Genetic dissection and QTL based modeling of vernalization and curd development in cauliflower (*Brassica oleracea* var. *botrytis*)

Von der Naturwissenschaftlichen Fakultät der
Gottfried Wilhelm Leibniz Universität Hannover

zur Erlangung des Grades

Doktor der Gartenbauwissenschaften (Dr. rer. hort.)

genehmigte Dissertation

von

M.Sc. Yaser Hasan,

geboren am 27.01.1974 in Hama, Syrien

2016

Referent: Prof. Dr. Ralf Uptmoor

Korreferent: Prof. Dr. Hartmut Stützel

Tag der Promotion: 19.08.2016

Zusammenfassung

Blumenkohl (*Brassica oleracea* var. *botrytis*) ist eine bedeutende Gemüsekultur, geerntet wird der aus weißen Infloreszenzmeristemen bestehende Kopf. Die Kopfbildung wird hauptsächlich durch die Temperatur beeinflusst und diese Arbeit soll die genetischen Hintergründe dieses Prozesses erklären. Zur Identifikation von quantitative trait loci (QTL), welche den Zeitpunkt der Kopfanlage und zugehörige Merkmale im Temperaturbereich von 12 - 27 °C steuern, wurde eine doppelhaploide (DH) Population erzeugt, die in dem Merkmal „Dauer der Kopfbildung“ spaltet. An Individuen dieser Population wurden die Tage bis zur Kopfanlage (DCI), die Blatterscheinungsrate (LAR) und die finale Anzahl Blätter (FLN) bestimmt. Composite interval mapping (CIM) zeigte QTL hotspot Regionen für DCI auf Chromosom sechs und neun. Einzelne QTL konnten 11 bis 41 % der phänotypischen Variation erklären, wobei derer geschätzte additive Effekt bei hohen Temperaturen größer ausfiel. Signifikante QTL × Umwelt-Interaktionen (Q×E) für FLN und DCI auf den Chromosomen sechs und neun deuten darauf hin, dass die hotspot-Regionen einen wesentlichen Einfluss auf die temperaturbedingte Kopfanlage hat. Rund 25 % der DH-Linien bildeten keinen Kopf bei Temperaturen über 22 °C. Die Verwendung eines binären Modells zeigte einen QTL mit LOD > 15 auf dem Chromosom sechs. Nahezu alle Linien, welche das Allel der früh reifenden Elternlinie (PL) an diesem Locus trugen, induzierten eine Kopfbildung bei hohen Temperaturen, während nur rund die Hälfte der DH-Linien, die das Allel der nicht-kopfinduzierenden PL trugen, die generative Phase erreichten. Die LAR wiesen eine hohe Variation aufund die QTL-Hotspots für LAR wurden auf chromosom eins, vier und sechs ermittelt. Die LAR QTL erklären 11 bis 29 % der phänotypischen Variation. Negative Korrelationen zwischen LAR und DCI sowie eine gemeinsame QTL-Lokalisation auf chromosom vier und sechs deuten darauf hin, dass die LAR einen Einfluss auf die Initiierung der Kopfanlage hat. Durch die Verwendung der Daten zur Kopfbildung von 151

