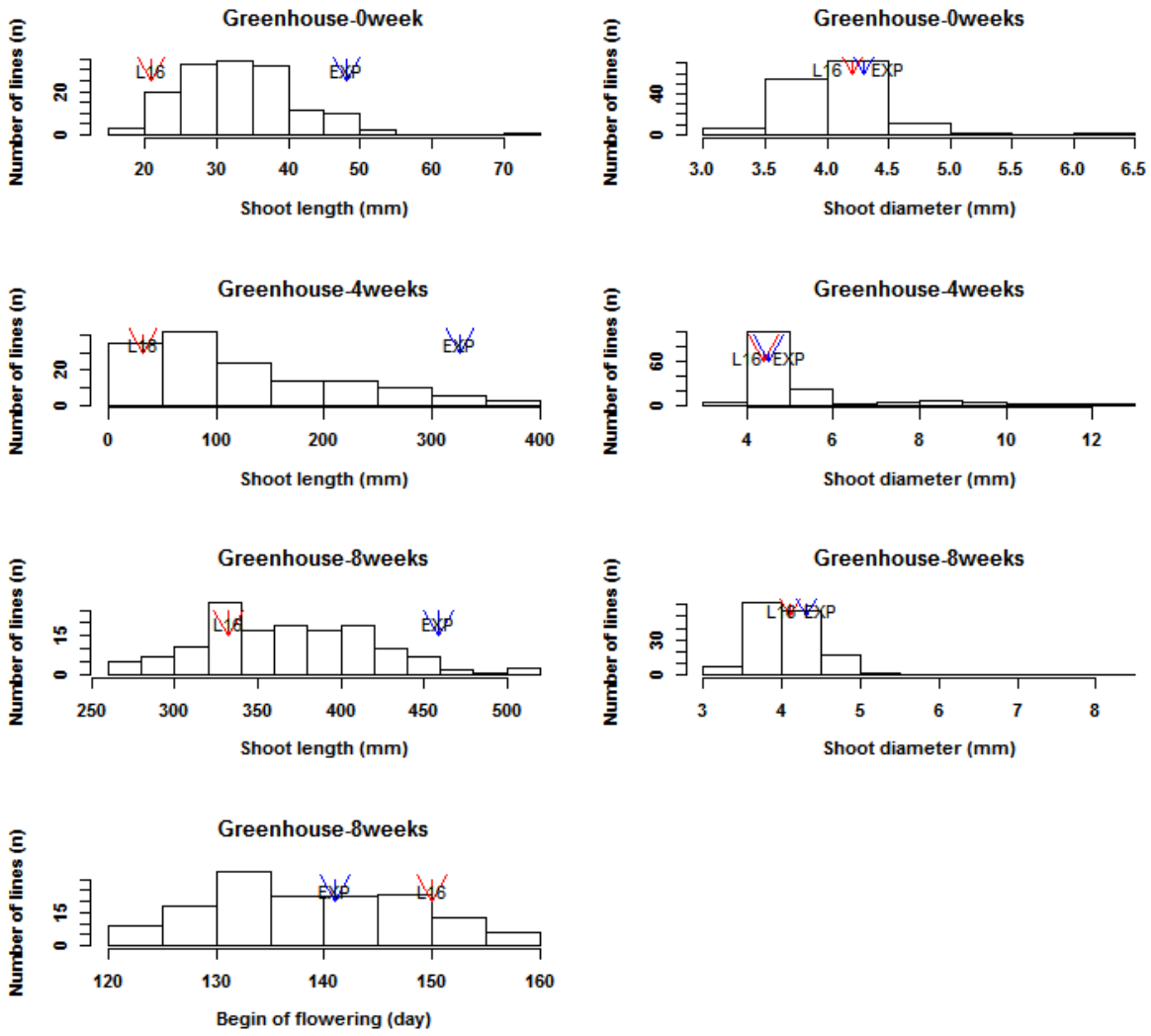

Figure 4.4.2: Frequency distribution of shoot length and shoot diameter in zero, four and eight weeks vernalization treatment. Begin of flowering in the eight weeks vernalization treatment in the DHLE population. Arrows indicate the parents mean across experiments.

For seed quality traits, significant variance components for genotype and experiment were observed in the DHLE population (Table 4.2.2). Broad sense heritability was high, ranging from 74 to 96% implying the large genetic variance that was promising for identification of loci affecting seed quality traits in the DHLE population. Frequency distribution along with descriptive statistics showed normal or near normal distribution with large phenotypic variation exceeding form the parents mean for majority traits. Nevertheless, glucosinolate content with bimodal frequency distribution was recognized from others, suggesting involvement a major gene for this trait (Table 4.3.3 and Figure 4.4.3). Parent L16 had significantly higher contents of glucosinolate (47.8 µmol/g) and protein of defatted meal, in contrast Express617 had significantly higher content of oil, oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) and thousand kernel weight (TKW).

Table 4.2.2: Variance components and heritability of the seed quality traits in the DHLE population (n=151)

Trait	Variance components			Heritability (%)
	Genotype (G)	Experiment (E)	G x E	
Oil[b]	1.25**	11.42**	1.11	81
PodM[b]	2.85**	17.31**	1.69	87
GSL(µmol/g)	34.6**	24.76**	3.44	96
C18:1[b]	0.59**	0.71**	0.41	84
C18:2[b]	0.63**	0.37**	0.68	88
C18:3[b]	0.26**	0.15**	0.12	90
NDFm[b]	1.08**	27.8**	1.54	74
ADFm[b]	0.98**	12.52**	0.95	81
ADLm[b]	0.68**	13.2**	0.87	76
TKW(g)	0.02**	0.11**	0.03	78

*and** denote significance at P<0.05 and P<0.01, respectively
[b] denote percentage (%)
PodM: protein of the defatted meal
GSL: glucosinolate content (µmol/g)
C18:1: oleic acid
C18:2: linoleic acid
C18:3: linolenic acid
NDFm: neutral detergent fibre of the defatted meal
ADFm: acid detergent fibre of the defatted meal
ADLm: acid detergent lignin of the defatted meal
TKW: thousand kernel weight (g)

Table 4.3.3: Descriptive statistics of the parents and the DHLE population (n=151) for the seed quality traits

Trait	Parents		Doubled haploid population (n=151)				
	L16	Express617					
	Mean		Min	Max	Mean	*F*-value	LSD 5%
Oil[b]	46.2	50.1	42.7	52.3	47.5	5.50**	1.5
PodM[b]	41.2	36.9	32.7	43.3	38.4	7.47**	1.81
GSL(µmol/g)	47.8	23.7	12.3	81.8	37.9	23.7**	9.9
C18:1[b]	62.5	63.5	60.3	65.5	63.1	6.02**	0.92
C18:2[b]	16.3	18.7	15.1	22.7	17.8	8.85**	0.82
C18:3[b]	9.6	10.5	8.1	11.1	9.6	9.7**	0.48
NDFm[b]	33.1	34.8	28.4	37.4	34.4	3.8**	1.73
ADFm[b]	22.4	25	21.1	26.3	23.7	5.1**	1.36
ADLm[b]	11.4	11.8	9.3	14.1	11.2	4.1*	1.3
TKW(g)	4.1	5.4	3.7	5.9	4.5	4.42**	0.25

*and** denote significance at P<0.05 and P<0.01, respectively
[b] denotes percentage (%)
PodM: protein of the defatted meal
GSL: glucosinolate content (µmol/g)
C18:1: oleic acid
C18:2: linoleic acid
C18:3: linolenic acid
NDFm: neutral detergent fibre of the defatted meal
ADFm: acid detergent fibre of the defatted meal
ADLm: acid detergent lignin of the defatted meal
TKW: thousand kernel weight (g)

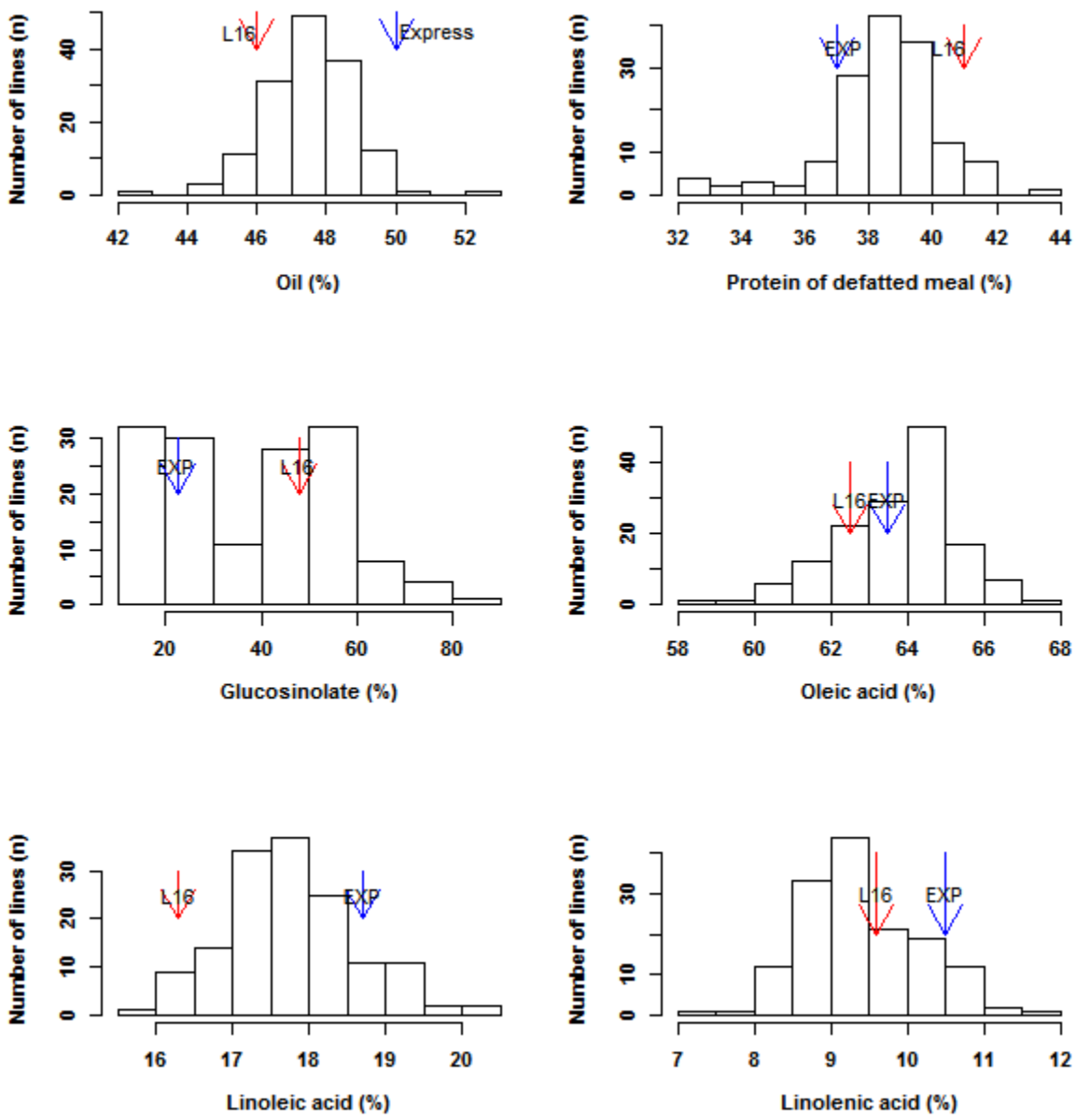

Figure 4.4.3: Frequency distribution of oil content, protein of the defatted meal, thousand kernel weight, glucosinolates, fatty acid composition, fibre seed fraction in the DHLE population. Arrows indicate the parents mean across the experiments. (*continued on the next page*)

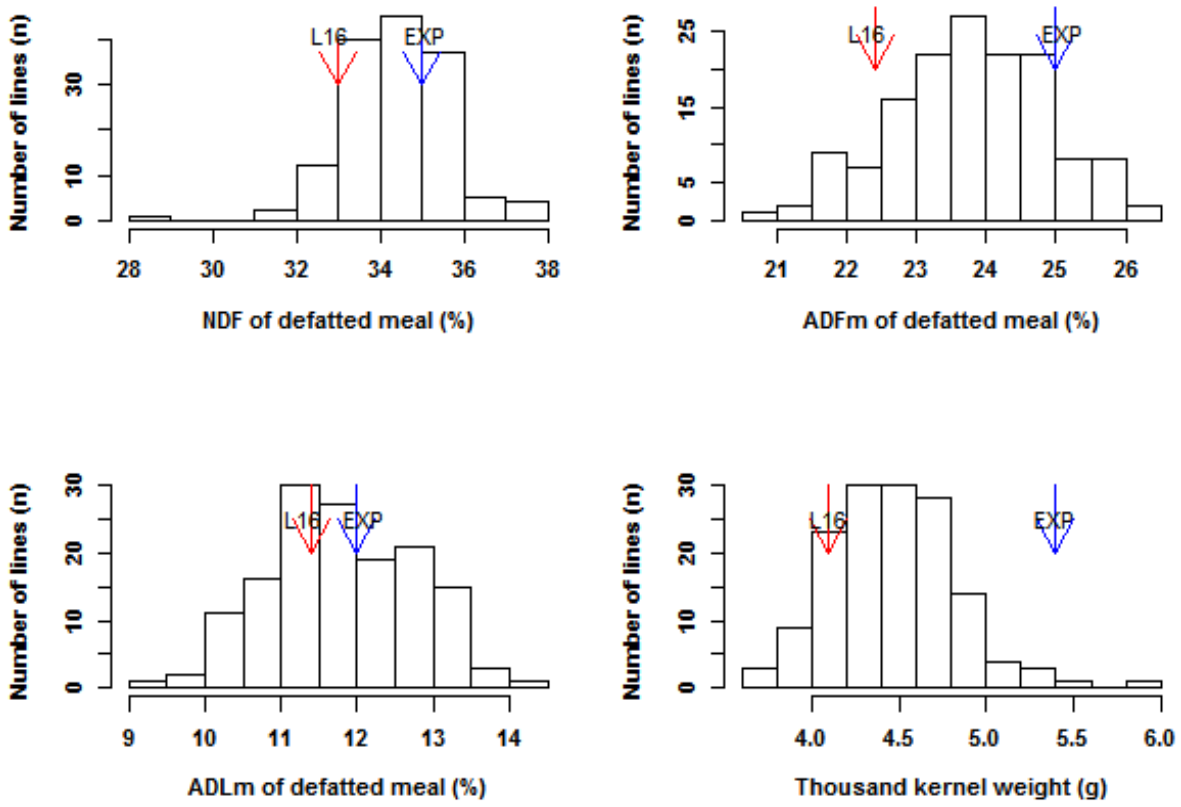

Figure 4.4.3 (*continued from previous page*) Frequency distribution of oil content, protein of defatted meal, seed weight, glucosinolates, fatty acid composition, seed fibre fraction, in the DHLE population. Arrows indicate parents mean across the experiments.

4.4.2 Correlation Analysis

Spearman's rank correlation ranged from 0.01 to 0.93 for the studied traits in the three environments (Table 4.4.1). Shoot length before winter was weakly, but significantly positive correlated with shoot length in the all greenhouse treatments. However, no significant correlation was observed between shoot length before winter and shoot length in the spring sown environment. Shoot length in the autumn sown environment was also not correlated with begin of flowering neither in the autumn sown nor in the greenhouse environment. In the autumn sown environment, begin of flowering was positively correlated with plant height at end of flowering (r_s=0.51**) which corresponds to the frequency distribution in which L16 with a late begin of flowering was longer than Express617 at end of flowering. Meanwhile, begin of flowering in the autumn sown environment had positive correlation (r_s=0.23**) with begin of flowering in eight weeks vernalized plants in the greenhouse and significant negative correlation with shoot length (r_s =-0.54**) and visible buds (r_s=-0.53**) in the spring sown environment. Shoot length in the spring sown environment had low to high positive correlations, ranging from 0.19* to 0.74** with shoot length in the zero, four and eight weeks vernalization treatment.

Table 4.4.1: Spearman's rank correlation of the traits in the DHLE population (n=151)

Environment	Trait	Autumn sown				Spring sown			Greenhouse 0week		Greenhouse 4weeeks		Greenhouse 8weeks		
		SL	SD	BOF	PH	SL	SD	Buds	SL	SD	SL	SD	SL	SD	BOF
Autumn sown	SD	0.10	1												
	BOF	-0.02	-0.08	1											
	PH	0.25**	-0.13	0.51**	1										
Spring sown	SL	0.11	0.09	-0.54**	-0.27	1									
	SD	-0.12	0.06	0.11	0.15	-0.29	1								
	Buds	0.08	0.08	-0.53**	-0.3**	0.93**	-0.29**	1							
Greenhouse 0week	SL	0.31**	0.01	-0.1	0.01	0.22**	-0.17	0.17	1						
	SD	-0.18*	0.17*	-0.12	-0.04	0.02	0.17	0.02	-0.06	1					
Greenhouse 4weeks	SL	0.17*	0.08	-0.4**	-0.21**	0.74**	-0.3	0.71**	0.37**	-0.03	1				
	SD	-0.05	0.16*	0.02	0.16*	-0.03	0.1	-0.04	-0.13	0.24**	0.23*	1			
Greenhouse 8weeks	SL	0.18*	0.22**	-0.06	0.06	0.19*	-0.04	0.18	0.16	-0.02	0.33**	-0.06	1		
	SD	-0.11	0.11	-0.06	-0.03	0.17*	-.003	0.2	-0.16	0.23**	0.04	0.07	0.14	1	
	BOF	-0.06	-0.08	0.23*	-0.01	-0.33**	0.14	-0.36**	-0.34**	-0.05	-0.26**	0.04	-0.36**	0.01	1

*and** denote significance at P<0.05 and P<0.01, respectively

SL= shoot length

SD= shoot diameter

BOF= begin of flowering (from first of January)

PH= plant height at the end of flowering

For the seed quality traits, Spearman's rank correlation ranged from -0.02 to 0.96 (Table 4.4.2). Oil content was positively correlated with oleic acid (C18:1), NDFm, ADFm and ADLm content in the defatted meal and negatively correlated with glucosinolates, linoleic acid (C18:2), linolenic acid (C18:2) and protein content in the defatted meal (PodM). Negative and significant correlation, ranging from -0.4^{**} to -0.7^{**} was found between PodM and seed fiber fraction. Oleic acid had negative significant correlation with both linoleic acid and linolenic acid, while linoleic acid and linolenic acid were positive correlated to each other ($r_s=0.54^{**}$). Thousand kernel weight (TKW) showed positive correlation with oil ($r_s=0.24^{**}$) and linolenic acid ($r_s=0.50^{**}$), yet it was negatively correlated with PodM ($r_s=-0.26^{**}$) and oleic acid ($r_s=-0.33^{**}$). Positive significant correlation ranging from 0.63^{**} to 0.96^{**} was found among NDFm, ADFm and ADLm, showing same genetic regulation for respective traits in the DHLE population.

Correlation between the seed quality traits with the studied traits in the three environment demonstrated positive correlation between shoot length before winter and contents of oil, protein of defatted meal, NDFm, ADFm, and ADLm. Shoot length and the percentage of buds in the spring sown environment, also, were correlated with oil content ($r_s=0.32^{**}$, $r_s=0.30^{**}$). Plant height at end of flowering was moderately positive correlated with oleic acid ($r_s=0.40^{**}$) and negatively correlated with linoleic and linolenic acid ($r_s=0.32^{**}$, $r_s=0.41^{**}$). Thousand kernel weight (TKW) was positively correlated with shoot length in the greenhouse environment with 0 week ($r_s=0.29^{**}$) and four weeks ($r_s=0.29^{**}$) vernalization treatment in the greenhouse environment.