DH-Linien, welche zuvor unter sechs verschiedenen Temperaturbedingungen im Gewächshaus evaluiert wurden, konnte ein lineares Modell mit zwei Phasen parametrisiert werden, welches die Kopfbildungsrate in Abhängigkeit zur Temperatur beschreibt. Die QTL-Analyse mittels CIM wurde auf die Modellparameter übertragen: SL parametrisiert die Empfindlichkeit gegenüber Temperaturen unter dem Optimum, SR die Empfindlichkeit gegenüber Temperaturen über dem Optimum, BP beschreibt den Wendepunkt und R_{max} beschreibt die maximale Entwicklungsrate bei optimaler Temperatur. Insgesamt wurden 20 QTLs für alle Parameter entdeckt. Ein entscheidener QTL wurde auf dem Chromosom sechs identifiziert, er macht 6,3 %, 6,1 % und 27 % der jeweiligen phänotypischen Variation von BP, SL und SR aus. Dieses QTL wurde in niedrigen sowie hohen Temperaturbereichen nachgewiesen, wobei das SR QTL einen fünfmal so hohen Effekt wie das SL QTL bei sehr hohem LOD score (LOD=11) aufwies, was darauf hindeutet, dass die Bedeutung dieser Region bei hohem Temperaturen zunimmt. In dieser Region konnte zudem kein QTL für R_{max} gefunden werden, was die zentrale Rolle des QTL für den Einfluss von suboptimalen Temperaturen weiter untermauert. Fünf QTL für Frühzeitigkeit konnten speziell für R_{max} detektiert werden. Durch das Schätzen von marker-basierten Werten für die phenologischen Modellparameter entsprechend der additiven Alleleffekte der identifizierten QTL, wurde ein QTL-basiertes Modell entwickelt, um die Kopfbildung zu simulieren. Dieses kombinierte Model erklärt 46 % der phänotypischen Variation des Merkmals Kopfbildung. Die Vorhersagequalität des Modells wurde in sechs Feldversuchen auf vier Standorten mittels unabhängigen Daten von DH-Linien validiert. Dabei war das QLT-basierte Modell in der Lage den Zeitpunkt der Kopfanlage mit einer Abweichung von drei bis acht Tagen vorherzusagen und 28 bis 65 % der Variation im Zeitpunkt der Kopfanlage zu erklären. Das QTL-basierte Modell konnte zudem in vier weiteren Experimenten auf zwei Standorten ähnliche Ergebnisse erzielen, wobei es die Kopfanlage mit einer Fehlergenauigkeit von drei bis neun Tagen vorhersagte und 24 bis 41 % der genetischen Variation des Zeitpunktes der

Kopfanlage erklärte. Diese Ergebnisse zeigen, dass QTL-basierte Modelle ein vielversprechendes Werkzeug für die Züchtung sein können, um die Zuchtprogramme zu beschleunigen, verschiedene züchterische Strategien zu testen und die Züchtung auf spezielle Umweltbedingungen hin zu optimieren.

Schlagworte: *Blüteninduktion von Blumenkohl*, *QTL kartierung*, *Kopplung von pflanzenbaulicher Modellierung und QTL Kartierung*

Abstract

Cauliflower (*Brassica oleracea* var. *botrytis*) is an important vegetable crop. It is grown for its arrested inflorescence meristem forming the curd. Temperature is a key environmental factor influencing curding time of cauliflower. To identify quantitative trait loci (QTL) controlling curding time and its related traits in a range of different temperature regimes from 12-27 °C, a doubled haploid (DH) mapping population segregating for curding time was developed and days to curd initiation (DCI), leaf appearance rate (LAR), and final leaf number (FLN) were measured. Composite interval mapping (CIM) revealed QTL hotspot regions for DCI on C06 and C09. Individual QTL explained between 11 and 41% of the phenotypic variation. The estimated additive effect was higher at high temperatures. Significant QTL × environment interactions (Q×E) for FLN and DCI on C06 and C09 suggest that these hotspot regions have a major influence on temperature mediated curd induction. 25 % of the DH-lines did not induce a curd at temperatures higher than 22 °C. Applying a binary model revealed a QTL with LOD >15 on chromosome C06. Nearly all lines carrying the allele of the early maturing parental line (PL) on that locus induced curds at high temperatures while only half of the DH-lines carrying the allele of the non-curd inducing PL reached the generative phase during the experiment. Large variation in LAR was observed. Main QTL hotspots for LAR were detected on C01, C04 and C06. LAR QTL explained between 11 and 29% of phenotypic variation. Negative correlations between LAR and DCI as well as QTL co-localizations on C04 and C06 suggest that LAR has also effects on development towards curd induction. Using the curding per se data for 151 DH lines which were previously evaluated under six different temperature regimes in greenhouse, two phase linear phenology model, describing curding rate to temperature was parameterized. QTL analysis by composite interval mapping was carried out on model parameters: SL which parameterizes the sensitivity to temperature below optimum, SR parameterizes the sensitivity to temperature above optimum, BP describe the point of rate change and also the computed R_{max} describe the