Table 4.4.2: Spearman's rank correlation of the seed quality traits in the DHLE population (n=151)

		Autumn sown													
Environment	Trait	SL	SD	BOF	PH	Oil	PodM	GSL	C18:1	C18:2	C18:3	NDFm	ADFm	ADLm	TKW
	Oil	0.3**	0.0	-0.15*	0.13	1									
	PodM	-0.4**	0.0	0.11	0.0	-0.4**	1								
	GSL	-0.24**	0.0	0.0	-0.1	-0.5**	0.20	1							
	C18:1	0.04	-0.04	0.13	0.40**	0.55**	0.15	-0.02	1						
	C18:2	0.17*	0.19*	0.09	-0.32**	-0.38**	0.19	-0.05	-0.5	1					
	C18:3	0.06	0.06	-0.29	-0.41**	-0.41**	-0.05	-0.11	-0.7**	0.54**	1				
	NDFm	0.44**	-0.04	-0.16	0.11	0.5**	-0.7**	-0.3	0.55**	-0.27**	-0.4**	1			
	ADFm	0.45**	-0.05	-0.2**	0.09	0.5**	-0.7**	-03	0.6**	-0.33**	-0.44**	0.84**	1		
	ADLm	0.35**	-0.08	-0.16	0.04	0.20*	-0.4**	-0.2	0.6**	-0.34**	-0.44**	0.63**	0.96**	1	
	TKW	0.11	0.18*	-0.1	-0.02	0.24**	-0.26**	0.07	-0.33**	0.22*	0.50**	-0.06	-0.05	-0.14	1
Spring sown	SL	0.11	0.09	-0.54**	-0.27	0.32**	-0.14	-0.11	0.02	-0.05	0.11	0.19*	0.26	0.21*	0.07
	SD	-0.12	0.06	0.11	0.15	0.0	0.04	0.03	-0.05	0.01	0.02	-0.08	-0.17	-0.21*	0.29**
	Buds	0.08	0.08	-0.53**	-0.3**	0.30**	-0.14	-0.1	0.01	-0.07	0.12	0.19*	0.26	0.21*	0.01
Greenhouse 0week	SL	0.31**	0.01	-0.1	0.01	0.04	-0.11	-0.08	0.10	0.14	0.17*	0.12	0.18	0.14	0.29**
	SD	-0.18*	0.17*	-0.12	-0.04	-0.12	0.11	0.0	0.01	0	0.0	-0.11	-0.13	-0.07	0.05
Greenhouse 4weeks	SL	0.17*	0.08	-0.4**	-0.21**	0.35**	-0.27**	-0.22*	0.05	-0.08	0.05	0.21	0.31	0.14*	0.18*
	SD	-0.05	0.16*	0.02	0.16*	0.24**	0.13	0.0	0.04	-0.08	0.05	-0.18*	-0.15	-0.15*	0.05
Greenhouse 8weeks	SL	0.18*	0.22**	-0.06	0.06	0.35**	-0.16*	-0.21*	01	0.02	0.09	0.16*	0.30	0.21**	0.29**
	SD	-0.11	0.11	-0.06	-0.03	0.24**	-0.10	-0.13	0.08	0.111	-0.08	0.19*	0.12	-0.02	0.01
	BOF	-0.06	-0.08	0.23*	-0.01	-0.15	0.11	-0.02	-0.05	0.08	0.02	-0.16	-0.21	-0.16	00

*and** denote significance at P<0.05 and P<0.01, respectively
SL= shoot length
SD= shoot diameter
BOF= begin of flowering
PH= plant height at the end of flowering

PodM: protein of the defatted meal
NDFm: neutral detergent fibre of the defatted meal
ADLm: acid detergent lignin of the defatted meal
TKW: thousand kernel weight (g)
ADFm: acid detergent fibre of the defatted meal

C18:3: linolenic acid
C18:1: oleic acid
C18:2: linoleic acid
GSL: glucosinolates

4.4.3 QTL Mapping

Multiple interval mapping (MIM) was performed from mean of genotypes over experiments, in each mega environment separately (Table 4.5.1 and Table 4.5.2). Moreover, QTL mapping was done separately in each greenhouse experiment with zero, four and eight weeks vernalization treatment (Table 4.5.3). In total 52 QTL on A-genome linkage groups and 57 QTL on C-genome linkage groups were localized for the 25 studied traits in the DHLE population.

4.4.3.1 Autumn sown environment

Eighteen main QTL and one epistatic QTL were detected for shoot length, shoot diameter, begin of flowering, and plant height at EOF (Table 4.5.1). Six main QTL for shoot length before winter were localized on linkage groups A07, A08, A09, C01, C04 and C09 explaining individually 3.6 to 15.2% of the phenotypic variance. Moreover, an epistatic QTL with additive effect of -2.1 mm was found between the QTL on A08 and C01, explaining 5.1% of the phenotypic variance that along with the six main QTL contributed 49.2% to the observed variance for shoot length before winter in the DHLE population. For shoot diameter before winter, two QTL with additive effect of 0.33 mm and -0.27 mm and coefficients of determination of 7.1% and 1.03% were found on linkage groups A07 and C04. Four QTL were identified for begin of flowering on linkage groups A02, A03(2) and C04. The QTL at position 52.2 cM on A02 was a major QTL (R^2=23.7%) that along with the three other QTL cumulatively explained 47.1% of the phenotypic variance for begin of flowering. Six QTL for plant height at end of flowering were mapped to linkage groups A02, C01, C03, C04, C05 and C08 with additive effect ranging from -20.2 to 33 mm. Additive effects of the mapped QTL for plant height indicated that alleles derived from L16 in the QTL mapped on C-genome increased plant height at end of flowering; in contrast alleles from L16 in the QTL mapped to A-genome decreased plant height. The six QTL together explained 47.8% of the phenotypic variance for plant height in the DHLE population.

Table 4.5.1: QTL mapped for the traits in the autumn sown environment in the DHLE population (n=151)

Trait	QTL.name	Linkage group	Position (cM)	CI[a] (cM)	LOD	Additive Effect[b]	R^{2c}	TR^{2d}
Shoot length	Wi-Len-1	A07	53.2	50.1-62.3	5.31	2.42	5.1	49.2
	Wi-Len-2	A08	43.12	35.8-46.5	2.78	-1.88	3.6	
	Wi-Len-3	A09	107.4	102.1-111.1	11.5	-3.55	15.2	
	Wi-Len-4	C01	5	1-7	3.96	-2.2	5	
	Wi-Len-5	C04	60.04	56.1-63.7	5.75	2.64	8.3	
	Wi-Len-6	C09	57.5	45-60.2	3.85	-2.1	7	
	Wi-Len-7	A08*C01	------	------	5.46	-2.02	5.1	
Shoot diameter	Wi-Dim-1	A07	67.2	66.2-69.2	3.73	0.33	1.03	8.13
	Wi-Dim-2	C04	1.01	0-6.1	2.43	-0.27	7.1	
Begin of flowering	Wi-Bflw-1	A02	52.2	51.3-54.9	9.24	-0.93	23.7	47.1
	Wi-Bflw-2	A03	6.7	3.5-8	3.79	-0.41	2.7	
	Wi-Bflw-3	A03	111.4	111.3-115.7	4.26	0.58	10.5	
	Wi-Bflw-4	C04	100.9	97-102.7	3.79	0.55	10.2	
Plant height at EOF	Wi-Het-1	A02	50.7	42.1-52.9	5.63	-29	10.8	47.8
	Wi-Het-2	C01	12.4	6-15	4.27	24	4	
	Wi-Het-3	C03	187.2	186.3-188.8	7.15	33	13.8	
	Wi-Het-4	C04	100.9	94.6-101.8	4.81	28.2	8.5	
	Wi-Het-5	C05	42.9	40.5-48.9	5.23	28.2	8.5	
	Wi-Het-6	C08	81.09	80.2-92.7	2.86	20.2	2.2	

[a] 1-LOD Confidence interval

[b] {+} or {-} indicates that the trait value is increased by the allele derived from L16 or Express617, respectively

[c] R^2 is the percentage of phenotypic variance explained by each QTL

[d] TR^2 is the percentage of phenotypic variance explained by all QTL

4.4.3.2 Spring sown environment

Twelve main QTL were mapped for shoot length, visible buds and shoot diameter in the spring sown environment (Table 4.5.2). As expected from three contiguous cohorts in the frequency plot of shoot length in the spring sown environment, two major QTL with R^2=35.5% and R^2=25.7% were localized on linkage group C09 and A02 respectively, which along with the QTL on A07 explained 68% of the phenotypic variance in the DHLE population. Additive effect of the two QTL with alleles derived from L16 were 189.6 and 73.7mm versus additive effect= -223.4 mm of alleles derived from Express617, which corresponds to position of parents in the frequency distribution in which L16 was significantly shorter than Express617 (see Figure 4.4.1 and Table 4.3.1). For shoot diameter, six QTL were identified on linkage groups A01, A06, A09, A10, C01 and C09 that together explained 48.4 % of the phenotypic variance. For the appearance of buds three QTL were mapped to linkage groups A02, A07 and C09 that together explained 60.1% of the phenotypic variance with additive effect ranging from -8 to -24%. The two major QTL mapped to A02 and C09 explained individually 22.3% and 33.4% of the observed variance that corresponds to three contiguous groups in the frequency distribution for the percentage of visible buds in the spring sown environment (see Figure 4.4.1).

Table 4.5.2: QTL mapped for the traits in the spring sown environment in the DHLE population (n=151)

Trait	QTL.name	Linkage group	Position (cM)	CI[a] (cM)	LOD	Additive Effect[b]	R^{2c}	TR^{2d}
Shoot length	Sp-Len-1	A02	52.7	44-52.7	20.8	189.6	25.7	68
	Sp-Len-2	A07	78.4	70.5-85.2	3.03	73.7	6.8	
	Sp-Len-3	C09	128.7	125-131	8.35	-223.4	35.5	
Shoot diameter	Sp-Dim-1	A01	3.74	3-8	2.45	-0.48	2.6	48.4
	Sp-Dim-2	A06	62.93	59-66	5.98	-0.74	11.7	
	Sp-Dim-3	A09	18.48	16-24.8	4.36	-0.65	10.7	
	Sp-Dim-4	A10	14.2	13.3-17.5	6.95	0.88	13.9	
	Sp-Dim-5	C01	13.4	10.3-15.5	3.82	0.58	6	
	Sp-Dim-6	C09	31.9	29-35	2.66	-0.47	4.4	
Buds	Sp-Bud-1	A02	52.9	52.3-54.1	20.15	22	22.3	60.1
	Sp-Bud-2	A07	90.26	80.8-91.3	4.01	-8	4.4	
	Sp-Bud-3	C09	128.7	127.5-130	16.96	-24	33.4	

[a]: 1-LOD Confidence interval

[b]: {+} or {-} indicates that the trait value is increased by the allele derived from L16 or Express617, respectively

[c]: R^2 percentage of the phenotypic variance by each QTL

[d]: TR^2 percentage of the phenotypic variance explained by all QTL

4.4.3.3 Greenhouse environment

QTL analysis revealed 19 main QTL and 2 epistatic QTL for the studied traits in the different vernalization treatments in the greenhouse environment (Table 4.5.3). In the non-vernalization treatment, two QTL were found for shoot length on A06 and A09 with opposite additive effects of -2.29 and 2.4 mm which together accounted for16.2 % of the phenotypic variance. The two QTL for shoot diameter were found on linkage groups A03 and C07 that individually explained 7.1 and 10.9% of the phenotypic variance in the non-vernalization treatment. QTL analysis for shoot length in the four weeks vernalization treatment identified 5 main QTL which individually explained 4 to 21% of the phenotypic variance, which collectively with two epistatic QTL A2*C09 and C04*C09 explained 68.5% of the observed variance. The two QTL for shoot diameter were detected on linkage groups A02 and A09 that collectively explained 17.1% of the phenotypic variance in the four weeks vernalization treatment. In the eight weeks vernalization treatment, two QTL for shoot length with opposite additive effects were mapped on A10 and C04 that together contributed 13.3% to the phenotypic variance. For shoot diameter two QTL were found on A07 and C04. The QTL on C04 was a major QTL that determined 62.4% of phenotypic variance for shoot diameter in the eight weeks vernalization treatment. For begin of flowering, four QTL were localized on linkage groups A10, C03(2) and C09, together explained 27.5% of the phenotypic variance in 8weeks vernalization treatment.

Overlapping QTL confidence intervals were observed in the genomic regions of the DHLE population for the studied traits within and between the three mega environments. QTL *Sp-Len-1* for shoot length in the spring sown environment overlapped with *Gh-Len-H1* on A02 with positive additive effect, showing alleles derived from L16 increased shoot length in the spring sown and greenhouse environment with four weeks vernalization treatment. Strong correlation between shoot length and visible buds in the spring sown environment was supported (see Table 4.4.1) by overlapping QTL intervals on A02, A07 and C09, suggesting dependent pathways regulate the two traits. Confidence interval of major QTL *Wi-Bflw-1* for begin of flowering coincided with QTL *Sp-Len-1* and *Wi-Het-1* on A02. This is in agreement with correlation coefficients found between the respective traits (see Table 4.4.1). The candidate gene *BnA2.FT* was identified within interval 48.5 to 50.7 cM in the vicinity of major QTL *Wi-Bflw-1* on linkage group A02. No overlapping confidence interval of QTL was found between shoot length before

winter and shoot length in the spring sown and greenhouse environment with zero and 8 weeks vernalization treatment. Only on A08 QTL *Win-Len-2* coincided with *Gh-Len-H3* for shoot length in the four weeks vernalization treatment. Furthermore, no collocation of QTL were found between begin of flowering in the autumn sown environment and begin of flowering in the eight weeks vernalized plants. In contrast, confidence intervals of QTL *Gh-Len-H-1* and *Gh-Len-F-2* collocated with the two QTL for begin of flowering in the autumn sown environment on A02 and C04, respectively. No colocalizing QTL were found between shoot length and shoot diameter within the three mega environments suggesting independent genetic pathways for shoot length and shoot diameter in the DHLE population.

Table 4.5.3: QTL mapped for the traits in the greenhouse environment in the DHLE population (n=151)

Treatment	Trait	QTL.name	Linkage group	Position (cM)	CI[a] (cM)	LOD	Additive Effect[b]	R^{2c}	TR^{2d}
Greenhouse 0 week	Shoot length	Gh-Len-N1	A06	49.4	46.6-55.5	2.73	-2.29	7.8	16.2
		Gh-Len-N2	A09	3.35	1-11	3.03	2.40	8.4	
	Shoot diameter	Gh-Dim-N1	A03	72.4	69-75	2.67	0.08	7.1	18
		Gh-DimN2	C07	46.7	42-51	3.98	-0.1	10.9	
Greenhouse 4weeks	Shoot length	Gh-Len-H1	A02	45.8	45.3-48.4	7.36	31.7	14.6	68.5
		Gh-Len-H2	A07	91.6	85-111	2.78	19.1	5.2	
		Gh-Len-H3	A08	37.5	35.2-38.7	3.6	-23.2	6	
		Gh-Len-H4	C04	75.9	74-85	2.8	-19.4	4	
		Gh-Len-H5	C09	134.2	131.3-134.3	11.9	-41.6	21	
		Gh-Len-H6	A02*C09	-----	-----	3.7	2.18	9	
		Gh-Len-H7	C04*C09	-----	-----	4.24	-23.5	8.7	
	Shoot diameter	Gh-Dim-H1	A02	52.9	41.5-52	4.21	0.97	10.3	17.1
		Gh-Dim-H2	A09	25.24	20-35	3.2	0.75	6.8	
Greenhouse 8weeks	Shoot length	Gh-Len-F1	A10	19.21	15.4-20	2.9	20.1	7	13.3
		Gh-Len-F2	C04	82.67	80.7-92	2.75	-18.6	6.3	
	Shoot diameter	Gh-Dim-F1	A07	24.5	15.2-26	16.26	-0.32	10.6	73
		Gh-Dim-F2	C04	33.6	25-35	28.1	-2.8	62.4	
	BOF	Gh-Flw-F1	A10	50.69	48-56	3.19	-2.7	6.3	27.5
		Gh-Flw-F2	C03	63.3	62.4-64	2.7	-2.86	2.9	
		Gh-Flw-F3	C03	92.87	87.9-93	4.94	4.04	9.5	
		Gh-Flw-F4	C09	133.2	131-134	3.2	2.8	8.8	

[a]: 1-LOD Confidence interval
[b]: {+} or {-} indicates that the trait value is increased by the allele derived from L16 or Express617, respectively
[c]: R^2 percentage of the phenotypic variance explained by each QTL
[d]: TR^2 percentage of the phenotypic variance explained by all QTL
BOF: begin of flowering (from sowing time)

4.4.3.4 Seed quality traits

For the seed quality traits 64 QTL for the 10 studied traits were mapped to different linkage groups (Table 4.6.4). For oil content four QTL were mapped to linkage groups A07, C02(2) and C09 with additive effects ranging from 0.32 to 0.5%, which together explained 41.8% of the phenotypic variance. The QTL mapped at position 46.5 on C02 individually accounted for18.4% of the phenotypic variance that was bigger than the other QTL for oil content. Six QTL were identified for protein content of the defatted meal (PodM) that collectively accounted for 56% of the phenotypic variance. For glucosinolate content, a major QTL on C02 explained individually 50% of the phenotypic variance with additive effect of 12.84 µmol/g derived from L16 that along with the five other QTL contributed 83.1% to the phenotypic variance. In total, 22 QTL were localized for the fatty acid profile on different linkage groups that explained 54.8%, 79.4% and 52.2% of the phenotypic variance for oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3), respectively. QTL mapping for thousand kernel weight (TKW) revealed six QTL with additive effects ranging from -0.08 to 0.22 g that explained 65.8% of the phenotypic variance. For seed fibre fraction, in total, 16 QTL found were distributed mainly over the C genome, contributed 52.7%, 49.1% and 32.7% to the observed variance for NDFm, ADFm and ADLm. The total additive effect of alleles coming from L16 was bigger than additive effect of alleles from Express617, which corresponds to higher means for NDFm, ADFm and ADLm in parent L16 (See Table 4.3.3). Multiple overlapping confidence intervals were found between QTL for fatty acid composition on A03, A04, A10, C01, C02 and C05 showing tight linkage between different genes or the pleiotropic effect of genes for the respective traits. Likewise, protein content of the defatted meal overlapped with opposite direction of additive effect for ADFm, NDFm and ADLm on A01, A07 and C02. Major QTL mapped for glucosinolates on C02 (*Wi-Gsl-4*) overlapped with *Wi-oil3*, *Wi-Ndf-4* and *Wi-Adf-5* with opposite effect suggesting that alleles derived from L16 increased GSL and simultaneously decreased oil content and the content of fiber fraction in defatted meal. The two mapped QTL for shoot length before winter overlapped with *Wi-Oil-1* on A07 and *Wi-Oil-4* on C09 in the same and opposite direction of additive effect, respectively. Furthermore, collocation of multiple QTL between NDFm, ADFm, ADLm and shoot length before winter and in greenhouse environment were found on A08, C01 and C04.