maximum development rate at optimum temperature. A total of twenty QTLs were detected for all parameters. A major QTL was identified on C06 accounted for 6.3 %, 6.1 % and 28.5 % of the phenotypic variation in BP, SL and SR respectively. Although this QTL was detected for both SL and SR, the SR QTL have five times the effect of SL QTL with very high lod score (LOD=11) which indicate that high temperatures enhance the role of this genomic region. At this genomic region no QTL for R_{max} was detected which further support the central role in suboptimal high temperature effect. Six QTLs were specific for R_{max} indicating earliness per se. A QTL based model was developed by estimating the marker-based value of each phenology model parameter from the additive allele effects of QTLs detected and incorporating into the original phenology model to simulate curding time. The combined QTL and crop model explained 46 % of the phenotypic variation in curding time of the parameterization set. The predictive quality of the model was validated in field trials on independent validation data set of DH lines at four locations in six experiments. In this set, the QTL-based model was able to predict the curding time with a root mean square error (RMSE) of prediction of 3 to 8 days explaining 28 to 65 % of the variation in curding time. The QTL based model was further used to predict the performance of a test cross at two locations in four experiments. The QTL-based model could predict the curding time with RMSE of 3 to 9 days and explain 24 to 41% of the genetic variation in curding time. This suggests that the QTL based model is a promising tool for plant breeders to accelerate their breeding programs, test different plant breeding strategies and to design ideotypes for contrasting target environments.

Keywords: *Cauliflower floral induction, QTL mapping, coupling of crop modeling and QTL mapping*

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BC	Backcross
BoAP-a, b, c	*Brassica oleracea* floral merstiem identity genes
BP	Breakpoint
bp	Base pair
CIM	Composite Interval Mapping
cM	Centimorgan
DCI	Days to curd initiation
DH	Doubled haploid
DR	Development rate
FLC	Flowering locus C
FLN	Final leaf number
FRI	FRIGIDA gene
FT	Flowering locus T
GDD	Growing degree day
GEI	Genotype by environment interaction
LAR	Leaf appearance rate
LOD	Logarithm of odds
MAS	Marker assisted selection
Mbp	Mega base pair
NIL	Near Isogenic Lines
PCR	Polymerase Chain Reaction
PV	Phenotypic variation
QTL	Quantitative trait loci
Q×E	QTL by environment interaction
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RIL	Recombinant Inbred Lines
R_{max}	Maximum development rate at optimum temperature

RMSE	Root Mean Square Error
SRAP	Sequence-Related Amplified Polymorphism
SSR	Single Sequence Repeat
SL	Slope left
SI	Self incompatibility
SIM	Simple Interval Mapping
SNP	Single nucleotide polymorphism
SR	Slope right
T	Actual temperature
t_{BP}	Temperature at Breakpoint

CHAPTER 1

General Introduction

1.1 The family Brassicaceae

Brassicaceae contains 338 genera and 3709 species (Warwick et al. 2006b). In this family, the genus *Brassica* comprises six cultivated species: three diploid species of *Brassica rapa* (AA genome, 2n=20), *Brassica oleracea* (CC genome, 2n=18) and *Brassica nigra* (BB genome, 2n=16), and three amphidiploids species: *Brassica juncea* (AABB, 2n=36), *Brassica napus* (AACC, 2n=38) and *Brassica carinata* (BBCC, 2n=34), which were formed through hybridization of their diploid genome counterparts (U, 1935).

Brassica oleracea includes most economic important cultivated vegetable *Brassica* species which are morphologically highly diverse with different crop forms such as the enlarged hypocotyls of kohlrabi, the floral meristem of cauliflower and broccoli, and the fresh leaves of kales and cabbages (Dixon, 2007 and Liu et al. 2013).

1.2 Cauliflower development and temperature effect

Cauliflower (*Brassica oleracea* L. var. *botrytis* L. 2n=2x=18) is an important vegetable crop. It is grown for its arrested inflorescence meristem forming the curd. This arrested stage precedes flowering (Dixon, 2007). It is cultivated worldwide and adapted to a wide range of environments ranging from tropic to temperate zone during most growing seasons (Sharma et al. 2004).

The development of cauliflower between emergence and curding is characterized by three distinct development phases: the juvenile phase during which plants are insensitive to vernalizing temperature, and vernalization sensitive stage in which plants require relatively cool temperatures for induction and finally the curd growth phase (Dixon, 2007).

Many Brassicas such as brussels sprouts (*B. oleracea* var. *gemmifera*) (Thomas 1980), cabbage (*B. oleracea* var. *capitata*) (Lin et al. 2005) and cauliflower (*B. oleracea* var. *botrytis*) (Hand and Atherton 1987; Booij and Struik 1990; Wurr et al. 1993) must pass through a juvenile phase before they become competent to perceive the vernalization stimulus. In cauliflower, the length of the juvenile phase is defined as the number of initiated leaves (Hand and Atherton 1987; Booij and Struik 1990; Wurr et al. 1993). Considerable genetic variation has been observed in the lengths of juvenile phase across a range of cauliflower genotypes (Hand and Atherton 1987; Booij and Struik 1990; Wurr et al. 1993; Wurr et al. 1994). Wiebe (1972a) found that the length of the juvenile phase was 4 and 8 leaves for cultivars Aristokrat and Sesam, respectively. Hand and Atherton (1987) reported that the ends of juvenile phase after the initiation of 13 to 15 leaves in cultivar Perfection and 17 to 19 leaves in cultivar White Fox. Similarly, Boiij and Struik (1990) found the length of juvenile phase to be 17 and 19 for cultivars Delira and Elgon, respectively.

Following the end of juvenility and during the vernalization sensitive stage, temperature is the main environmental factor influencing *B. oleracea* curding time (Salter 1969; Sadik 1967; Hand and Atherton 1987). The relatively cool temperatures which promote curd initiation are termed as vernalization. Frequent high temperature occurrence during the vernalization sensitive stage delays curd initiation and increases the total number of leaves produced (Booij and Struik 1990; Fujime and Okuda 1996; Fellows et al. 1999).

Genetic variation in cardinal temperatures for vernalization has been observed across a range of cauliflower genotypes. Pearson et al. (1994) reported that the rate towards curd initiation in cauliflower increased up to a mean temperature of 14 °C but declined thereafter. Wurr et al. (1995) reported maximum vernalization rate in the range 9 to 9.5 °C which declined to zero below 9 °C and above 21 °C for the cultivar White Fox. Wurr and Fellows (1998) indicated an optimum temperature of 14 °C in winter cauliflower. Wurr and Fellows (2000) reported

that under UK conditions, the optimum temperature for curd initiation of all maturity types ranged between 9 °C and 14 °C. Temperature optimum of most spring and summer cultivars of the temperate region ranges between 10 to 16 °C (Wiebe 1972abc; Hand and Atherton 1987; Wurr et al. 1990; Booij and Struik 1990). However, temperatures above 23 °C are assumed inhibitory for curd initiation (Nieuwhof 1969; Fellows et al. 1999; Kage and Stützel 1999; Guo et al. 2004).

Temperature is also the reason for two morphological abnormalities that can attribute to cauliflower curd quality defects "bractiness" and "riciness". High temperature enhances the development of the bracts in the axis of the inflorescence primordium through the curd surface (Fujime and Okuda 1996; Grevsen et al. 2003). Riciness which refers to the development of small flower buds on the curd surface in plants which exposed to low temperature at curd initiation representing a kind of over vernalization (Wiebe, 1973).

After curd initiation, the rate of curd growth is influenced by temperature (Wiebe 1975; Wurr et al. 1990). Rahman et al. (2007a) studied the effect of temperature on growth and development of cauliflower after curd initiation and found that cauliflower curds increased in size with the increase in mean growing temperature after curd initiation and the optimum temperature for curd growth was 21-22 °C.

1.3 Flowering time, Brassica genus and Arabidopsis

Flowering time is a major development switch that is controlled by complex regulatory pathways which integrates endogenous and environmental cues (Koornneef et al. 2004). Six main flowering pathways have been described in *Arabidopsis*: the photoperiod pathway, vernalization pathway, the autonomous pathway, the gibberellin pathway (Simpson and Dean 2002) age pathway, and ambient temperature pathway (Fornara et al. 2010).