Table 4.6.4: QTL mapped for seed quality traits in the DHLE population

Trait	QTL.name	Linkage group	Position (cM)	CI (cM)	LOD	Additive Effect	R^2	TR^2
Oil	Wi-Oil-1	A07	56.2	55-69	2.95	0.32	7.1	41.8
	Wi-Oil-2	C02	48.4	37-58	3.68	-0.38	12	
	Wi-Oil-3	C02	127.2	125-128.2	8.9	-0.5	18.4	
	Wi-Oil-4	C09	46.5	41-50	2.84	0.5	4.3	
PodM	Wi-Pdm-1	A01	14.5	10-17	4.63	-0.37	4	56
	Wi-Pdm-2	A07	28.2	26-29	8	0.95	17.6	
	Wi-Pdm-3	C02	97.3	95-98	6.32	0.57	8.8	
	Wi-Pdm-4	C04	21.7	19-25	3.1	-0.45	6.8	
	Wi-Pdm-5	C05	25	20-26	3.3	0.38	8.7	
	Wi-Pdm-6	C08	8.2	6-13	6.3	0.54	10.1	
GSL	Wi-Gsl-1	A03	109.1	109-123	4.4	-4.4	5.6	83.1
	Wi-Gsl-2	A07	88.3	79-91	2.9	-2.40	0.5	
	Wi-Gsl-3	A08	51.1	41-60	3.3	1.35	1	
	Wi-Gsl-4	C02	123.4	123-124	38	12.84	50	
	Wi-Gsl-5	C06	29.9	26-32	2.83	2.25	2	
	Wi-Gsl-6	C09	28	27-29	17	11	24	
C18:1	Wi-C181-1	A03	74	73-77	6.4	0.3	19	54.8
	Wi-C181-2	A04	50	40-52	5	-0.27	5.2	
	Wi-C181-3	A10	26.8	22-28	10	0.2	8	
	Wi-C181-4	C02	90	89-108	3	0.15	4	
	Wi-C181-5	C03	36.7	20-37	3.1	0.25	6.6	
	Wi-C181-6	C05	61	57-62	7	0.3	12	
C18:2	Wi-C182-1	A03	75	73-78	6.3	-0.34	16.2	79.4
	Wi-C182-2	A06	1	0-3	3.2	-0.23	7.8	
	Wi-C182-3	A07	6	5-9	3.3	-0.24	8.4	
	Wi-C182-4	A09	8.9	3.4-11.2	3	0.24	8.2	
	Wi-C182-5	C02	120	119-121	3.5	-0.26	9.6	
	Wi-C182-6	C03	12	9-17	3.5	-0.26	9.7	
	Wi-C182-7	C05	75	73-81	3	-0.24	8.2	
	Wi-C182-8	C09	42	31-47	4	-0.28	11.3	
C18:3	Wi-C183-1	A04	63.7	56-67	3	0.12	3.8	52.2
	Wi-C183-2	A06	59.3	57-63	2.8	0.11	2.4	
	Wi-C183-3	A09	75.4	65-79	3.3	-0.11	3	
	Wi-C183-4	A10	26.7	25-28	6	-0.21	12	
	Wi-C183-5	C01	52.6	45-54	5.5	0.13	9	
	Wi-C183-6	C02	73.1	70-89	3.4	-0.16	7.2	
	Wi-C183-7	C03	12.1	5-28	2.9	-0.11	6.6	
	Wi-C183-8	C05	59.6	55-63	6	-0.15	12	
TKW	Wi-Tkw-1	A05	1	0-12	10	-0.22	23.7	65.8
	Wi-Tkw-2	A06	48	46-49	6	-0.17	10	
	Wi-Tkw-3	A09	73.4	64-75	4.9	-0.09	8.4	
	Wi-Tkw-4	C01	13	5-15	4.5	0.12	11	
	Wi-Tkw-5	C02	119	105-122	4	-0.08	6.1	
	Wi-Tkw-6	C09	5.3	1-13	4	-0.09	6.6	
NDFm	Wi-Ndf-1	A07	28	15-30	5	-0.45	14	52.7
	Wi-Ndf-2	C01	10	6-14	3.7	-0.34	6	
	Wi-Ndf-3	C01	71	70-72	8.6	0.47	9	
	Wi-Ndf-4	C02	127	120-128	3.9	-0.3	8.5	
	Wi-Ndf-5	C04	68	66-75	3.2	0.2	5.2	
	Wi-Ndf-6	C06	71	65-75	3.2	-0.4	10	
ADFm	Wi-Adf-1	A01	14.5	11.7-17.3	2.9	0.29	5.5	49.1

	Wi-Adf-2	A08	43.1	40-50	3.2	-0.37	10.8	
	Wi-Adf-3	C01	10.5	6-14	7	-0.4	11	
	Wi-Adf-4	C01	71	70-75	3.3	0.3	5.2	
	Wi-Adf-5	C02	125	120-128	2.9	-0.25	4.8	
	Wi-Adf-6	C04	66	60-76	2.8	0.25	4	
	Wi-Adf-7	C05	107	100-115	3.1	0.34	7	
ADLm	Wi-Adl-1	A03	42.8	36-45	2.9	0.25	7.7	32.7
	Wi-Adl-2	A08	43.1	42-50	5	-0.34	11.3	
	Wi-Adl-3	C02	61.6	56-64	5	-0.38	7.7	
	Wi-Adl-4	C02	100	99-105	2.8	0.3	6	

[a]: 1-LOD Confidence interval

[b]: {+} or {-} indicates that the trait value is increased by the allele derived from L16 or Express617, respectively

[c]: R^2 percentage of the phenotypic variance by each QTL

[d]:TR^2 percentage of the phenotypic variance explained by all QT

4.5 Discussion

4.5.1 Phenotypic analysis

Parent L16, in this study, is a semi-resynthesized line that significantly differed from Express617 for shoot length in the three mega environments, giving large phenotypic variance in the DHLE population. It is believed that some desired genes are lost due to selective bottlenecks for oil quality and oil content in modern oilseed rape varieties (Girke *et al.* 2012).Therefore, using of the artificially resynthesized *B. napus* is a breeding strategy to broaden genetic variability and introgression exotic genes in the current genepool of *B. napus* (Becker *et al.* 1995) Significant difference between L16 and Express617, also, was observed in the mean comparisons of 19 oilseed genotypes for shoot length in the autumn sown and spring sown environment (See Chapter 3). Importance of shoot growth in the autumn on winter survival is shown in the results of a study with different sowing dates (Darby *et al*. 2013). They discussed that very much growth prior to winter cessation can determine the overwintering of crop and causes killing of the crown and growth of disease in oilseed rape. That is, winter type crops with enhanced shoot length are very prone to frost damage due to less photosynthetic activity and less accumulated carbohydrates in shoot apex (Rapacz *et al.* 2001, Rapacz 2002b, Prásil *et al.* 2004, Velicka *et al.* 2010, Asghari *et al.* 2014, Balodis and Gaile 2015). Also, successful overwintering of oilseed rape plants was observed after they developed a root crown diameter of 8-10 mm and a height of apical bud not exceeding 30 mm in autumn (Cramer 1990). In the present study population mean for shoot length before winter was 42 mm that was longer than optimum shoot length suggested for the successful overwintering. However, no frost damage was observed during implementation of the project in years 2014 and 2015.

Heritability was higher than 70% for begin of flowering and plant height at end of flowering in the autumn sown environment, in contrast low heritability was observed for shoot length and shoot diameter before winter which is explained by high standard error due to measuring error, a few number of plants (five samples) or effect of sowing conditions (single seed drilling vs. normal seed drilling) and different sowing date at four locations. For instance, population mean for shoot length before winter was 72 mm at location Peine, sown on 21 August 2014, while

population mean for shoot length was 26 mm at location Einbeck, sown on 4 September 2014. Velicka *et al*. (2010) explained that sowing date, autumnal growth and meteorological parameters such as air temperature and precipitation significantly influenced height and diameter of apical buds in oilseed cultivars.

Greenhouse results illustrated the all the DH lines flowered within 139 days after being incubated 8 weeks in the cold chamber (Table 4.3.2), while begin of flowering in the autumn sown delayed until other ambient cues were met in the following spring. The long gap between vernalization saturation and begin of flowering raises the question whether flowering time is a proper feature to measure vernalization requirement in the autumn sown field experiments or no? One alternative method to determine the vernalization requirement is, cultivation of winter oilseed rape in spring. This is done routinely by the German Federal Plant Variety Office (BSA, www.bundessortenamt.de) when testing varieties for Distinctness, Uniformity and Stability (DUS) in field plots. Inflorescence formation of winter oilseed rape cultivars in the year of spring sown field experiments is a much valued DUS-trait, because otherwise very similar cultivars usually can be distinguished by this trait (E. Thiemt, BSA Hannover, personal communication to C. Möllers).The tendency to form inflorescence in the year of spring sowing field experiments indicates that few cold days and nights at end of March and beginning of April are obviously sufficient for some winter oilseed rape genotypes. Obviously, increasing day length and temperatures in March/April positively influence bolting and flowering. The parents used for developing the DHLE population were selected from their contrasting characteristics that were in in compliance with results of the spring sown field experiments, where Express617 showed significant higher shoot length and low vernalization requirement than L16, giving a near bimodal distribution along with transgressive segregation for shoot length and visible buds. Quantitative nature of vernalization requirement is observed in most winter type crops (Hawkins *et al.* 2002).

4.5.2 Correlation analysis

Our results showed no significant phenotypic correlation between shoot length before winter and vernalization requirement, determined by shoot length in the spring sown environment.

Likewise no significant correlation was observed between shoot length before winter with other traits influenced by vernalization such as begin of flowering in the autumn sown environment and tendency to form floral buds in the spring sown environment. Dechaine *et al.* (2013) observed no significant correlation between vegetative traits and reproductive traits in a recombinant inbred lines of *Brassica rapa* in the field and glasshouse due to different magnitude of genetic variation of traits in the different developmental stage. The lack of correlation between shoot length before winter as relevant trait for winter hardiness and vernalization requirement is in accordance with some published findings that illustrated there is no simple relationship between winter hardiness and the degree of vernalization (Fowler *et al.* 1966b, Teutonico *et al.* 1993, Markowski *et al*. 1994)**.**

Negative correlation between vernalization requirement determined by shoot length in the spring sown environment and begin of flowering in the autumn sown and greenhouse environment (r_s=-0.54** and r_s=-0.33**) indicated that begin of flowering was accelerated in genotypes whose vernalization requirement is low. Hence, variation observed for elongation of shoot in the spring is influenced by different vernalization requirement and could be a more relevant trait than begin of flowering, which is routinely used by researchers, to measure vernalization requirement in the plant material. As a further evidence, highly positive correlation between shoot length, visible buds in the spring sown environment and shoot length of plants incubated for four weeks in the greenhouse environment (r_s=0.74**) proved that shoot length in the spring sown environment is strongly differentiated by the different vernalization requirement of DH lines. Therefore, shoot length in non-complete vernalized genotypes could be consider as a fast method to screen genotypes according to vernalization requirement. Positive correlation between plant height and begin of flowering in the autumn sown environment corresponds to the frequency distribution in which parent L16 with late flowering had longer plant height (See Figure 4.4.1). Mei *et al.* (2009) found positive correlation of r_s=0.29** and r_s=0.48** between plant height and flowering time in a segregating *Brassica napus* population with 145 $F_{2:3}$ lines in two growing seasons.

Highly broad sense heritability with significant variance for genotypes was observed for the seed quality traits in the DHLE population suggested that genetic gain could be achieved through phenotypic selection of the superior lines. However, complex relationship among seed quality traits impedes improvement of oil nutrition quality in oilseed rape varieties. Oil content had

positive correlation with oleic acid (r_s=0.55**) and negative correlation with poly unsaturated fatty acids linoleic (r_s=-0.38**) and linolenic (r_s=-0.41*) that is promising for the development of ‘‘HOLLi’’ oilseed rape cultivars with a high content of oleic acid (18:1) and a low content of linolenic acid (18:3) in the seed oil (Abbadi and Leckband 2011). For human nutrition, negative correlation between oil content and polyunsaturated fatty acids and positive correlation between oil content and both oleic acid is beneficial due to better oxidative stability of the oil (Teh and Möllers 2016). Negative correlations were observed between fibre-related traits and protein content of defatted meal, while oil content was positively correlated with fibre traits in defatted meal and negatively correlated with protein content in the defatted meal. The results confirm that an increase of oil content in the seed leads to an increase fibre content in the defatted meal. The negative correlations between oil content and protein content of the defatted meal indicates that the increase in oil content occurred at the expense of protein content instead of fibre content. This interpretation is supported by positive correlations between fibre traits of defatted meal and oil content. Si *et al.* (2003) have also observed a negative correlation between oil and protein content of the defatted meal of canola quality material tested at 7 to 9 locations across 4 states in southern Australia. They suggested that direct selection for high seed oil content and high protein concentration of meal would reduce the proportion of seed residue (fibre content). Furthermore common carbon signaling pathway involved in the synthesis of oil and seed fibre fraction is described as main reason for positive correlation between oil and NDFm, ADFm and ADLm in oilseed rape (Suprianto 2015). Correlation between the seed quality traits and the studied traits in the three mega environment are not well known, only genetic correlation between flowering time and seed quality traits have been mainly investigated in a few studies (Zhao *et al.* 2006, Chen *et al.* 2010) but result shows no strong correlation between flowering time and seed quality traits in oilseed rape populations. Also in this study, no notable correlation was found between the seed related traits and begin of flowering in the autumn sown and greenhouse environment. Weak to moderate correlations were often found between shoot length in three environment and some quality traits such as oil content, protein content of defatted meal, NDFm, ADFm and ADLm content, suggesting that variation in vernalization requirement and shoot development before winter might influence genes regulating seed quality traits in oilseed rape.

4.5.3 QTL Mapping

In total, 105 QTL were localized on 19 linkage groups for the 25 studied traits over three environments in the DHLE population. A general view of the position of all QTL on linkage groups demonstrated collocation of QTL for different traits were more frequently observed than individual isolated QTL. The overlapping QTL along with phenotypic correlation can shed light on genetic association between the traits measured in the different environments.

As already discussed, shoot length and visible buds in the spring sown environment are highly correlated with vernalization requirement. Therefore, QTL mapped for these traits are more likely linked to regulator genes for vernalization. It is shown in *B. napus* that the MADS box transcription factor gene *FLOWERING LOCUS C* (*FLC)* is the key regulator of vernalization requirement and is down-regulated by vernalization, thus enabling promotion of flowering by *FLOWERING LOCUS T* (*FT)* (Zou *et al.* 2012, Raman *et al.* 2016). Two out of nine paralogues of *FLC* (*BnFLC.C9a* and *BnFLC.C9b*) were physically identified on chromosome C09 of *B. napus* (Zou *et al.* 2012). Physical position of a copy of *FLC* (*BnFLC9b)* was found in a genomic region from 125 to 131 cM on CO9 that was within confidence intervals of the QTL for shoot length, visible buds in the spring sown environment, shoot length in four weeks vernalized plants and begin of flowering in eight weeks vernalized plants in greenhouse environment (Figure 4.5). Therefore, it can be speculated that *BnFLC9b* is the candidate gene controlling 35.5% of the phenotypic variance for shoot length in the spring sown environment. On linkage group A02 a QTL hotspot from 48.5 to 55.1cM encompassed QTL for begin of flowering and plant height in the autumn sown environment, shoot length and visible buds in the spring sown environment, shoot length and shoot diameter in the 4 weeks vernalization treatment in greenhouse (Figure 4.6). Surprisingly all the QTL were the largest or the second largest QTL for respective traits. The QTL on A02 is constantly reported in the flowering time-related studies, using informative molecular markers for genotyping, in oilseed rape segregating populations. Long *et al.* (2007) found a stable QTL for flowering time, budding time and bolting time on linkage group N02 across 11 field environments in *B. napus*. Lou *et al.* (2007) showed responsible QTL for flowering time, plant height, leaf number, turnip length on linkage R02 in three different segregating populations of *B. rapa*. A copy of gene *FT* (*BnA2.FT*) was identified in a genetic position of 51.3 cM within the hotspot on A02 (Figure 4.6). Our finding was in accordance with

a *FT* paralogue (*BnA2.FTa*) associated with a major QTL for flowering time in the results reported by Wang *et al.* (2009) in *B. napus*. In Arabidopsis, the input from the vernalization and photoperiod pathway is integrated by floral integrator gene *FLOWERING LOCUS T (FT)* that is strong promoter of flowering (Michaels 2009). Multiple copies of *FT*, with different cis-intronic sequence, exist and appear to operate harmoniously within *Brassica napus* (AACC), a member of the same plant family as *Arabidopsis* (Wang *et al.* 2009). Kole *et al.* (2001) suggested that alleles affecting flowering time in oilseed *Brassica* species might also affect winter survival. In the present study, QTL *Sp-Len-1* mapped on A02 for shoot length in the spring sown environment coincided with negative additive effect of QTL *Wi-Bflw-1* detected for begin of flowering and QTL *Wi-Het-1* for plant height in the autumn sown environment, indicating alleles derived from L16 on A02 caused lower vernalization requirement and earlier begin of flowering. This finding is in contrast with the finding that L16 with shorter shoot length in the spring sown environment showed stronger vernalization (Table 4.3.1), yet a glance to other QTL for shoot length and visible buds proved that the biggest QTL for shoot length in the spring sown environment was mapped at position 128.7 cM on C09, overruling the additive effect of QTL mapped to A02 and A07. Interrelation between different loci affecting quantitative traits are studied in Arabidopsis. Caicedo *et al*. (2004) represented that in *Arabidopsis thaliana,* the *FRI* and *FLC* genes mechanistically interact to control flowering time and they showed that this epistatic interaction also contributes to genetic architecture of evolutionary diversification within the species. Epistatic relation between two candidate genes *BnFLC9b* and *BnA2.FT* in the vicinity of two major QTL with opposite additive effects on C09 and A02 for shoot length in the spring sown environment could be questionable. However, allelic combinations of flanking markers in the position of two major genes differentiated majority of genotypes into four genotypic classes (Figure 4.7), indicating that there is no apparent epistatic effect between the two major genes for shoot length in the spring sown environment.

Our results indicated that no overlapping QTL confidence interval was found between vernalization requirement and shoot length before winter as relevant trait for winter hardiness that was in accord with no significant correlation between them. Previous QTL mapping for freezing tolerance suggested that different linkage groups exist in both *B. rapa* and *B. napus* for capacity to attain freezing tolerance and vernalization (Ferreira *et al.* 1995, Teutonico *et al.* 1995). However, Chen *et al.* (2010) found three major QTLs controlling variation in

developmental process, and each of them was tightly associated with a known flowering gene. QTL mapping, in our study, revealed that two QTL *Wi-Len-1* and *Wi-Len-6* were in the same linkage groups of QTL mapped for shoot length in the spring sown environment (A07 and C09). Furthermore, the QTL *Win-Len-5* on linkage group C04 was at the same linkage group with the two QTL *Gh-Len-H4* and *Gh-Len-F2* for shoot length in the four and eight weeks vernalization treatment in greenhouse.

QTL for protein content of defatted meal (PodM), oil content, fatty acid composition, glucosinolate content, thousand kernel weight, NDFm and ADFm were cumulated on linkage group C02, in which QTL associated with oil content in a high-density map on *Brassica napus* also was reported in a different bi-parental population (Wang *et al.* 2013). Oil and protein synthesis in the seed share basic resources in the metabolic pathways and are partly controlled by the same genes, which causes significant negative correlation between the two traits (Zhao *et al.* 2006). Gül (2003) identified six QTL for seed oil content and four of them showed a close linkage with QTL for protein content. In three cases the allele increasing oil content was in coupling phase with the allele decreasing protein content and vice versa, explaining the negative correlation between the two traits. Interestingly, no QTL from the traits related to shoot elongation and vernalization requirement were found at this region which might be applicable for improved seed quality without significant impact on vegetative and generative traits. In this study, a major QTL explaining 50% of the variance for the glucosinolate content was found on C02 that also was observed in oilseed rape populations (Howell *et al*. 2003, Liu *et al.* 2016). Brandes (2016) also reported a major QTL for glucosinolate content on C02 in the DHLE population. QTL mapping revealed linkage groups that comprised genetic regions influencing both morphological and seed quality traits in the DHLE population. Linkage groups A07 and C09 encompassed more QTL from the both categories. The QTL *Wi-Len-1* in a genomic region from 10 to 27 cM on A07 increased shoot length before winter while it was collocated with *Wi-Oil-1* for oil content with positive additive effect. In the second region from 67 to 79 cM on A07, another overlapping QTL between shoot length in spring and glucosinolate content was discovered that led to a reduction of both vernalization requirement and GSL content. C09 was the second linkage group with overlapping genomic regions between oil content, shoot length before winter and oleic acid content with opposite effects. In other words, QTL at this region increased oil content and at the same time decreased shoot length before winter and oleic acid

content. The two out of four QTL detected for oil content coincided with the QTL for shoot length before winter with positive and negative additive effects that should be taken in account, breeding for reduced shoot length before winter might influence oil profile. Rapacz and Markowski (1999) reported that modern double zero cultivars are characterized by lower frost resistance than traditional varieties. In addition, result of genetic variation in 19 oilseed genotypes (See chapter 3) revealed that the two resynthesized line R53 and L16 had a reduced shoot elongation, compared to the modern cultivars in the autumn sown and spring sown environment.