Molecular bases of flowering time have been extensively studied in *Arabidopsis*. More than 180 genes have been identified which play a role in flowering time (Fornara et al. 2010). Comparative genomics studies have shown substantial genomic collinearity and conservation between *Brassica* and *Arabidopsis* genomes (Lagercrantz et al. 1996; Parkin et al. 2005) which can facilitate the utilization of *Arabidopsis* genomic tools in *Brassica* research (Lagercrantz et al. 1996; Parkin et al. 2005).

In both genera, *FLOWERING LOCUS C* (*FLC*) is a major determinant of vernalization requirement. *FLC* acts as repressor of flowering (Michaels and Amasino 1999) and is downregulated during vernalization. Five copies were described in *B. rapa* and *B. oleracea*, and nine copies in *B. napus* (Lagercrantz et al.1996; Tadege et al. 2001; Schranz et al. 2002; Okazaki et al. 2007; Yuan et al. 2009; Zhao et al. 2010).

In *Arabidopsis*, *FRIGIDA* (*FRI)* locus is also a repressor of flowering by up regulating *FLC*. Variation in flowering time in *Arapidopsis* was shown to be associated with (*FRI)* alleles (Clarke and Dean 1994). Plants with dominant allelic genotype at (*FRI*) locus confer late flowering, and change into early flowering by vernalization (Johanson et al. 2000). Two *FRI* homologs in *B. oleracea* have been mapped to regions on C03 and C09. Four *FRI* homologs were described in *B. napus* and one gene co-localized to a major flowering time QTL (Wang et al. 2011a).

Interactions between meristem identity genes (MIGs) and temperature effects have been studied in *Arabidopsis* (Bowman et al. 1993) and *B. oleracea* (Anthony et al. 1996). Two functional and one non-functional *APETALA1* (*AP1*) orthologs were isolated in the *Brassica* genome: *BoAP1-a* (Anthony et al. 1996), *BoAP1-c* (Smith and King 2000), and *BoAP1-b* (Lowman and Purugganan 1999), respectively. The expression of *BoAP1* in cauliflower plants which were grown at temperatures higher than 25 °C was switched off during the vegetative reversion (Anthony et al. 1996). Kop et al. (2003) suggested that *BoAP1-a* involved in

controlling bract development, which essentially occurs at high temperatures during curd development.

Quantitative trait loci for flowering time were detected in different *Brassica* mapping populations and some QTL hotspots overlapped with *FLC* orthologs (Lagercrantz et al. 1996; Osborn et al. 1997; Bohuon et al. 1998; Rae et al. 1999; Sebastian et al. 2002; Schranz et al. 2002; Brown et al. 2007; Long et al. 2007; Zhao et al. 2010; Uptmoor et al. 2012). However, it was also shown that several of the flowering time QTL did not co-segregate with the *FLC* loci in *B. oleracea* (Razi et al. 2008) or that the underlying *FLC* gene was not functional (Okazaki et al. 2007).

1.4 QTL mapping

1.4.1 Molecular markers and genetic mapping

Molecular markers reveal polymorphism at the DNA sequence level (Jones et al. 1997). Different molecular detection techniques are used to reveal the variation in DNA sequence. These techniques can be grouped into three major classes: (1) low throughput, hybridization based markers such as restriction fragment length polymorphism (RFLP); (2) medium throughput, PCR based markers such as random amplified polymorphic DNA (RAPD), amplified length polymorphism (AFLP) and simple sequence repeat or microsatellite (SSR) (3) high throughput sequence based markers such as single nucleotide polymorphism (SNP) (Collard et al. 2005; Mammadov, 2012). In this study SNPs were used to detect the polymorphisms on the DNA level. SNPs are individual nucleotide differences between DNA sequences which allow the detection of variation between individuals or within populations. As a nucleotide base is the smallest unit of inheritance, and can occur in coding and non-coding regions of the genome and at different frequencies in different genomic regions, SNPs provide the ultimate form of molecular genetic markers and have been increasingly used for

QTL mapping studies because they are widely distributed through the genome. Typically, SNP frequencies in a range of one SNP every 100-300 bp in plants, therefore, they can provide higher resolution of the map compared to other markers system. Moreover, they are co-dominant markers, amenable to automation and can be rapidly detected, with a high efficiency for detection of polymorphism (Edwards et al. 2007).