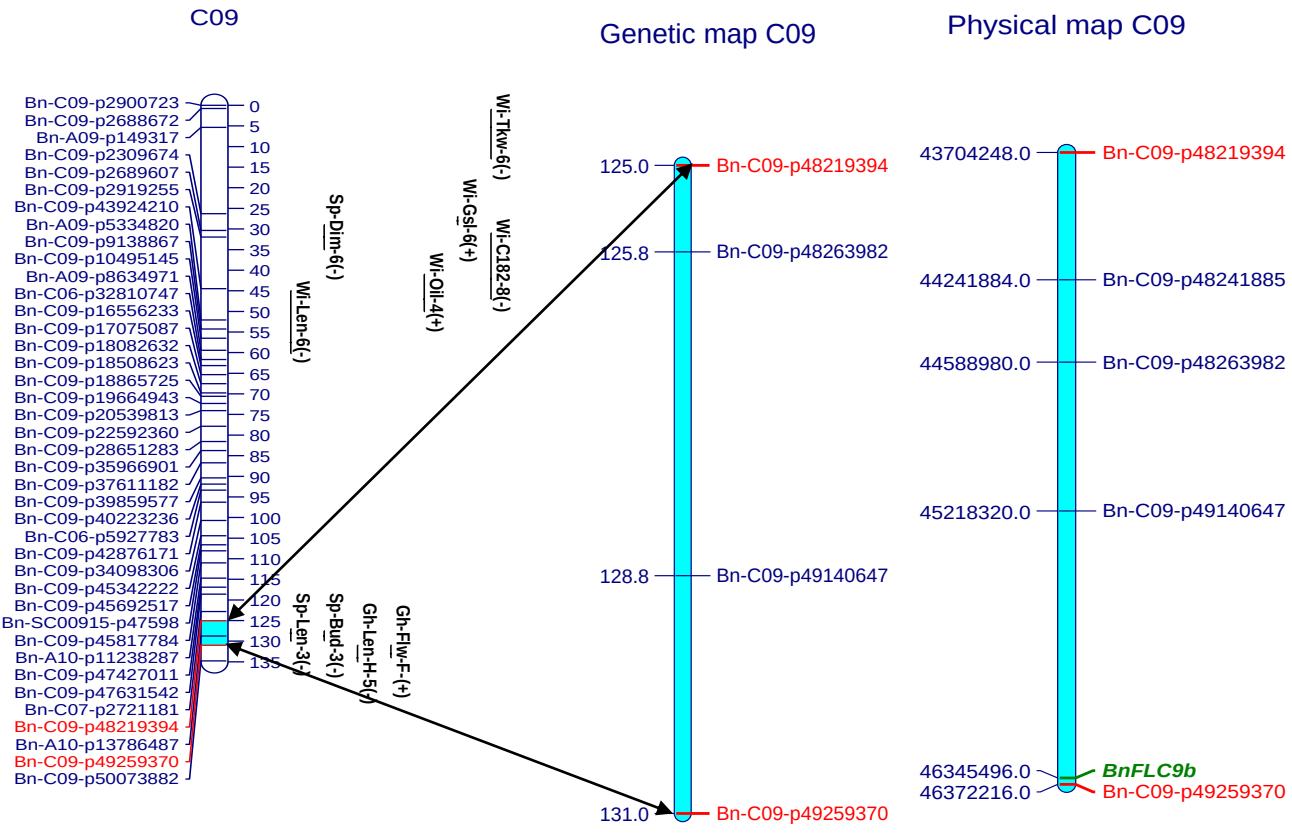

Figure 4.5: Genetic and physical map positions of markers within the QTL genomic region (125-131cM) on C09 in the DHLE. Left: QTL for shoot length and percentage of visible buds in the spring sown, shoot length and in the 4 week vernalization treatment and begin of flowering in the greenhouse with eight weeks vernalization treatment. Middle: Additional markers mapped within the QTL genomic region in the full map. Right: The corresponding physical positions of additional markers and the candidate gene (*BnFLC9b*) in *B. napus* genome

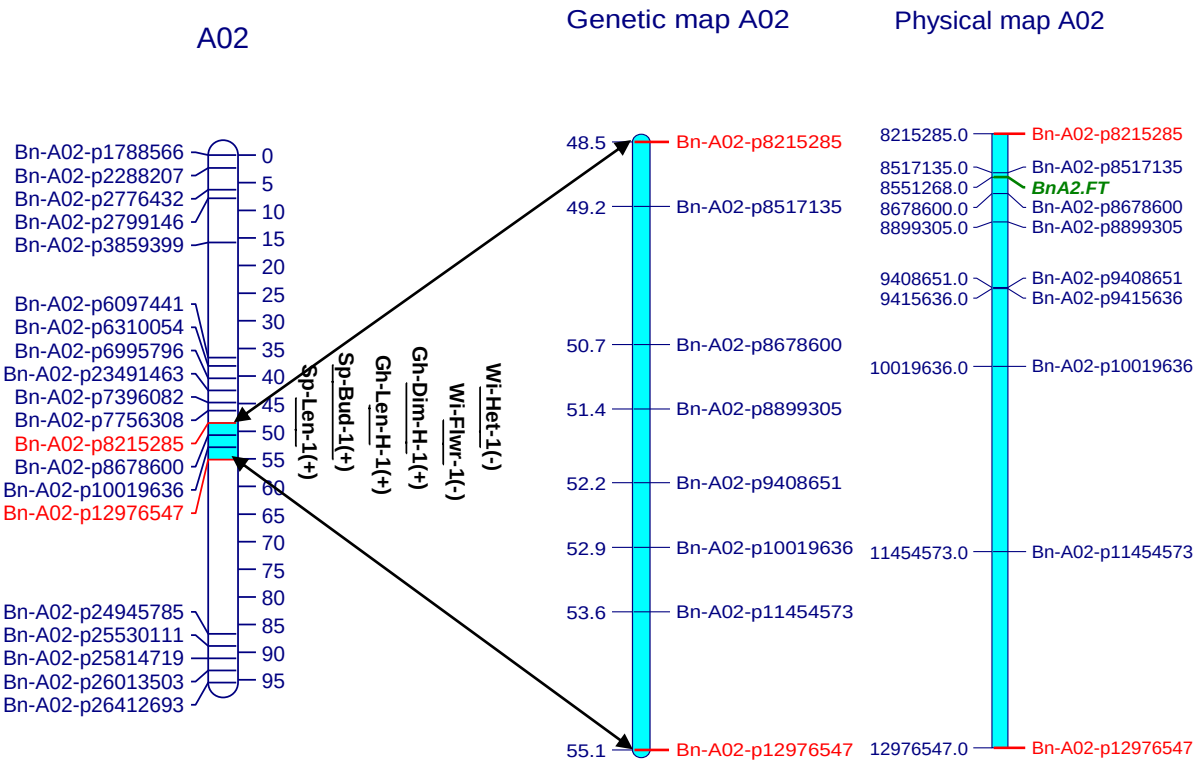

Figure 4.6: Genetic and physical map positions of markers within the QTL genomic region (48.5-55.1cM) on A02 in the DHLE. Left: QTL for begin of flowering and plant height at end of flowering in the autumn sown, shoot length Figure 4.7: Additive effect of two flanking markers *Bn-A02-p8215285* and *Bn-C09-p482119394* linked to the two major QTL on A02 and C09 for the shoot length in the spring sown environment in the DHLE population.

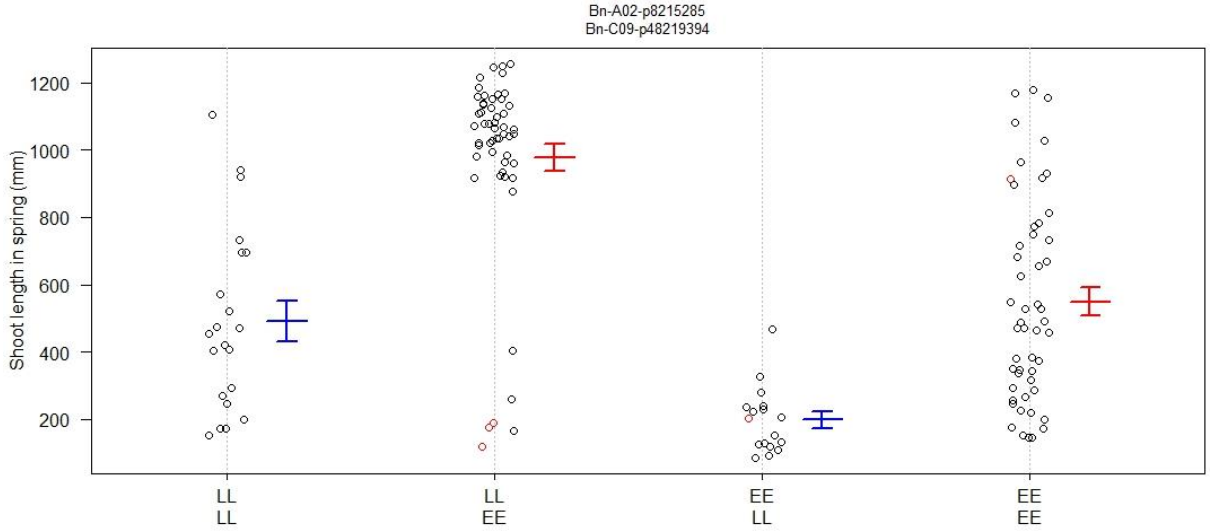

Figure 4.7: Additive effect of two flanking markers *Bn-A02-p8215285* and *Bn-C09-p482119394* linked to the two major QTL on A02 and C09 for the shoot length in the spring sown environment in the DHLE population. Red points indicate missing data. Red boxplot shows standard error and higher phenotypic mean and blue boxplot shows standard error and lower phenotypic mean for shoot length in the spring sown environment in each genotypic combination.

Chapter 5

Genetic analysis and inheritance of shoot elongation before winter and its relation with other traits in a doubled haploid population Sansibar x Oase (*Brassica napus* L.)

5.1 Abstract

Winter seasons are getting warmer with more rain due to global warming and increasing temperature. However, extreme freezing temperature are occasionally occurring in temperate regions, causing frost damage and yield loss. Cessation of shoot growth before and during winter in winter oilseed rape is a tolerance strategy to avoid winterkill by freezing temperatures. The present study aimed to analyze genetic architecture of shoot elongation before winter and its correlation with the other interacting traits in winter hardiness of oilseed rape. QTL mapping was performed in a segregating doubled haploid population derived from a cross of the two winter oilseed rape cultivars Sansibar and Oase, named DHSO population. The DHSO population and the parental lines were characterized in the three mega environments: autumn sown and spring sown and greenhouse. In the autumn sown environment, shoot length and shoot diameter were measured before winter, while begin, end, duration of flowering and plant height were measured in spring the following year. Likewise, shoot length, shoot diameter were measured around three months after sowing time in the spring sown environment. Non vernalized plants were cultivated in the greenhouse and shoot length and shoot diameter were measured three months after sowing time. High broad sense heritability was found for shoot length in the spring sown and greenhouse environment, in contrast shoot length before winter had medium heritability. Spearman's rank correlation revealed significant negative correlation between shoot length in the spring sown environment with begin of flowering, end of flowering and plant height in the autumn sown environment. Positive correlation (r_s=0.31**) was found between shoot length before winter and vernalization requirement determined by shoot length in the spring sown environment. Multiple interval mapping detected five main QTL, explaining collectively 23.5% of the phenotypic variance for shoot length before winter. For shoot length in the spring sown environment a major QTL on A02 explained 70% of the phenotypic variance. Three QTL for shoot length in greenhouse contributed 26% to the observed variance. The candidate gene *BnFLC2* on linkage group A02 was identified in the confidence interval of the major QTL for shoot length in the spring sown and a minor QTL for begin of flowering and shoot length in the autumn sown environment. Furthermore, a copy of candidate gene *FT* (*Bn.C6.FT.b*) on linkage group C06 was identified in the vicinity of a major QTL for begin, end and duration of flowering in the autumn sown environment.

5.2 Introduction

Oilseed rape varieties are cultivated in different regions across the world, including Central and Western Europe, Canada, China and other parts of the world. The large adaptation has been achieved by both spring and winter types, enabling genotypes to grow in diverse climates. (Downey 1990). This classification is based on vernalization requirement and not on their level of frost tolerance. However, winter types are generally assumed to have better winter survival (Teutonico *et al*. 1993). Winter survival is defined by Blum (1988) as "the final integrated plant response to a multitude of stresses involved during and after freezing stress, including both external-physical and biotic stresses.", therefore, even if plants are not winter-killed, they can be affected by freezing temperatures that may damage the leaf, causing reduction in leaf area, delayed growth, and plant debilitation (Sãulescu and Braun 2001). Selection for improved winter hardiness is a difficult task, because many interacting factors can vary greatly among regions causing winterkill at one location but having no impact at other locations. For instance, in the Ukraine, an analysis of data from the last 100 years showed that winterkill was caused by low temperatures in 35% of cases, by alternate freezing and thawing in 26% of cases, and by ice encasement in 22% of years when significant winter damage occurred (Poltarev *et al.* 1992). Shoot elongation before winter is one of the contributing factor in overwintering of plants which are sown in late summer or begin of autumn. It is shown that promotion of elongation growth leads to consumption of accumulated photosynthetic products, e.g. sugars and loss of frost tolerance (Levitt 1972). Rapacz *et al*. (2001) stated that decrease in frost tolerance observed in spring type plants was associated with the beginning of elongation growth of petioles and epicotyl and expansion of leaf area. With respect to the genetic basis of mechanisms regulating shoot development in winter type crops, few studies have been conducted. In the model plant *Arabidopsis*, the growth and development of the plant may be divided into the vegetative phase, the first-inflorescence phase and the second-inflorescence phase (Ratcliffe *et al.* 1998). These developmental phases are believed to be controlled by a group of flowering time genes comprising gibberellins, vernalization, photoperiod, and autonomous pathways (Ratcliffe *et al.* 1998, Sung and Amasino 2004). Therefore, understanding genetic mechanism controlling flowering time and vernalization requirement might be valuable to enhance level of winter hardiness in winter oilseed rape. The onset of flowering in plants is a critical life-history trait that

has been shaped by natural and artificial selection to maximize reproductive success (Amasino 2010). Widespread researches have been launched to dissect genetic structure of flowering time in different growth types of oilseed rape, resulting in a general frame of flowering time pathway and its underlying genes (Ferreira *et al.* 1995, Long *et al.* 2007, Lou *et al.* 2007, Jung and Muller 2010, Luo *et al*. 2014, Schiessl *et al.* 2015). It is shown in Arabidopsis that some underlying genes in flowering time pathway contribute to developmental process and frost tolerance. For instance, *FLOWERING LOCUS C* (*FLC*) is a key gene in the onset of flowering time that is down regulated by vernalization in winter crops. However, it is shown that *FLC* binds to 505 DNA sites, mostly located in the promoter regions of genes, *FLC* targets are involved in developmental pathways throughout the life history of the plant, many of which are associated with reproductive development (Deng *et al.* 2011). Flowering-time gene *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*) negatively regulates the expression of the cold induced genes *CBF*s in *Arabidopsis thaliana*. As a results, overexpression of cold-inducible *CBFs* caused late flowering through increased expression of *FLC*, an upstream negative regulator of *SOC1*. In *B. napus* correlation between floral related genes and the economic traits oil content and grain yield are questioned in natural or bi parental populations. It was suggested that selection of genes involved in post-transcriptional and epigenetic regulation of flowering time may play a potential role in adaptation of *B. napus* to highly divergent environments (Schiessl *et al.* 2015). However, due to complex network of flowering time function, gene effects are not stable over different environments and grain yield might not be increased with regard to flowering genes (Schiessl *et al.* 2015).

Correlation between flowering time and oil content in oilseed rape is contradictory and it varies greatly between populations. In a RIL population of *B. napus*, negative correlation was found (r= -0.32) between oil content and days to flowering (Chen *et al.* 2010). In contrast, Javed *et al.* (2016) reported a positive phenotypic correlation($r=0.30^{**}$). The magnitude of the correlation was supported by collocation of two late flowering time QTL with QTL for oil, linoleic acid and linolenic acid content. In the present climate conditions with thermal fluctuations and warm winters, flowering time genes and especially vernalization related genes are gaining more importance, because winter oilseed rape has to be able to re-establish frost tolerance if freezing temperature follows after spring-like temperatures during winter. This situation has been observed in Northern Europe. For example, in 2012 following a rather normal winter, extreme

low temperatures of up to −25 °C occurred in February in North Western Germany (https://www.wunderground.com, site visited March 3, 2016). Since at that time crops were not covered by snow, this caused severe frost damage. Also in January 2016 after two weeks above 10 °C in Dec 2014, suddenly temperature dropped to -14 °C in January that damaged plants with enhanced shoot length (own personal observation). To date, little is known about the genetic basis of shoot elongation before winter and its relation to vernalization requirement, bolting and beginning of flowering in spring. Therefore, a DH population constructed from the two winter oilseed rape cultivars, Sansibar and Oase, was used in this study to investigate the inheritance of shoot elongation before winter and its relation to vernalization requirement, flowering time and seed quality traits. It is anticipated that by the use of the DHSO population whose parents differed in shoot length before winter, QTL mapping could detect quantitative traits loci that are involved in genetic regulation of shoot elongation before winter as a relevant trait for winter hardiness.

5.3 Materials and Methods

5.3.1 Plant material

A doubled haploid bi-parental population consisting of 226 inbred lines, derived from a cross between the two winter oilseed rape cultivars Sansibar and Oase. The population was developed from F1-microspore culture technic in the Division of Plant Breeding at Georg-August-University Göttingen and was named DHSO population (Doubled Haploid of Sansibar and Oase; Teh and Möllers 2016). Both parents were characterized as "double low" quality in Europe and "canola" in Canada, meaning that they have less than 2% erucic acid in the oil and less than 25 $\mu molg^{-1}$ glucosinolates in the seed. The two parents differed for shoot elongation before winter in which Sansibar is shorter shoot length before winter and Oase is longer shoot length before winter (See Figure 3.1.1 in Chapter 3).

5.3.2 Field sown and greenhouse experiments

The DHSO population and the parental lines were evaluated in field and greenhouse experiments. Field experiments were performed at two different seasons. Those included the normal sowing time at end of August/beginning of September and spring sowing time at end of March/beginning April. Since the greenhouse, autumn and spring sown experiments represented very different environments; they were called mega environments.

5.3.2.1 Autumn sown environment

The DHSO population and its parental inbred lines were sown at four locations in North Western Germany during growing seasons 2012/13 and 2015/16. In 2012/13 the locations were Einbeck (KWS Saat SE), sown on 1 September and Göttingen-Reinshof, sown on 3 September. In 2015/16 the locations were Peine (Limagrain GmbH), sown on 20 August, and Göttingen-Reinshof, sown on 28 August. Hundred seeds from each line were sown in small field-plots with

double rows in Peine and Göttingen with 2 m length, 0.5 m space between plots and plant-to-plant distance was 10cm. In Einbeck seeds were sown as one row with 3 m length and 8 cm space where plant-to-plant distance was 6 cm. Three to four months after sowing, five representative plants were selected and harvested by cutting the stem below the root neck (crown). Harvesting time in 2012/13 for Einbeck and Göttingen were 5 and 8 January 2013, respectively and in 2015/16 for Peine and Göttingen were 30 November 2015 and 11 January 2016, respectively. Shoot length from root neck to apex and shoot diameter at the root neck were measured using a slide gauge and a metering rule and they were called shoot length and shoot diameter before winter. Additionally, mean data for begin of flowering, end of flowering, duration of flowering and plant height at end of flowering were kindly provided by Lishia Teh (Teh and Möllers 2016) for field experiments performed in 7 environments from 2011 and 2012.

5.3.2.2 Spring sown environment

The DHSO population and its parental inbred lines were sown at four spring sown locations in North Western Germany. Three locations were Göttingen-Reinshof sown on 19 April 2013, 30 April 2013 and 20 March 2014. The fourth experiment was in Einbeck (KWS Saat AG), sown on 4 April 2014. All plants were grown by sowing 100 seeds in 2 m long double rows and 50 cm space between plots and 10 cm between plants in the same row in Göttingen and 3 m single row and 80 cm space between plots and plant to plant distance was 6 cm in Einbeck. Three to four months after sowing (Göttingen 18.07.2013, 7/8.08.2013, 02/03.07.2014; Einbeck 25/26.06.2014), seven representative plants were selected and harvested by cutting the stem below the root neck (crown). Shoot length from root neck to apex and shoot diameter, at the root neck, were measured using metering rule and slide gauge. Tendency to form inflorescence was scored as 100% for visible buds or flower and 0% for lack of buds for each of the seven plants.

5.3.2.3 Greenhouse environment

The DHSO population and its parental lines were sown in 96-multipot trays, in compost soil. Four seeds, with one seed in each slot, were sown for each genotype. Three independent experiments were performed in non vernalization treatment. DH lines were sown on 5 October,

18 October and 6 November 2012 and were kept constantly in the greenhouse at around 20 °C without vernalization treatment. The three experiments were respectively harvested on 18 January, 30 January and 11 February 2013 and four plants *per* genotype were harvested by cutting the stem below the root neck (crown). Shoot length from root neck to apex and shoot diameter at the root neck were measured using metering rule and slide gauge.

5.3.3 Seed quality traits

Data for six quality seed traits including oil content, protein of the defatted meal, oleic acid, linoleic acid and linoleic acid was kindly provided by Lishia Teh (Teh and Möllers 2016) to calculate Spearman's rank correlation between the seed quality traits and the studied traits in three mega environments.

5.3.4 Linkage map

Linkage map was constructed by Teh and Möllers (2016) using MAPMAKER/EXP 3.0 (Lincoln *et al.* 1992). The linkage map for the DHSO population has 1642 markers mapped to 23 linkage groups and covered 2350.2 cM of linkage map with a mean interval distance of 2.0 cM between markers (Table 5.1). The 23 linkage groups represented 19 chromosomes from A01 to C09 in *B napus*, additional four linkage groups (A08-II, C02-II, C0-II, and C04-II) were formed due to loose or no linkage to their main linkage groups. The map has an average density of 0.70 marker *per* cM with distribution of markers varied from 0.20 to 1.37 marker/cM across the linkage groups. The A genome comprised more markers (987) as compared to the C genome (655), with a mean interval distance between markers of 1.6 cM in the A genome and 2.4 cM in the C genome. The number of markers mapped in an individual linkage group ranged from 7 (A08-II) to 164 (C07). Also pairwise recombination and LOD scores indicted that markers were well allocated to linkage groups based on LOD and recombination frequency (Figure 5.1). For QTL mapping, a subset of 317 markers were manually selected from the high-fidelity markers with an average density about 1 marker *per* 7.4 cM. (Figure 5.2 and Figure 5.3). The term framework map was used to refer to the map used for QTL mapping.

Table 5.1: Marker distribution, size and marker density between markers of each linkage group in the linkage map of DHSO population. (data taken from Teh and Möllers 2016)

Linkage group	No. of markers per linkage group								Size (cM)	Marker Density (cM^{-1})
	AFLP	CG[a]	DART	KASP	Silico-DART	SNP	SSR	Total		
A01	2		6	1	58	4	4	75	98	0.77
A02	3		5		26	3		37	40.7	0.91
A03	7		19	3	107	15	1	152	224.1	0.68
A04	2		14		84	5	2	107	194.2	0.55
A05	2		7	2	63	5	3	82	163.8	0.5
A06	4		18	2	95	5		124	128	0.97
A07	1	3	9	2	134	15		164	133.7	1.23
A08	2		9	1	31	2		45	37.8	1.19
A08-II			1		6			7	9.7	0.72
A09	3		8	2	49	2	1	65	130.2	0.5
A10	8		11		107	3		129	94..1	1.37
C01			4		24	4	2	34	77.5	0.44
C02			1	1	11	1		14	48.2	0.29
C02-II	1		1		16	1	1	20	97.9	0.2
C03	2		5	2	72	3		84	111.3	0.75
C03-II	3		5	1	45	6	1	61	134.8	0.45
C04			1		19	2	2	24	79.5	0.3
C04-II	5		3	4	75	6		93	101	0.92
C05	2		1	1	45	4		53	92.1	0.58
C06	2		5	1	49	7		64	95.2	0.67
C07	8		8	2	91	7	5	121	142.4	0.85
C08	4				27	2		33	52.5	0.63
C09	1	1	3		44	4	1	54	63.5	0.85
A genome	34	3	107	13	760	59	11	987	1254.3	0.79
C Genome	28	1	37	12	518	47	12	655	1095.9	0.60
Whole Genome	62	4	144	25	1278	106	23	1642	2350.2	0.7

[a]: Candidate Gene-based markers for phytosterol biosynthesis.

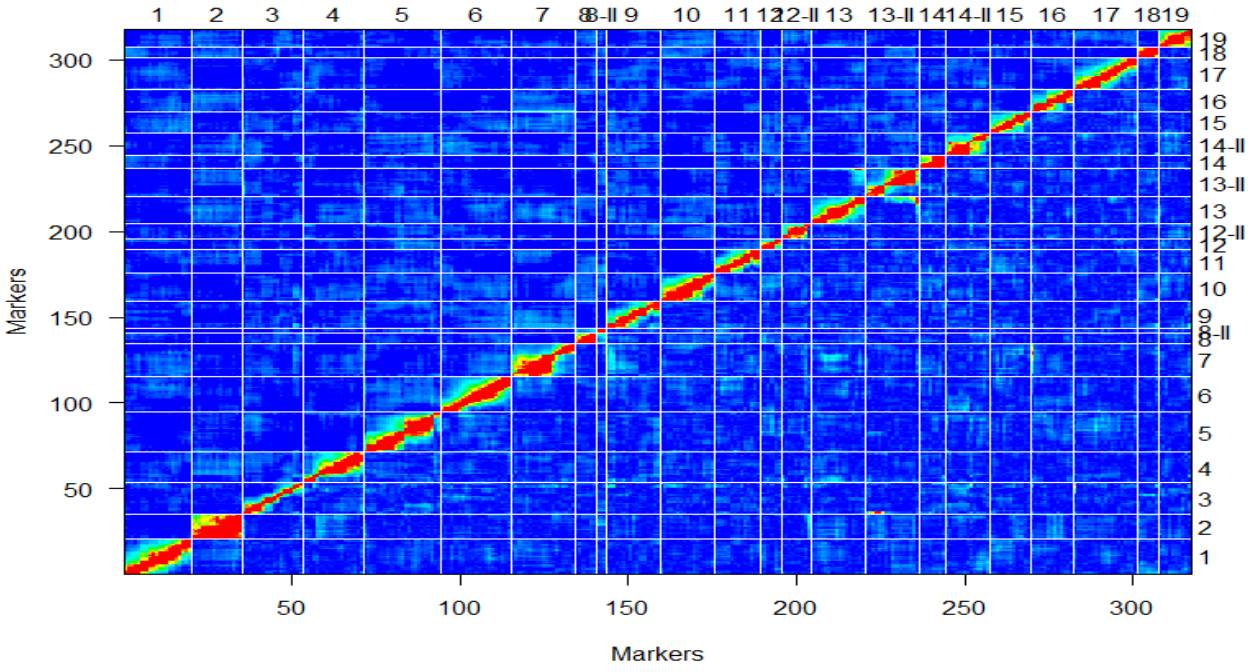

Figure 5.1: Estimated recombination fractions (upper left) and LOD scores (Lower right) for all pairs of markers in the DHSO population. Dark red indicates pairs of markers that appear to be tightly linked (very low recombination), dark blue indicates pairs that are not completely linked (very high recombination). Green points (combination of red and blue points) indicated marker's pairs ranging from very high recombination to very low recombination.

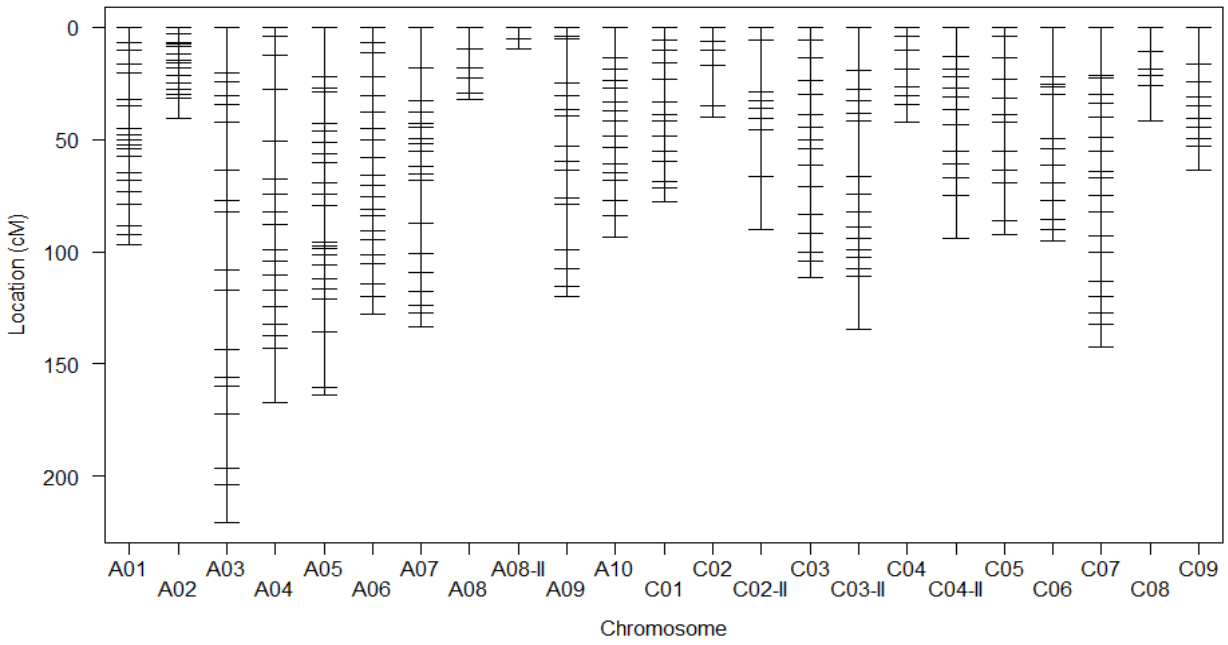

Figure 5.2: Genetic framework map of the DHSO population, with average density 1 marker *per* 7.4 cM

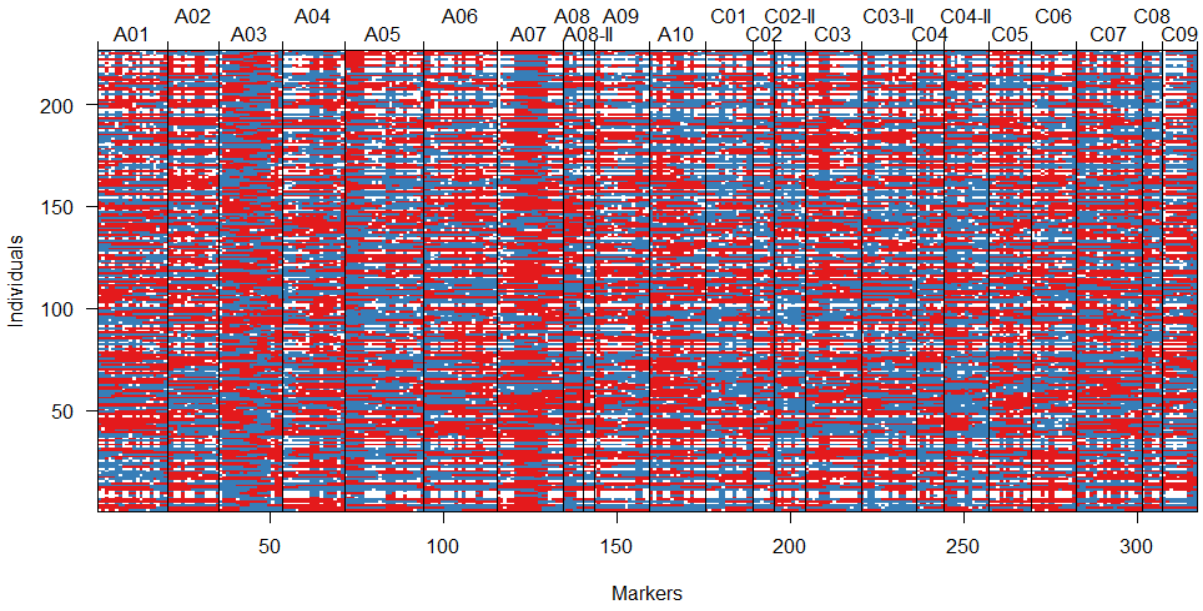

Figure 5.3: Genotype data for the DHSO population. Red and blue pixels correspond to alleles coming from Sansibar and Oase, respectively. White pixels indicate missing genotype data.

5.3.5 Statistical analysis

Analysis of variance, descriptive statistic, frequency distribution and Spearman's rank correlation analysis were performed for the studied traits in the DHSO population as described for the DHLE population in section 4.3.5 in Chapter 4.

5.3.6 QTL mapping and Identification of candidate genes

QTL mapping and identification of candidate genes in the vicinity of major QTL for the some traits of interest were performed as described in section 4.3.6 in Chapter 4.

5.4 Results

5.4.1 Phenotypic analysis

Analysis of variance represented highly significant genotypic effects for all traits recorded in the three mega environments: autumn sown, spring sown and greenhouse (Table 5.2.1). Furthermore, the variance components of the experiment (E) and interaction between genotype and experiment (G x E) were significant and larger than genotypic variance for some traits in the three environment. Estimation of broad sense heritability classified traits in two groups. First group consisted of traits with high heritability ranging from 70 to 91% including plant height at end of flowering, begin and duration of flowering in the autumn sown environment, shoot length in the spring sown and greenhouse environment. Second group consisted of traits with weak to medium heritability ranging from 18 to 62% including shoot length, shoot diameter and end of flowering in the autumn sown experiments, shoot diameter in the spring sown and greenhouse environment.

Table 5.2.1: Variance components and heritability of the DHSO population in the three mega environments.

Environment	Trait	Variance components			Heritability (%)
		Genotype (G)	Experiment (E)	G x E	
Autumn sown	Shoot length[a]	14.7**	202.7**	65.65**	46
	Shoot diameter[a]	0.22**	0.067**	2.59**	18
	BOF	4.1**	259.4**	3.5*	86
	EOF	1.77**	93.9**	4.37**	62
	DOF	3.7**	4.4**	24.6**	78
	Plant height at EOF[a]	82.4**	162.9**	54.9*	86
Spring sown	Shoot length[a]	898.1**	257.8*	355.1*	90
	Shoot diameter[a]	2.68**	12.4**	10.9**	43
	Buds[b]	0.11**	0.07*	0.06	87
Greenhouse 0 week vernalization	Shoot length[a]	171.8**	62.1**	191.2**	69
	Shoot diameter[a]	0.08**	0.07**	0.20**	43

*and** denote significance at P<0.05 and P<0.01, respectively.
[a] and [b] denote millimeter (mm) and percentage (%), respectively.
BOF: begin of flowering (from firs of January)
EOF: end of flowering (from firs of January)
DOF: duration of flowering

In the autumn sown environment, parental differences for shoot length before winter, begin of flowering and end of flowering were significant and more pronounced than other traits (Table 5.3.1). Oase showed significant higher values for shoot length before winter and begin and end of flowering were almost four days earlier. Large phenotypic variation with normal or near-normal distributions were found for shoot length before winter, shoot diameter before winter, begin, end and duration of flowering and plant height at end of flowering (Figure 5.4.1). Furthermore, transgressive segregation was observed for all the traits with extreme values at both ends of the distributions exceeding the mean values of both parents.

Table 5.3.1: Descriptive statistics of the parents and the DHSO population in the autumn sown environment

Environment	Trait	Parents		Doubled haploid population (n=226)				
		Sansibar	Oase					
		Mean		Min	Max	Mean	*F*-value	LSD 5%
Autumn sown	Shoot length[a]	29	40	9	58	31	1.9**	9.8
	Shoot diameter[a]	10	11	8	14	11	1.2**	2.9
	BOF (days)	128.4	124.4	120	130.6	125.4	7**	2.3
	EOF(days)	141	137.5	135.8	144.8	141	2.6**	2.9
	DOF(days)	26	26.5	25	34.5	29	4.4*	3
	Plant height at EOF[a]	1375	1425	950	1550	1337	7**	100

* and ** denote significance at P<0.05 and P<0.01, respectively.
[a] and [b] denote millimeter (mm) and percentage (%), respectively.
LSD5%: least significant difference at level of P< 0.05
BOF: begin of flowering (from first of January)
EOF: end of flowering (from firs of January)
DOF: duration of flowering

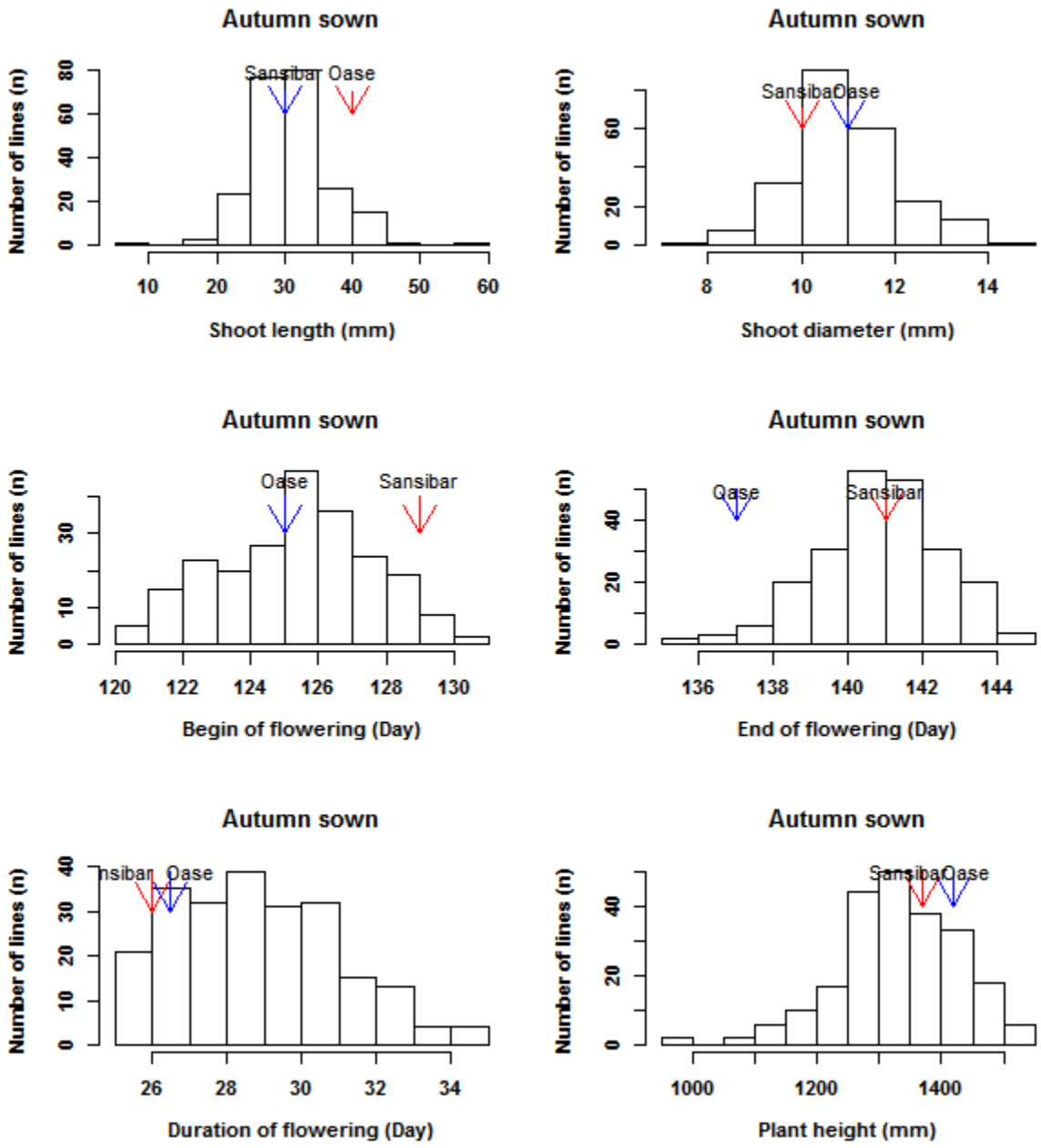

Figure 5.4.1: Frequency distribution of shoot length, shoot diameter, begin of flowering, end of flowering, duration of flowering and plant height at end of flowering in the autumn sown environment in the DHSO population. Parental mean values are indicated by arrows

In the spring sown environment, large phenotypic variation was observed for shoot length which ranged from 209 mm to 1238 mm, resulting in a bimodal distribution (Table 5.3.2 and Figure 5.4.2).The bimodal frequency distribution suggested involvement of one major gene in inheritance of this trait. Parent Sansibar was with 721 mm shoot length significantly longer than Oase with 442 mm shoot length. Visible buds was second trait with the bimodal distribution and the number of DH lines with 100% visible buds were almost 4 times as many as number of DH lines with no visible buds, representing low vernalization requirement in majority DH lines. The two parents Sansibar and Oase showed almost the same values for buds percentage (Table 5.3.2). Comparison of frequency distributions between shoot length and visible buds indicated that shoot length in the spring sown environment was correlated to the appearance of buds and only 9% of the DH lines (25 lines out of 226), whose shoot length was less than 300 mm, didn't show any visible buds across the spring sown experiments.