Genetic map construction requires the development of an appropriate mapping population; screen the mapping population for marker polymorphism with suitable molecular marker system. Once the individuals have been genotyped with a set of molecular markers, the linkage map can be constructed by arranging the markers in a linear order on the basis of recombination frequencies and estimated genetic distance (Jones et al. 1997).

1.4.2 Mapping populations

Mapping populations are usually developed by crossing two inbred lines that differ in the target trait. Different mapping populations are used in QTL mapping studies including F_2 popualtion, backcross (BC), recombinant inbred lines (RILs), near isogenic lines (NILs) and double haploids (DH). Each of the above mentioned populations has its own advantages and disadvantages. F_2 and BC are the simplest types of mapping populations because they are easy to construct. However, F_2 and BC populations are considered to be temporary populations. In contrast, recombinant inbred lines (RILs), near isogenic lines (NILs) and doubled haploid (DHs) are immortal populations (Collard et al. 2005).

1.4.3 Quantitative trait loci and detection methods

The advent and use of molecular markers for the detection of DNA polymorphisms at specific locations in the genome enables identification of genetic loci controlling variation in quantitative traits. Several statistical methods have been used in QTL mapping. The most basic approach for detecting an association between a molecular marker and a trait is to conduct single marker analysis one at a time and split the progenies according to the genotype

at this marker, then comparing the phenotypic means of two classes of progeny using t-test or ANOVA to deduce the marker linked to a QTL (Doerge, 2002).

Lander and Botstein (1989), based on the advent of complete linkage maps, proposed the Interval Mapping method (IM) and carried out a systematic scan of the whole genome through the use of flanking markers when searching for a single QTL in each map interval. This method is more powerful than single marker analysis. However, it has some drawbacks. Since it is a one QTL model, the QTL position will be biased when more than one QTL is located at the same chromosome (Haley and Knott 1992). To solve this problem, Zeng (1994) proposed a more sophisticated, precise and effective method called Composite Interval Mapping (CIM). The principle of this method is combining interval mapping and multiple regressions (Haley and Knott 1992). CIM has proved a better performance than interval mapping and represents the most commonly approach for mapping QTL (Li et al. 2010).

Although QTL studies provide useful information about the genetic control of a trait, there are some limitations. Due to the differences in polymorphisms pattern at different sets of markers among the different population, the location and effect size of QTL are specific to the the studied population. QTL analysis detects genomic regions, not genes, controlling the trait. Such genomic regions encompass hundreds of genes, so it is difficult to infer the most influencing gene (Kearsey, 2002).

1.4.4 Mapping populations and flowering time QTLs in *Brassica oleracea*

Several mapping populations have been developed in *B. oleracea* using different markers system and QTL have been identified for a wide range of crop traits. For instances, a DH population of a cross between A12 (rapid cycling Chinese Kale line; *B. oleracea* ssp. *alboglabra*) and GD (Calabrese; *B. oleracea* ssp. *italica*) using RFLP markers (Bohuon et al. 1998); F_2 population of a cross between cabbage (*B. oleracea* ssp. *capitata*) and broccoli (*B.*

oleracea spp. *italica*) using RFLP and RAPD markers (Camargo et al. 1997); DH population of a cross between cauliflower (*B. oleracea* ssp. *botrytis*) and brussels sprouts (*B. oleracea* spp. *gemmifera*) based on RFLP, AFLP and SSR markers (Sebastian et al. 2002); F_2 population of a cross between brocooli (*B. oleracea* ssp. *italic*a) and cauliflower (*B. oleracea* spp. *botrytis*) using 1,062 sequence-related amplified polymorphism markers (SRAP), 155 cDNA SRAP markers, 26 SSR markers, 3 broccoli BAC end sequences and 11 known *Brassica* genes (Gao et al. 2007).

In previous studies, many QTLs affecting flowering time in *B. oleracea* have been identified. Camargo and Osbron (1996) identified two QTLs in F_3 families of a across between cabbage by broocoli on C07 and C08.

Bohuon et al. (1998) using DH mapping population provide evidence of five flowering time QTLs, one each on C02, C03 and C05 and two on C09. Rae et al. (1999) using substitution lines derived from the same parental lines used by the latter study have detected additionally two QTLs on C01, one on C03 and two on C09. On the same mapping population developed by Bohuon et al. (1998), Uptmoor et al. (2008) identified eleven QTLs for floral induction and flowering tow each on C02 and C05, three each on C03 and C09, and one on C06.

Okazaki et al. (2007) in F_2 population of a cross between *B. oleracea* var. *italica* and *B. oleracea* var. *capitata* revealed six flowering time QTLs; two on C02 and one each on C03, C06, C08 and C09. Sebastian et al. (2002) identified two QTLs one each on C07 and C08 in a cross of cauliflower and brussels sprout.

1.4.5 Marker assisted selection

Conventional plant breeding is typically based on phenotypic selection of individuals with best performance for certain trait. Although, conventional breeding methodologies have extensively proven successful in improvement of plant cultivars, considerable difficulties are

often encountered during the process. However, the efficiency of conventional plant breeding has been enhanced by the rapid development of molecular markers and the large number of quantitative trait loci (QTLs) mapping studies which allow the use of marker assisted selection (MAS) in different crop species. MAS is carried out on the basis of a marker instead of the phenotypic trait itself (Pérez-de-Castro et al. 2012). The successful application of MAS relies on the tight association between the marker and the major gene or QTL responsible for agronomical important trait in crop plants. Markers linked to a QTL of interest can be used in MAS whereby desirable phenotypes are selected based on the presence of the favorable allele at one or more marker near the QTL.

Compared with conventional plant breeding, MAS has significant advantages. MAS allows the selection for a trait to be carried out during the seedling stage and thus reduce the time cost. MAS is not affected by environment allowing the selection under any environment. For traits controlled by multiple genes; favorable alleles can be selected simultaneously and thus used for gene pyramiding. Selection based on molecular markers can be faster, cheaper and more accurate than conventional phenotypic assays. However, integration of MAS in plant breeding program has few successful stories for many reasons such as accuracy of detected QTL, reliability of marker trait association, lack of commonly valid QTL marker associations among different sets of breeding material, loss of linkage between marker and QTL due to the recombination which might occur between the marker and QTL (Jiang, 2013).

1.5 Responses to temperature in crop models

Many crop growth models have been developed to describe plant response to environmental factor, such as temperature. In modeling development rate to temperature, current models have adopted various functions. Both crop models, Sirius (Jamieson et al. 1998b) and CERES-Wheat (Ritchie and Otter, 1985) assume a simple linear relationship between temperature and development rate. Although of the simplicity of linear model using only two

parameters, it fails to account for the fact the temperatures greater than optimal delay growth (Yan and Hunt 1999; Parent and Tardiue 2014). To accommodate for the invalidity of linear function beyond optimum temperature, several crop models have adopted either bilinear model such as STICS (Brisson et al. 2008), and CropSyst (Stöckle et al. 2003) or three linear model in APSIM-maize (Hammer et al. 2010) and CropSIM (Hunt and Pararajasingham 1995). Although, the three linear-models is more close to reality than bilinear models, the greater number of parameters used, results in calibration error, which renders the application of this function (Yan and Hunt 1999). Since the segmented linear model consists of a combination of linear equations, which introduces abrupt changes, curvilinear response temperature response functions have been introduced in several crop models such as beta function with four parameters in GECROS (Yan and van Laar, 2005).

1.6 Responses to temperature in cauliflower crop models

In cauliflower, several phenology models have been developed with major focus on scheduling and predicting harvest time. These models are mainly driven by temperature. Several studies divided the development of cauliflower from transplanting to harvest into three distinct phases: juvenile phase, vernalization phase and curd growth phase (Wiebe 1972abc; Wiebe 1973; Wurr et al. 1981; Booij and Struik 1990a; Grevsen and Olsen 1994; Kage and Stützel 1999; Wurr et al. 2004). Different temperature response functions were used for the different development phases. Grevsen and Olesen (1994a) developed a model for cauliflower from transplanting to curd initiation. They described the duration of juvenile phase by a simple temperature sum model and the duration of curd induction phase by bi-linear function of temperature which was symmetrical below and above an optimal temperature with three cardinal temperatures of 0, 12.5 and 25.6 °C. Wurr et al. (1993) assuming the juvenility ends at 17 leaves in cultivar White Fox, they examined different segmented temperature response function forms to predict the time of curd initiation and