In the greenhouse environment, in which genotypes were treated under non-vernalized conditions, normal frequency distribution was found for shoot length which ranged from 26 to 148 mm and ranged from 16 to 34 mm for shoot diameter (Figure 5.4.2). Parent Sansibar was shorter and thinner for shoot compared to Oase, however the difference was not significant (Table 5.3.2). Comparing the parental performance in the three mega environments showed that parent Oase was longer than Sansibar before winter and in the greenhouse, while it became shorter than Sansibar in spring. To find out the strange performance of parents the two parents were incubated under four and eight weeks vernalization conditions in the greenhouse environment. Results illustrated parent Oase was longer and had 3 days earlier begin of flowering under the eight weeks vernalization conditions, in contrast Sansibar was longer under four weeks vernalization treatment (data are not shown). The results confirmed that Sansibar has longer shoot length in the non-complete vernalization conditions .i.e., spring sown and greenhouse environment with four weeks vernalization treatment

Table 5.3.2: Descriptive statistics of the parents and the DHSO population (n=226) in spring and greenhouse

Environment	Trait	Parents		Doubled haploid population (N=226)				
		Sansibar	Oase					
		Mean		Min	Max	Mean	*F*-value	LSD 5%
Spring sown	Shoot length[a]	721	442	209	1238	763	10.5**	27.1
	Shoot diameter[a]	24	22	16	34	22	1.74**	5.3
	Buds[b]	82	75	0	100	65	7.9**	36
Greenhouse 0 week vernalization	Shoot length[a]	36	44	26	148	55	3.2**	24.6
	Shoot diameter[a]	4	4.6	2.2	5.8	4.5	1.8**	0.9

* and ** denote significance at P<0.05 and P<0.01, respectively.
[a] and [b] denote millimeter (mm) and percentage (%), respectively.
LSD5%: Least significant difference at level of P< 0.05

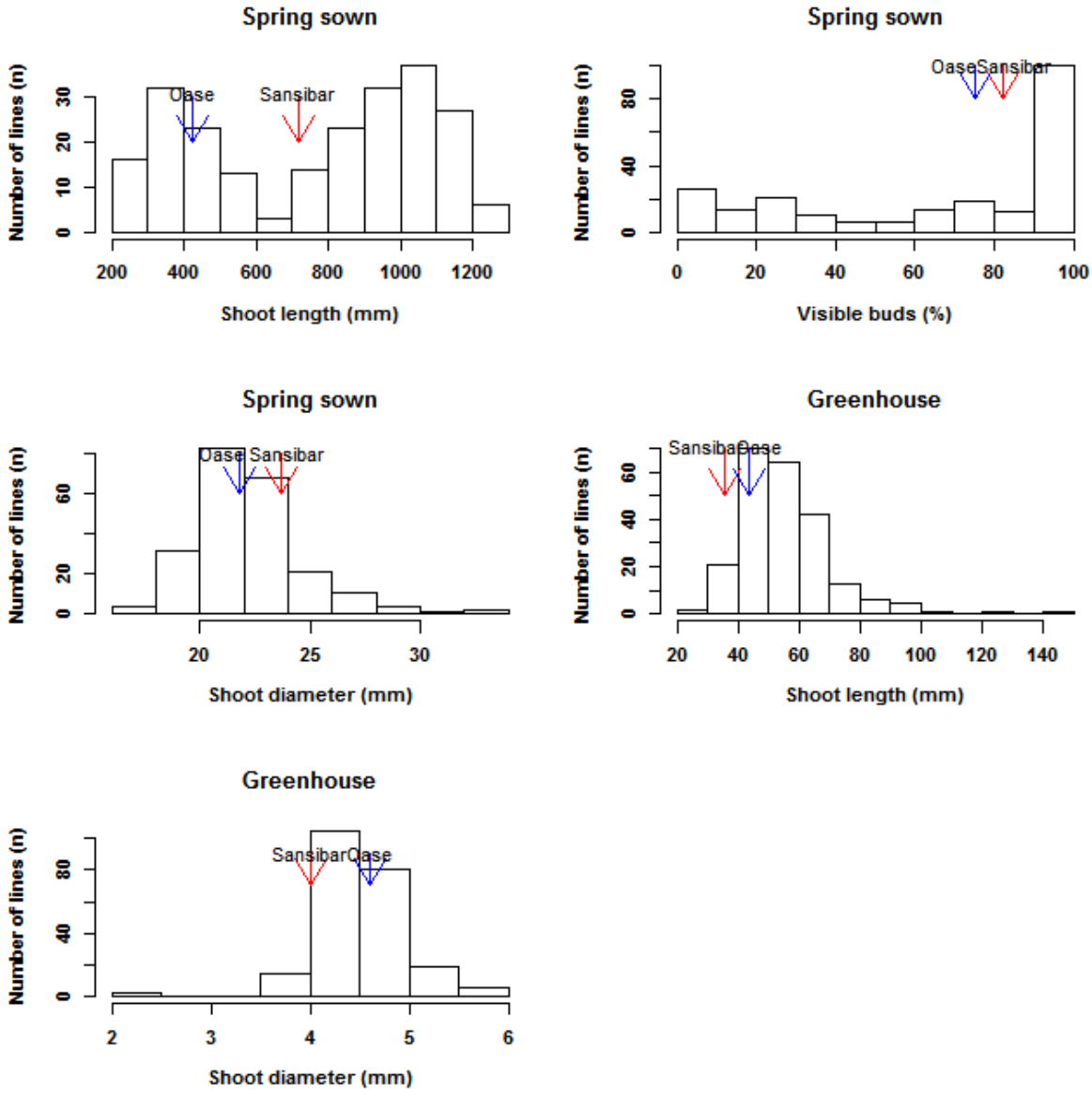

Figure 5.4.2: Frequency distribution of shoot length, visible buds and shoot diameter in the spring sown environment and shoot length, shoot diameter in the greenhouse environment in the DHSO population. Parental mean values are indicated by arrows

5.4.2 Correlation Analysis

Correlations ranging from -0.07 to 0.90 were found for the studied traits in three different environments (Table 5.4.1). Shoot length before winter was positively correlated with shoot length in the spring sown (r_s=0.31**) and greenhouse environment (r_s=0.32**). Shoot length in the spring sown environment was positively correlated with the presence of visible buds (r_s=0.90**), duration of flowering in autumn sown (r_s=0.46**) and shoot length of non-vernalized plants in the greenhouse environment (r_s=0.42**). Begin of flowering was negatively correlated with shoot length before winter (r_s=-0.29**) and shoot length in the spring sown experiments (r_s=-0.52**) but had positive correlation with plant height at end of flowering (r_s=0.62**). Highly significant correlations were found among flowering time traits in which begin of flowering was highly positive correlated with end of flowering (r_s=0.93**) and negatively with duration of flowering (r_s=-0.91**).

Table 5.4.1: Spearman's rank correlation of the studied traits in the DHSO population (n=226)

Environment	Trait	Autumn sown						Spring sown			Greenhouse
		SL	SD	BOF	EOF	DOF	PH	SL	SD	Buds	SL
Autumn sown											
	SD	0.13	1								
	BOF	-0.29**	-0.1	1							
	EOF	-0.19	-0.07	0.93**	1						
	DOF	0.31**	0.16	-0.91**	-0.74**	1					
	PH	-0.08	-0.05	0.62**	0.50**	0.60**	1				
Spring sown	SL	0.31**	0.1	-0.52**	-0.51**	0.46**	-0.25*	1			
	SD	-0.2	0.26*	0.08	0.15	-0.04	0.12	-0.2	1		
	Buds	0.26*	0.09	-0.51**	-0.50**	0.5**	-0.3*	0.9**	0.09	1	
Greenhouse	SL	0.32**	0.12	-0.17	-0.12	0.23*	-0.2	0.42**	-0.2	0.38**	1
0 week vernalization	SD	0.1	-0.03	-0.16	-0.16	0.11	-0.1	0.05	0.06	0.03	-0.3**

*and** denote significance at P<0.05 and P<0.01, respectively.
SL: shoot length
BOF: begin of flowering (from first of January)
EOF: end of flowering (from first of January)
SD: shoot diameter
DOF: duration of flowering
PH: plant height at end of flowering

The studied traits in three different mega environments were either not or only moderately correlated with seed quality traits (Table 5.4.2), suggesting no or little share of a regulatory pathways in the DHSO population. Nevertheless some correlation might be notable such as correlation between plant height and oil content (r_s=0.45**) and correlation between duration of flowering and thousand kernel weight (r_s=0.43**). Correlation between seed quality traits *per se* are well shown and discussed by Teh and Möllers (2016).

Table 5.4.2: Spearman's rank correlation of seed quality traits in the DHSO population (n=226)

Trait	Autumn sown						Spring sown			Greenhouse 0 week vernalization	
	SL	SD	BOF	EOF	DOF	PH	SL	SD	Buds	SL	SD
Oil	0.07	-0.28*	0.18	0.07	-0.26*	0.45**	-0.2	0.02	-0.23	-0.12	-0.03
PodM	0.07	-0.02	-0.10	-0.06	0.16	0.07	0.2	-0.06	0.18	0.27*	-0.05
C18:1	-0.03	-0.07	0.13	0.14	-0.13	-0.11	-0.16	0.18	-0.15	0.08	-0.1
C18:2	-0.01	0.17	-0.14	-0.15	0.14	0.01	0.11	-0.14	0.15	-0.02	0.09
C18:3	0.12	-0.06	-0.08	-0.11	0.08	-0.2	0.18	-0.18	0.14	-0.08	0.15
TKW	0.01	0.19	-0.3**	-0.18	0.43**	-0.23*	0.42**	-0.1	-0.36**	0.27*	-0.05

*and** denote significance at P<0.05 and P<0.01, respectively.
PodM: protein of the defatted meal
C18:1: oleic acid
C18:2: linoleic acid
C18:3: linolenic acid
TKW: Thousand kernel weight

SL: shoot length
SD: shoot diameter
BOF: begin of flowering (from first of January)
EOF: end of flowering (from first of January)
DOF: duration of flowering
PH: plant height at end of flowering

5.4.3 QTL Mapping

To assess the utility of the DHSO population and linkage map for the genetic analysis of the traits, multiple interval mapping was performed from genotype's mean over the replicated experiments in each mega environment. In total 38 main QTL were detected for 11 traits in the three environments. (Table 5.5.1 and Table 5.5.2). The QTL were distributed over 18 linkage groups as shown in Figure 5.2. Collocation of QTL for different traits was observed more frequently than individual isolated QTL.

5.4.3.1 Autumn sown environment

In the autumn sown environment, 24 main QTL were mapped for shoot length, shoot diameter, begin, end and duration of flowering and plant height at EOD in the DHSO population (Table 5.5.1). Five main QTL identified for shoot length before winter were localized on linkage groups A02, A03, A05, A06 and C05 explaining collectively 23.5% of the observed variance. QTL *Wi-Len-5* at position 4.1 cM on C05 was the biggest QTL with alleles derived from Oase, contributing 9.4% to the shoot length variance. For shoot diameter, only one QTL with additive effect of 0.38 mm and coefficient of determination of 11.1% was found on linkage group A07. QTL mapping for begin of flowering identified one major QTL at position 27.5 cM on C06 which along with 5 further QTL were distributed on linkage groups A02, A05, A10, C05, C06, and C09, accounting for 71.6% of the phenotypic variance. Around 65% of the phenotypic variance for end of flowering was explained by five mapped QTL on linkage groups A02, A03, A10, C06 and C09 with additive effects ranging from 0.3 to 1.04 days. Three and four QTL identified for duration of flowering and plant height at end of flowering were distributed on linkage groups A02, C04, C05, C06 and C09, explaining collectively 54.1 and 34% of the phenotypic variance. The two QTL mapped to A02 and C06 were identical between plant height and flowering traits with the biggest additive effect for the respective traits.

Table 5.5.1: QTL mapped for the studied traits in the autumn sown environment in the DHSO population.

Trait	QTL name	Linkage group	Position (cM)	CI[a] (cM)	LOD	Additive effect[b]	R^{2c} (%)	TR^{2d} (%)
Shoot length[d]	Wi-Len-1	A02	18	15-25	2.9	1.3	7	23.5
	Wi-Len-2	A03	180	176-199	2.8	1.1	3.1	
	Wi-Len-3	A05	46	42-51	2.9	-1.24	4	
	Wi-Len-4	A06	60	58-68	2.9	-1.15	3	
	Wi-Len-5	C05	4.1	0-13	3.2	-1.4	9.4	
Shoot diameter[d]	Wi-Dim-1	A07	127	124-132	5.6	0.38	11.1	11.1
Begin of flowering[c]	Wi-BFlw-1	A02	21	17-23	8	-0.8	12.7	71.6
	Wi-BFlw-2	A05	126.1	121-135	4.3	-0.4	5.4	
	Wi-BFlw-3	A10	60.7	47-65	3.9	0.4	3.7	
	Wi-BFlw-4	C05	13.7	6-19	2.8	0.28	1.8	
	Wi-BFlw-5	C06	27.5	22-30	28.7	1.5	46.9	
	Wi-BFlw-6	C09	51.7	45-59	3.2	0.3	2.2	
End of flowering[c]	Wi-EFlw-1	A02	17.3	17-22	13.5	-0.64	13	64.4
	Wi-BFlw-2	A03	88	68-104	3	0.3	3.1	
	Wi-EFlw-3	A10	53.5	48-60	6.6	0.43	6.3	
	Wi-EFlw-4	C06	28.5	20-30	30.3	1.04	37.6	
	Wi-EFlw-5	C09	49.7	47-52	3.6	0.31	3.3	
Duration of flowering[c]	Wi-DFlw-1	A02	16.9	15-19	11.26	1.4	13.2	54.1
	Wi-DFlw-2	C05	13.7	5.6-22	3.22	-0.3	2.9	
	Wi-DFlw-3	C06	28.5	26.7-32	32.5	-1.4	38	
Plant height[d] at EOD	Wi-Het-1	A02	21.3	15-24	4.8	-265	7.1	34.1
	Wi-Het-2	C04	19	17-23	2.8	-183	3	
	Wi-Het-3	C06	27.5	16-32	11.4	458	20.6	
	Wi-Het-4	C09	27.2	15-30	2.9	178	3.4	

[a] 1-LOD Confidence interval
[b] {+} and {-} indicate that the trait value is increased by the allele Sansibar and Oase, respectively.
[c] and [d] denote days and millimeter for additive effects, respectively.
EOD: end of flowering
[c] R^2 is the percentage of phenotypic variance explained by each QTL
[d] TR^2 is the percentage of phenotypic variance explained by all QTL

5.4.3.2 Spring sown environment

In the spring sown environment, 10 main QTL were mapped for shoot length, visible buds and shoot diameter in the DHSO population (Table 5.5.2). For shoot length *Sp-Len-1* on A02 with R^2=70% along with two minor QTL on A10 and C07 explained 75.7% of the phenotypic variance which is also reflected by the large and the bimodal frequency distribution for shoot length in the spring sown (Figure 5.4.2). Positive additive effect of *Sp-Len-1*, indicated that alleles coming from Sansibar had a notable increasing effect of 256 mm for shoot length which was in compliance with the descriptive statistics of the parents, showing longer plants for Sansibar compared to Oase in the spring sown environment (Table 5.3.1). QTL mapping for shoot diameter revealed three QTL that explained 24.5% of the phenotypic variance. Negative effect of *Sp-Dim-1* mapped on A02 decreased shoot diameter, while alleles coming from Sansibar at position 74.3 cM on A05 and at position 127.2 cM on A07 increased shoot diameter in the spring sown environment. For the appearance of visible buds a major QTL with additive effect of 27% and coefficient of determination of 70% was localized on A02 that along with QTL mapped on A10, C06 and C09 together explained 76.1% of the phenotypic variance.

5.4.3.3 Greenhouse environment

Three main QTL for shoot length and a QTL for shoot diameter in non-vernalized plants were mapped to different linkage groups (Table 5.5.2). Three QTL for shoot length were found on A02, A06 and A07 with additive effects ranging from -5 to 5.23 mm which together explained 26% of the phenotypic variance. In total, additive effects of alleles derived from Oase increased shoot length at two positions 61.2 cM, on A06, and 77 cM on A07, whereas Sansibar allele increased shoot length in Gh-Len-N1 that might corresponds to the longer plants of Oase compared to Sansibar in the greenhouse experiment (Table 5.3.2).

Table 5.5.2: QTL mapped for the studied traits in the spring sown and greenhouse environment in the DHSO population.

Environment	Trait	QTL name	LG	Position (cM)	CI[a] (cM)	LOD	Additive effect[b]	R^{2c} (%)	TR^{2d} (%)
Spring sown	Shoot length[d]	Sp-Len-1	A02	16.9	15-17	16.9	256	70	75.7
		Sp-Len-2	A10	59.7	53-64	3.26	-48.1	3.1	
		Sp-Len-3	C07	93	85-96	3	34.7	2.5	
	Shoot diameter[d]	Sp-Dim-1	A02	15	8.7-17	3	-0.5	5.1	24.5
		Sp-Dim-2	A05	74.3	66-79	3.6	0.6	5.5	
		Sp-Dim-3	A07	127.2	122-130	8.2	0.2	14	
	Buds[c]	Sp-Bud-1	A02	16.9	16-18	18	27	70	76.1
		Sp-Bud-2	A10	82	77-91	2.8	-0.5	2.2	
		Sp-Bud-3	C06	29	23-37	3.4	-0.5	2.1	
		Sp-Bud-4	C09	48	42-52	2.8	-0.4	1.2	
Greenhouse 0 week vernalization	Shoot length[d]	Gh-Len-N1	A02	18.3	17-20	7	5.23	11.2	26
		Gh-Len-N2	A06	61.2	55-64	2.9	-3.55	4.8	
		Gh-Len-N3	A07	77	65-90	5	-5	10	
	Shoot diameter[d]	Gh-Dim-N1	A10	37	30-39	5.2	-0.07	2.4	2.4

[a] 1-LOD Confidence interval
[b] {+} and {-} indicate that the trait value is increased by the allele Sansibar and Oase, respectively.
[c] and [d] denote percentage and millimeter for additive effects, respectively.
[c] R^2 is the percentage of phenotypic variance explained by each QTL
[d] TR^2 is the percentage of phenotypic variance explained by all QTL

5.4.4 Identification of candidate genes

Overlapping of QTL confidence intervals were observed for the studied traits within and between the three mega environments (Table 5.5.1 and Table 5.5.2). A genomic region from 14.6 to 23.6 cM was found on A02 with collocation of QTL for shoot length before winter ,shoot length in the spring and greenhouse environment, second biggest QTL for begin, end and duration of flowering and plant height in the autumn sown environment (Figure 5.5). The sequence of the two flanking Silico DArT markers linked at the position 14.6cM (3091433|F|0) and 23.6 cM (3080289|F| 0) were blasted against complete genome sequence of *B. napus* (http://www.brassicadb.org/brad/) resulting in the roughly 50 kbp interval between physical position of two flanking markers. Searching for flowering genes within the mentioned interval detected the candidate gene *BnFLC2* (*BnaA02g00370D*) in the interval 15.3 to 15.9 cM on A02. In addition, additive effect of the DArT marker 3110832|F|0 linked to *BnFLC2* at position 15.9 cM showed that allele derived from Sansibar increased dramatically shoot length in the spring sown environment (Figure 5.6). Apart from A02, the overlapping QTL confidence intervals were observed on C06 between QTL *Sp-Bud-3* and the biggest corresponding QTL for begin, end, duration of flowering and plant height in the autumn sown environment (Figure 5.7). Physical positions of two flanking Silico DArT markers were used to search for relevant flowering time genes within the genomic region from 28542458 to 30304316bp (22.1 to 29.7 cM) on linkage group C06. The candidate gene *Bn.C6.FTb* (*BnaC06g27090D*) is a copy of *FT* gene found in the genetic interval 25.1 to 29.7 cM on linkage group C06 that might be the causal gene for respective traits. For shoot length in the greenhouse environment, QTL mapped to A02 and A06 had overlapping confidence intervals with QTL for shoot length before winter which corresponds to moderate correlation between them ($r_s=32^{**}$).

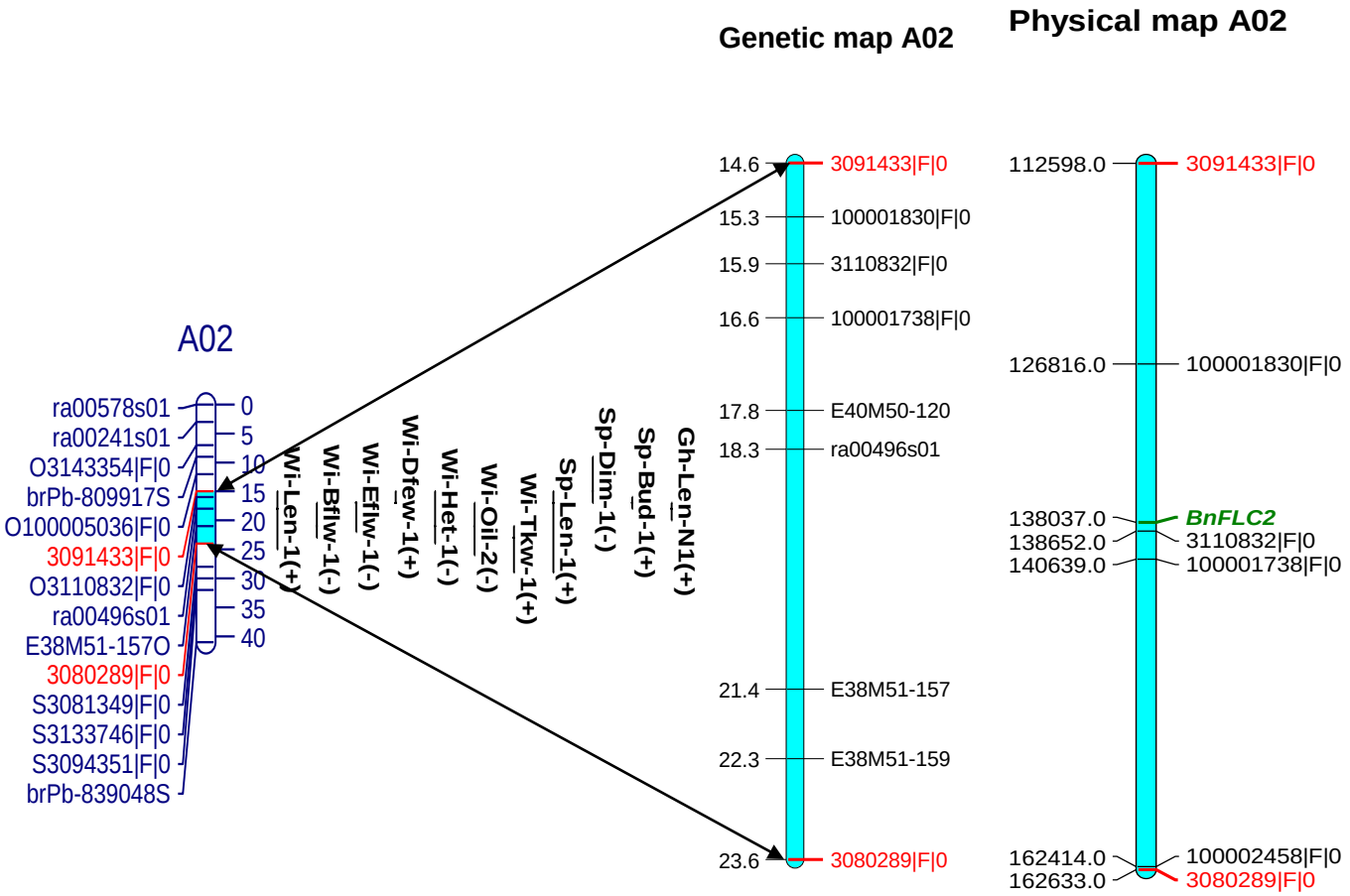

Figure 5.5: Genetic and physical map positions of markers within the QTL genomic region (14.6-23.6 cM) on A02. Left: QTL for shoot length, shoot diameter, begin of flowering, end of flowering, duration of flowering, plant height at end of flowering, percentage of visible buds, oil content and thousand kernel weight in the DHSO population. Middle: Additional markers mapped within the QTL genomic region in full map of the DHSO population Right: The corresponding physical positions of additional markers and the candidate gene (*BnFLC2*) in *B. napus* genome

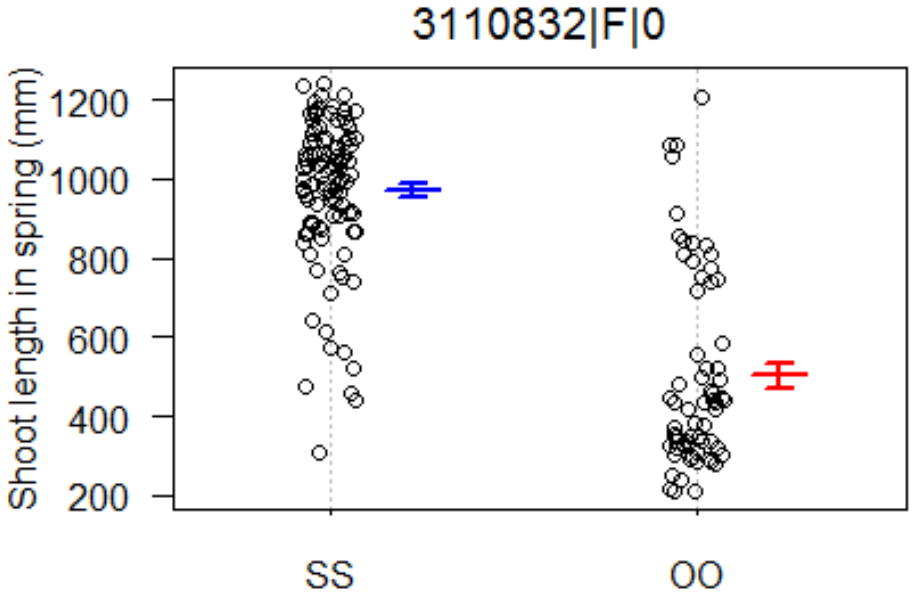

Figure 5.6: Additive effect of flanking marker 3110832|F|0 linked to the major QTL on A02 for the shoot length in the spring sown environment in the DHSO population. Red boxplot shows standard error and lower phenotypic mean and blue boxplot shows standard error and higher phenotypic mean for shoot length in the spring environment.

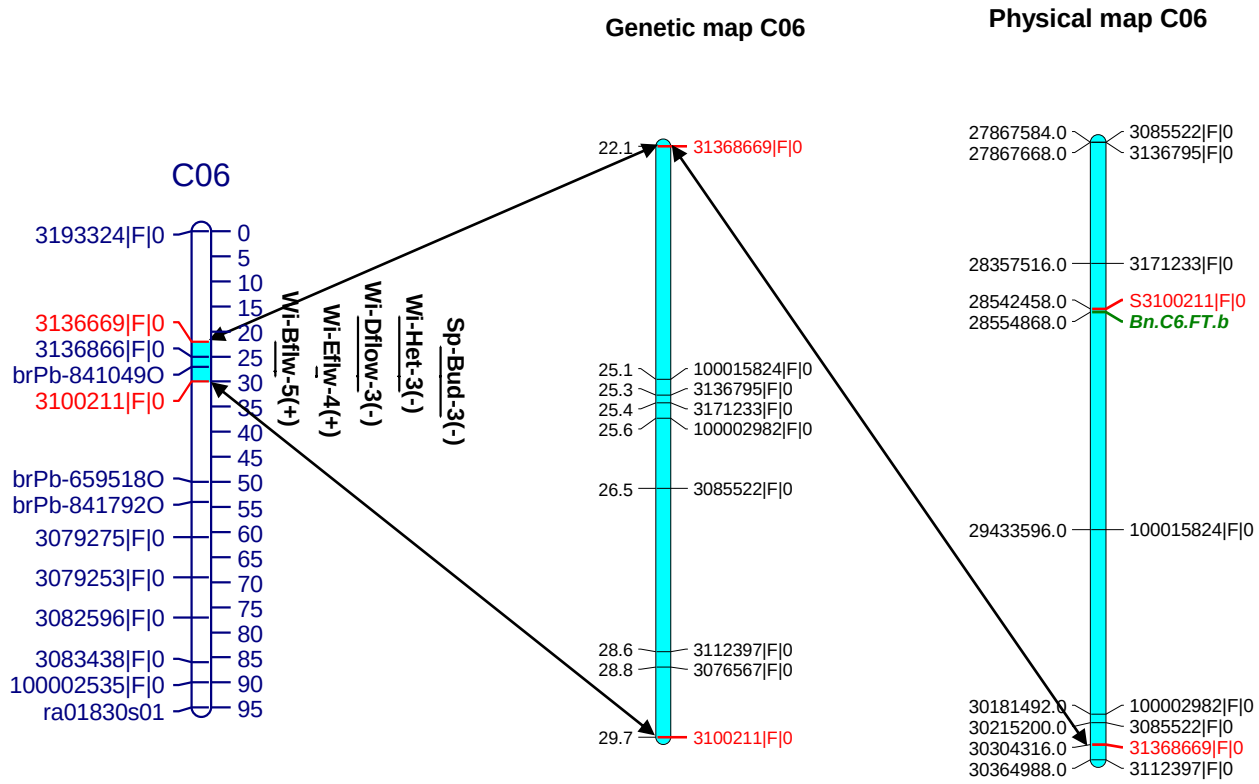

Figure 5.7: Genetic and physical map positions of markers within the QTL genomic region (22.1-29.7 cM) on C06. Left: QTL for begin of flowering, end of flowering, duration of flowering, plant height at end of flowering, percentage of visible buds in the DHSO population. Middle: Additional markers mapped within the QTL genomic region in full map of the DHSO population Right: The corresponding physical positions of additional markers and the candidate gene (*Bn.C6.FT.b*) in *B. napus* genome

5.5 Discussion

5.5.1 Phenotypic analysis

Successful plant overwintering of oilseed rape depends on plant preparation before winter in two aspects: (1) meteorological conditions for oilseed rape growth during autumn and (2) plant condition (development and chemical composition) in autumn (Balodis *et al.* 2015). The meteorological conditions are not predictable and they are changing from year to year and location to location. But, plant development before winter can be optimized by breeding to reduce risk of frost damage in regions with freezing temperatures during winter. In the present study the population mean for shoot length and shoot diameter before winter were 31 and 11 mm that was within the optimum range recommended for successful overwintering (Cramer 1990). However, extreme cold temperature (-22 °C) was experienced in February 2012 in Göttingen (https://www.wunderground.com) which may have caused some frost damage in some genotypes of the DHSO population (Christian Möllers, personal communication). In addition, in growing season 2015-16 following the two warm weeks in December, leading to bud formation and elongation of shoot in some genotypes (own personal observation) suddenly temperature dropped down -14 °C and it remained below -10 °C for a few days (https://www.wunderground.com). As a result, frost damage was observed in some genotypes in Göttingen and Peine especially for those with enhanced shoot length (data not shown). In alfalfa, it was stated that warm temperatures during cold season causes genotypes to resume growth too early and these genotypes are prone to frost damage if temperature drops again below zero (Hesterman and Durling 1991). In the present study, population mean for shoot length was bigger at two locations Göttingen and Peine in growing season 2015-16 (data are not shown) that might be the reason for frost damage at the two-mentioned location. High heritability for flowering time traits indicated the precision of field trials at four locations and high genotypic stability despite ambient changes. The genetic stability for the flowering time traits is consistently reported by other researchers (Long *et al*. 2007, Wang *et al.* 2009, Raman *et al.* 2013). Comparison of frequency distribution between shoot length and the percentage of visible

buds in the spring sown environment represented that only 9% of population didn't show any visible buds, while 65% of the population showed shoot length longer than 600 mm and more than 50% tendency to form inflorescence. Thus, although Sansibar and Oase are winter type cultivars, high tendency to form inflorescence in majority DH lines might be due to spring or semi winter alleles that exists in the parents' genome. The high tendency to form buds in spring sown environment raised up two hypothesis that either vernalization is partially met when night temperatures drop close to zero in March and April in Germany or vernalization competence does not necessarily rely on chilling treatment and just being under long days is enough to resume shoot growth in the 65% of DH lines. To get an answer, DHSO population was kept under non-vernalizing conditions in the greenhouse with 20 °C and 16 hours light. Results illustrated although large phenotypic variation was observed for shoot length ranging from 62 to 192 mm, no tendency to form inflorescences was found in the non-vernalization condition, rejecting the assumption that day length alone induces flowering in winter oilseed rape. Waalen *et al*. (2014) reported that long day conditions don't affect plants that are not fully vernalized. Furthermore it has been found in model crops that vernalization requirement is essential mechanism for vegetative/reproductive transition and promoting reproductive growth in the spring (Kim *et al.* 2009, Zografos and Sung 2012).

5.5.2 Correlation analysis

Rather low correlation (r_s=31**) was observed between shoot length before winter and vernalization response determined by shoot length and the percentage of visible buds in the spring sown environment, suggesting that only a certain share of genetic regulation exists between vernalization requirement and shoot length before winter in the DHSO population. The certain share can be seen in wheat, in which genes controlling the development and growth operate in three pathways: vernalization, photoperiod, and earliness *per se* (Chen et al. 2009). Common genetic pathway between flowering time and vernalization is expected by the negative correlation between shoot length in the spring sown and begin and end of flowering in the autumn sown environment (r_s=-0.52**). The genetic analysis of flowering time pathway showed that vernalization and photoperiod are main inputs inducing development of shoot apex and acceleration of flowering in winter crops (Jung and Müller 2009). Weak correlation was found

between shoot length before winter and shoot length in the greenhouse (r_s=0.32**) that means shoot length under natural (field) conditions is modulated by environmental cues that are likely to be absent or only partly relevant under controlled greenhouse conditions (El-Soda *et al.* 2014).

5.5.3 QTL Mapping

To date, no effort has been published regarding the QTL mapping for shoot length before winter in oilseed rape which marks the current study as first report of QTL mapped in association with shoot elongation before winter. We found five QTL that accounted for 23.5% of the phenotypic variance for this medium heritable trait. The data used for QTL mapping was mean of genotypes across four locations with 46% genetic variance (h^2) for shoot length before winter, therefore it can be declared that QTL are constitutive QTL with consistent effects across environments. Constitutive QTL are the main targets for breeding programs because such QTL can be used to improve crop performance in all regions where the crop can be grown (El-Soda *et al.* 2014).

The QTL *Wi-Len-1* on A02 overlapped with QTL for flowering time traits and a major QTL for shoot length in the spring sown environment. Positive additive effect of *Wi-Len-1* and *Sp-Len-1* indicated that allele coming from Sansibar yielded longer shoot length before winter and lower vernalization requirement. Candidate gene *BnFLC.A2* was identified in the vicinity of a QTL hotspot on A02 that might be the causal gene affecting the regulation of respective traits in the DHSO population. The MADS box transcription factor gene *FLOWERING LOCUS C* (*FLC*) is the key regulator of vernalization requirement and is down-regulated by vernalization, enabling promotion of flowering by *FT*. In *B. napus* nine homologues of *FLC* were identified to six of 19 chromosomes that were relatively conserved in the coding region (Zou *et al.* 2012). Javed *et al.* (2016) reported a QTL on A2 that was in the vicinity of a known Brassica vernalization gene *FLC* explaining 43.2% of the trait variation. Raman *et al.* (2013) found QTL associated with flowering time on A02, A03, A07, and C06 which may represent homologues of known flowering time genes in Arabidopsis.

Teh and Möllers (2016) reported two QTL (*DE-Oil.2* and *E-SW.1*) for oil content and thousand kernel weight (TKW) in the DHSO population that coincided with the QTL hotspot on A02 in our study. A positive correlation between oil content and flowering time was supported by

colocation of two late flowering time QTL with an oil content QTL in a DH population of *B. napus* (Javed *et al.* 2016). The candidate gene *BnFLC2* on A02 may influence oil profile and TKW because of importance of *FLC* in biochemical pathways. Deng *et al.* (2011) demonstrated that *FLC* binds to more than 500 target sites in the *Arabidopsis* genome, potentially regulating genes involved in vegetative and reproductive pathways throughout the life history of the plant, many of these genes are associated with plant development.

C06 was the second linkage group with a genomic region from 22.1 to 29.7 cM with the biggest QTL for begin and end of flowering in the autumn sown environment and a QTL for visible buds in the spring sown environment with opposite additive effects. No additional QTL for shoot length in the spring sown environment was found at this position. It can be speculated that candidate gene at this position is more related to flowering time and other ambient cues than vernalization. A copy of *FLOWERING LOCOUS T* (*BnC6.FT.b*) was identified within the hotspot on C06 in the DHSO population. Wang *et al.* (2009) reported two *FT* paralogues (*BnA2.FTa* and *BnC6.FT.b*) were associated with two major QTL clusters for flowering time in *B. napus*. *FLOWERING LOCUS T* (*FT*), along with *SUPPRESSOR of OVEREXPRESSION of CONSTANS* (*SOC1*) and *LEAFY* (*LFY*) are the major integrators of flowering time in *Arabidopsis thaliana* (Bäurle and Dean 2006). *FT* induces flowering in response to long day and is a direct target of the nuclear protein *CONTANS* (*CO*) in leaves (Li *et al*. 2008). It is well known in plants that *FT* is down regulated by *FLC* (Jung and Müller 2011), hence it can be speculated that two candidate genes reported on A02 (BnaA02g00370D*)* and C06 (*BnaC06g27090D*) are involved in flowering time pathways, in which vernalization is regulated by *FLC* on A02 and induces begin of flowering by *FT* on C06.

Overlapping QTL confidence intervals is an advantage when loci with desired alleles are linked, making breeding methods faster for QTL pyramiding but on the other hand, it is a threat when desired and undesired alleles are tightly linked. To conclude the chapter it should be pointed out that low vernalization requirement was observed in the DHSO population and in the parental lines. Furthermore, shoot elongation before winter is mainly regulated by independent genomic regions from flowering time and vernalization requirement and no strong correlation was found between vernalization and shoot elongation before winter.

Chapter 6

General discussion

Long term meteorology data show tendency of winter warming in the regions with cold temperatures during winter, as a consequence of global warming (Intergovernmental Panel on Climate Change IPCC 2007). Despite increasing temperature in the northern hemisphere, nothing changed for the extreme low temperatures that occasionally occur during winter (Imai *et al*. 2012), for instance, in 2012 after a normal winter in Germany, temperature dropped to -25 °C, causing frost damage on winter crops (Europe Mars Bulletins 2012). Therefore, breeding for improved winter hardiness is still an important breeding aim in the current winter crop genotypes. Yet, complexity of winter hardiness and the hardship of field test implementation has slown down breeding programs for this goal (Sãulescu and Braun 2001). Plant development before winter and especially shoot elongation before winter, as an important organ for accumulation of essential assimilates, is considered by canola breeders as a relevant trait for the successful overwintering (Schulz 2007). Unfortunately, we did not find any report regarding the genetic variation and genetic regulation of shoot elongation before winter in oilseed rape or other winter crops. It seems that the trait has been neglected by researchers for improved winter hardiness. Therefore, the present study is the first report of genetic variation and inheritance of shoot length before winter in *B. napus*. The two DH populations and a collection of 19 winter oilseed rape genotypes were tested in replicated field and greenhouse trials in three different environments: autumn and spring sown field experiments and greenhouse trials.

6.1 Phenotypic analysis

In the all plant materials, large phenotypic variation with significant genotypic effect was found for shoot length in the autumn sown and spring sown environment. Shoot length before winter ranged from 23 to 66 mm among 19 winter oilseed genotypes that was relatively the same as the phenotypic variation observed in the two DH populations. This large variation among a small sample of winter oilseed genotypes shows large existing diversity in the genepool of *B. napus*.

Segregating populations developed from the oilseed rape material are able to broaden the genetic variation for target traits. For instance, DH lines whose shoot length before winter were 71 mm in the DHLE populations and DH lines with 9 mm shoot length before winter in the DHSO population give large variation to select genotypes with desired shoot length for further breeding studies. Population mean for shoot length in the DHLE was 42 mm that was bigger than the DHSO population with 31 mm. This difference can be due to different sowing time and different growing seasons during implementation of field trials in the two DH populations. Because results of genetic variation in 19 winter oilseed rape genotypes showed that mean for shoot elongation of two parents Sansibar and Oase (36 mm) was larger than mean of Express617 and L16 (31 mm). The significant genotype x experiment effect for shoot length before winter becomes visible by the intermediate heritability ranging from 46 to 62% across the three plant materials.

Tendency to form inflorescence and shoot length in the spring sown environment represented significant variation for vernalization requirement. Results showed same phenotypic variation with high heritability for shoot length in the spring sown environment over the three plant materials. Bimodal or near bimodal frequency distribution was found in the both DH populations suggesting involvement of one or two major genes for shoot length and visible buds in the spring sown environment. The two parents L16 and Express617 showed clear differentiation for vernalization requirement, whereas Sansibar and Oase differed only slightly. Furthermore, among 19 oilseed rape genotypes, the two resynthesized lines were distinguished for strong vernalization requirement while traditional lines and cultivars showed large variation ranging from strong to week vernalization requirement.

Phenotypic variation and population mean for shoot length under the non-vernalized condition in the greenhouse environment was larger in the DHSO population (55 mm) compared to the DHLE population (33 mm).

Broad sense heritability was higher than 70% for beginning of flowering and plant height in the autumn sown environment in the both DH populations. Begin of flowering and plant height are well evaluated in oilseed rape and high heritability often has been reported for them in different breeding materials (Mei *et al.* 2009, Chen *et al.* 2010, Javed et al. 2016). Phenotypic variation for beginning of flowering was larger in the DHSO population, while larger phenotypic variation was found for plant height in the DHLE population. We noticed that in the two DH populations magnitude of parental variation for the related traits changed in different mega environments. For

instance in the DHLE population, shoot length for parent L16 was halved of Express617 (34 mm versus 61 mm) in the spring sown environment, but such a large variation was decreased between L16 and Express617 for plant height and begin of flowering in the autumn sown environment (See table 4.3.1). These oscillations are explained by effect of genotype x environments in phenotypic plasticity that are categorized in five genetic models; Overdominance, Pleiotropy, Epistasis, Epigenesis (El- Soda *et al.* 2014)

6.2 Correlation analysis between traits

Vernalization requirement along with shoot length before winter are involved in the complex architecture of winter hardiness. Hence, correlation between vernalization and shoot length before winter has been one of the main objectives in the three plant materials. Our results indicated that, no strong correlation was found between shoot length before winter and shoot length in the spring sown environment in the three studies. However, weak and medium correlations were found between the two traits in the DHSO population ($r_s=0.31^{**}$) and among 19 oilseed genotypes ($r_s=0.48^{**}$), suggesting certain share of genetic pathway controlling shoot length before winter and vernalization requirement in winter oilseed rape. This changing correlations have also been reported between vernalization requirement and frost tolerance in cereals and oilseed rape (Prásil *et al*. 2004, Waalen *et al.* 2014). Begin of flowering showed negative correlation, around $r_s=-0.50^{**}$, with shoot length in the spring environment in the two DH populations. This correlation was expected according to known genetic pathway of flowering time that is down regulated by vernalization genes in oilseed rape (Osborn and Lukens 2003).

6.3 QTL mapping in the two DH populations

QTL mapped for same traits in the three environments were compared between the two DH populations (Table 6.1). For shoot length before winter, six main QTL and one epistatic QTL explained 49.2% of the phenotypic variance in the DHLE population while 23.5% of the phenotypic variance was explained by five QTL in the DHSO population. Larger phenotypic difference between parent L16 as resynthesized line and parent Express617 could be the main

reason for higher coefficient of determination for shoot length before winter in the DHLE population. The major QTL for shoot length and the percentage of visible buds in the spring sown experiment were localized on linkage group A02 in the both populations. Likewise, for shoot length in the non-vernalized conditions in the greenhouse environment a QTL on A06 was found in the both DH populations. For begin of flowering time and plant height in the autumn sown environment a QTL on A02 was found in the both DH populations, However, physical position of flanking markers linked to the mentioned QTL showed that no QTL with similar physical positions were found between the two DH populations, indicating different regulator genes are involved for the trait variation in the two DH populations

Table 6.1: Number of QTL mapped in the DHLE and DHSO for the studied traits in three mega environments.

Environment	Trait	DHLE	DHSO	Total no. of QTL[a]
Autumn sown	Shoot length	7	5	12
	Shoot diameter	2	1	3
	Begin of flowering	4	6	10
	Plant height at EOF	6	4	10
Spring sown	Shoot length	3	3	6
	Buds	3	4	7
	Shoot diameter	6	3	9
Greenhouse 0 week vernalization	Shoot length	2	3	5
	Shoot diameter	2	1	3

EOF: end of flowering

[a] Total number of QTL mapped in the two DH populations.

The two interesting QTL hotspots were found on A02 and C09 in the DHLE population and on linkage groups A02 and C06 in the DHSO populations. Identification of candidate genes on the QTL hotspots showed two different copies of candidate genes *FLC* and *FT* in the two DH populations. One copy of *FLC* (*BnFLC.C9b*) was mapped to the genomic region from 128 to 131 cM on C09 in the DHLE population. The second copy of *FLC* (*BnFLC2)* was in the DHSO population in the genomic region from 15.3 to 15.9 cM on A02. One copy of candidate gene *FT* (*BnA2.FT*) was identified in the genomic region from 49.2 to 50.7 cM on A02 in the DHLE population. The second copy of *FT* (*Bn.C6.FTb*) was identified in the interval 25.6 to 29.7 cM on linkage group C06 in the DHSO population.

In the both DH populations, candidate gene *FLC* was found in the vicinity of major QTL for shoot length and visible buds in the spring sown environment, meaning *FLC* has a decisive role in vernalization requirement. Whereas, candidate gene *FT* was found in the genomic region of major QTL for begin of flowering and plant height in the autumn sown environment. Both *FLC* and *FT* are responsible in the timing of flowering in *B. napus.* Candidate gene *FT* is a central regulator whose protein product is a major component of the mobile signal that initiates flowering time in *B. napus* (Shavorskaya 2004). As soon as vernalization and photoperiod pathway allow, *FT* is translocated to the shoot apex, triggering flowering time (Schiessl *et al.* 2014). *FLC* and *FRIGIDA* (*FRI*) are important determinants of variation in the requirement for vernalization. *FLC* represses the initiation of flowering and prevents changes that convert the apical meristem to the reproductive structures (Shindo *et al.* 2005). Genome wide association mapping on big diversity set of canola (n=188) for flowering time revealed that different homologues of *FT* and *FLC* are responding to vernalization and photoperiods and their effect may be seen only in some conditions (Raman *et al.* 2016).

Comparison of QTL mapped for seed quality traits in the vicinity of candidate gene *FLC* and *FT* in the two DH populations indicates that confidence intervals of QTL for oil and thousand kernel weight collocated with the genetic position of *BnFLC.A2* in the DHSO population, while no QTL from seed quality traits were found in the vicinity of *FLC* in the DHLE population. Pleotropic effects of *FLC* and *FT* in pathway of different traits are described in some crops. Deng *et al.* (2011) showed that more than 500 genes for developmental pathways are potentially regulated by *FLC* in the *Arabidopsis thaliana*. In tomato and oilseed rape mutations within the paralogues of *FT* had large effects on fruit yield and yield components, showing *FT* protein binds

to pathways of seed traits (Kriege *et al.* 2010, Gou *et al.* 2014). Also tight linkage between underlying genes of oil content, thousand kernel weight and *FLC* might be the reason for overlapping confidence interval of the mapped QTL for the seed quality traits and the studied traits in three mega environments.

Chapter 7

Summary

Oilseed rape (*Brassica napus* L.) is a major oilseed in many parts of the world with well adaptation to cold, dry and moist growing conditions. Despite large adaptation, winter survival is a major limiting factor to success of the crop in the regions with extreme freezing temperatures. Selection for improved winter hardiness is a difficult task, because firstly, efficient selection can only be performed in extreme winters, which in principle are not accessible and predictable in all regions. Secondly, winter hardiness is a complex trait, which consists of other interacting stresses. Cessation of shoot elongation before and during winter, has been considered one of the main requirements for successful overwintering in winter plants. Therefore, shoot length before and during winter is particularly considered by oilseed rape breeders as a contributing trait for selection of winter-hardy genotypes. Vernalization requirement, also, is a main winter survival mechanism that reduces the rate of shoot development and extend the vegetative phase to prevent frost damage by prohibiting floral transition during long warm periods before and during winter.

To analyze the genetic variation for shoot elongation before winter and vernalization requirement, field experiments were performed with a set of 19 European winter oilseed rape genotypes. Moreover, to understand the genetic architecture of shoot elongation before winter and its correlation with vernalization requirement in winter oilseed rape, QTL mapping was implemented in two bi-parental doubled haploid (DH) populations of *Brassica napus* (DHLE and DHSO). The DHLE population consists of 151 doubled haploid lines, derived from a cross between the resynthesized line L16 and the winter oilseed rape line Express617. The DHSO population consists of 226 doubled haploid lines, derived from a cross between the two winter oilseed rape cultivars Sansibar and Oase. The two DH populations and the parental lines were phenotypically characterized in series field and greenhouse experiments.

The two field environments included the normal sowing time at end of August/beginning of September, called autumn sown environment, and the spring sowing time at end of March/ beginning of April, called spring sown environment. Since the greenhouse, autumn and spring sown experiments represented very different environments; they were called three mega

environments**.** Shoot length, shoot diameter were measured around three months after sowing time in the all three environments. In addition, data from flowering time, plant height, and seed quality traits were used to study their phenotypic and genetic association with the studied traits in the two DH populations.

In the set of 19 European winter oilseed rape genotypes, results showed large phenotypic variation with significant genotypic variance components for shoot length in the autumn sown and spring sown environment. Broad sense heritability was high for shoot length in the spring sown environment (h^2=97%) while medium heritability (h^2=62%) was observed for shoot length before winter. Spearman's rank correlation revealed a medium positive correlation (r_s= 0.48*) between shoot length before winter and shoot length in the spring sown environment. Scatter plot of spearmen's rank correlation distributed the 19 genotypes mainly in two clusters; first cluster consisted of genotypes with short shoot length in the autumn sown and spring sown environment, including R53 and L16, Mohican, Lorenz and Sollux, Zenith, Apex and Akela. Second cluster consisted of cultivars which were short before winter but long in the spring sown environment including Montego, Tenor, Adriana, Sansibar, Oase, Express 617, SGEDH13, SGDH14, King 10 and hybrid cultivar Visby. Gaoyou was the only cultivar with the longest shoot length in the both field environments. Low vernalization requirement was characterized in genotypes with significant elongation of shoot in the spring sown environment.

In the DHLE population, large and significant genotypic variance components were found for shoot length and shoot diameter in the all three mega environments. Express617 was significantly longer than L16 for shoot length before winter (61 vs. 34) and begin of flowering was around five days earlier in the autumn sown environment. In the spring sown environment, parent L16 was with 218 mm length significantly shorter than Express617 with 855 mm shoot length. A near bimodal frequency distribution was found for shoot length in the spring sown environment with large phenotypic variation that ranged from 87 mm to 1255 mm. In addition, comparison of frequency distribution of shoot length and percentage of visible buds in the spring sown environment indicated that genotypes shorter than L16 almost did not show any tendency to form an inflorescence, whereas genotypes longer than Express617 showed almost 100% tendency to form an inflorescence.

Correlation analysis in the DHLE population indicated no significant correlation between shoot length before winter and shoot length in the spring sown environment. Shoot length before

winter was also not correlated with begin of flowering neither in the autumn sown nor in the greenhouse environment upon eight weeks vernalization treatment. Significant negative correlation of r_s =-0.54** was found between begin of flowering in the autumn sown environment and shoot length, visible buds in the spring sown environment. Also, significant correlation was found between shoot length in non-vernalized plants in the greenhouse and shoot length in the autumn (r_s = 0.31**) and spring sown environment (r_s = 0.22**).

Multiple interval mapping in the DHLE population, detected six main QTL for shoot length before winter that contributed 49.2% to the phenotypic variance. For shoot length in spring sown environment a major QTL with R^2=35.5% was localized on linkage group C09 which along with two QTL mapped to A02 and A07 explained 68% of the phenotypic variance. In the non-vernalized greenhouse environment, two QTL were found for shoot length on A06 and A09 which together explained 16.2% of the phenotypic variance. No collocation of QTL was found between shoot length before winter, shoot length in the spring sown and non-vernalized greenhouse environment. Two candidate genes *BnA2.FT* and *BnFLC9b* were identified in the genomic regions with overlapping confidence intervals of QTL on A02 and C06. The candidate gene *BnA2.FT* is a paralogue of gene *FT* that collocated with major QTL on A02 for begin of flowering and plant height in the autumn sown environment. The candidate gene *BnFLC9b* is a paralogue of gene *FLC* found within a hotspot of linkage group C09 in which major QTL for shoot length and visible buds were localized in the spring sown environment.

In the DHSO population large and significant genotypic effects were found for shoot length in the three mega environments. High heritability was found for shoot length in the spring sown (h^2=90%) and greenhouse environment with non-vernalized plants (h^2=69%), in contrast shoot length before winter had rather medium heritability (h^2=46%). Parent Oase showed significant higher value than Sansibar for shoot length before winter (40 ver. 29), while Sansibar was significantly longer than Oase in the spring sown field experiments (721 ver. 442). Tendency to form inflorescence in the spring sown environments was higher than 80% in Sansibar and Oase, showing low vernalization requirement in the parental lines. Spearman's rank correlation reveled negative correlation between shoot length in the spring environment and begin of flowering, (r_s=-0.52**), plant height (r_s=-0.25**) in the autumn sown environment. Shoot length in the spring sown environment was positively correlated with the appearance of visible buds (r_s=0.90**) and shoot length in non-vernalized greenhouse environment (r_s=0.42**). Significant correlation was

found between shoot length before winter and vernalization requirement determined by shoot length (r_s=0.31**) and visible buds (r_s=0.26*) in the spring sown environment. Likewise, shoot length before winter was negatively correlated with begin of flowering (r_s=-0.29**). In the DHSO population, multiple interval mapping detected five main QTL for shoot length before winter, explaining collectively 23.5% of the phenotypic variance. Three QTL were found for shoot length in the spring sown environment. The major QTL on A02 explained 70% of the phenotypic variance for shoot length in the spring environment. Three QTL for shoot length in non-vernalized plants in the greenhouse contributed 26% to the phenotypic variance. QTL mapping for begin of flowering identified one major QTL on C06 which along with 5 additional QTL accounted for 71.6% of the phenotypic variance. On linkage group A02 a hotspot was found with the QTL for shoot length before winter, begin of flowering, end of flowering, duration of flowering and plant height in the autumn sown environment and the major QTL for shoot length and visible buds in the spring sown environment. Surprisingly, all the QTL were the biggest or almost the biggest QTL of respective traits. A copy of candidate gene *FLC* (*BnFLC2)* was found on the genomic region from 15.3 to 15.9 cM on A02. C06 was the second linkage group with overlapping confidence intervals of the biggest QTL for begin and end of flowering in the autumn sown environment and a QTL for visible buds in the spring sown environment with opposite additive effects in the DHSO population. A copy of *FT* (*Bn.C6.FTb*) was identified in the interval 25.6 to 29.7 cM within the QTL hotspot on C06.

In the both populations, different copies of candidate genes *FLC* were identified in the vicinity of major QTL for shoot length in the spring sown environment, indicating that elongation of shoot is significantly influenced by vernalization induced genes. Furthermore, different copies of candidate gene *FT* were identified in the vicinity of the major QTL for flowering time, showing key role of *FT* in regulation of flowering time. Identification of QTL mapped for some seed quality trait in the extent of *FLC* in the DHSO population suggests pleiotropic effect of *FLC* on different traits or tight linkage between genes involved in respective traits.

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Curriculum vitae

PERSONAL DETAILS

Name	Mohammad Ghanbari
Place of birth	Zahedan, Iran
Date of birth	21 March 1981
Address	Roodaki 28, Zahedan9816944445
Email	Mghanbari.msc@gmail.com
Nationality	Iranian
Marital status	Married, one child

Education Back ground

Oct 2013 - May2016	**Ph.D:** Plant Breeding, Georg August University Götingen. Germany
Sep2003 – March 2006	**Master:** Plant Breeding, University of Tehran, Tehran, Iran
Sep 1999- April 2003	**Bachelor:** Agronomy and Plant Breeding, University of Zabol, Iran

Work Experience

Aug 2009- Sep 2013	Researcher & Lecturer Department of Biotechnology and Plant breeding. University of Zabol, Zabol, Iran
Oct 2007 – Aug 2009	Plant breeder Agriculture research institute Ministry of agriculture, Iran.

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Honours, Awards, Distinctions

2013	PhD Fellowship at Göttingen University, Germany Erasmus Mundus
2011	Outstanding Researcher University of Zabol, Zabol, Iran
2010	Post-Graduate Fellowship Ministry of Science, Research and Technology, Iran
2003	Fourth Rank Student Out of 804 candidates Ministry of Science, Research and Technology, Iran

Publication

Ghanbari M, Molashahi M 2015 Study the response of mung bean to drought stress by multivariate analysis. International Journal of agriculture Innovations and Research. 3(4) 2319-1473.

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Workshop and practical course

Next Generation Sequencing (NGS) - Analysis of RNAseq and ChIPseq data, Nov 2015. Göttingen University, Germany

Systematic review and Meta-analysis, Dec 2015. Göttingen University, Germany

Linear statistical Model with R, Sep 2015. Göttingen University. Germany

Technique and teaching skills. Dec 2012. University of Zabol, Iran.

Application SPSS Software in Biometrical Analysis. May 2012. University of Zabol, Iran.

Molecular Markers Based PCR. Dec 2010. University of Zabol, Zabol, Iran.

Creativity and Innovation (invention). University of Zabol, Iran.

Introduction Research and Seed Propagation in Cotton. Mar 2009. Research Institute for Cotton and Fibrous Crops, Gorgan, Iran.

Familiarity with planning and value engineering and its application in drought management. Mars 2008.1th International Conference of water Crisis. Zabol, Iran.