indicated that a three-stage linear temperature function with four cardinal temperatures of 9, 9, 9.5 and 21 °C was appropriate for this variety. Kage and Stützel (1999) developed cauliflower harvest prediction model. The model is a combination of empirical relationships derived from field data of two varieties Fremont and Linday. The juvenile phase was described by expolinear function for the relation between temperature sum and leaf number. The vernalization process started after end of juvenile and was simulated according to Wiebe (1972b) using a three segmented linear model with for cardinal temperatures -2, 10, 13, and 28 °C. On the other hand, other authors used simple models predicted the time of harvest based on curd samples after initiation which are used to predict the number of days that are required to reach a certain size using the relation between curd diameter and temperature sum (Salter 1996; Wurr et al. 1990ab; Jensen and Grevsen, 2005). Wurr et al. (1990b) developed a model using quadratic function between the logarithm of curd diameter and accumulated degree days to predict when a curd of specified size will be produced. Web-based forecast of cauliflower harvest time use a second order polynomial relationship between the natural logarithm of the curd diameter and temperature (Jensen and Grevsen 2005).

Although these models have the potential to predict the phenotype of a certain genotype under wide range of environments, they are derived empirically (Kage and Stützel, 1999) or through calibration (Wurr et al. 1993) and designed specifically for limited number of genotypes on the basis of phenotypic observations and not the genotype in terms of genetic makeup. Thus, they are restricted to the environments and genotype tested (Reymond et al. 2003) and lack real genetic information which interact with environment to produce the phenotype (Baenziger et al. 2004).

1.7 Integration of crop modeling and QTL mapping

In quantitative genetics a phenotype is the result of the expression of the genotype, the environment and the interactions between genotype and environment. Genotype by environment interaction (GEI) is a common phenomenon in complex agronomic trait where changes in the relative performance of a genotype across different environments can be observed. Despite the identification and the potential of numerous quantitative trait loci involved in the control of different plant traits in different plant species, the implementation in plant breeding is not straightforward and greatly hinders by GEI. The incidence of GEI effect influences the selection of superior genotypes for a target population of environments. Plant breeders traditionally take into account this effect by the use of multi-environmental trials to assess cultivar adaptation within a target population of environments. The performance of a genotype across environments determines the significance of the interaction. In the absence of GEI, the performance of a genotype remains stable across environments, whereas the presence of the GEI confirms the superiority of a particular genotype in particular environments. As a result, many QTLs are only detected in narrow range of environmental conditions and their use is restricted to the corresponding environments. To overcome this difficulty, a growing interest in the use of ecophysiological model (Yin et al. 2003). Ecophysiological model have been proved to be able to predict phenotypic trait of a genotype under wide range of environmental conditions, and therefore it has the potential to resolve genotype-by-environment interactions (Yin et al. 2003; Hammer et al. 2005). The physiological model describes the multi phenotypic responses of a given genotype to diverse environment by a set of parameters. Values of these parameters can explain the differences among genotypes on the basis of their genotypic specific parameters. Such parameters are often referred to as ‘genetic coefficient’ (Baenziger et al. 2004), indicating that the variation of these parameters is under genetic control (Reymond et al. 2003). However, these genetic coefficients are estimated from phenotypic observations designed specifically for their

estimation which likely include some impact of environment and lack the incorporation of direct genetic information (Baenziger et al. 2004). Given the potential that model parameters are genetically controlled, the variability in parameters values in a breeding population can be used to dissect the genetic basis of the model parameters (Yin and Struik, 2010). In the view of added value of crop modeling to classical quantitative genetics, combining ecophysiological model and quantitative trait loci has been suggested as an approach to understand and predict complex traits variation across environments (Reymond et al. 2003; Yin et al. 2004; Hammer et al. 2006; Collins et al. 2008). Ultimately, this approach offer in *silico* prediction of a phenotype through the genotype (Hammer et al. 2006; Bertin et al. 2010).

The principle of this approach is to develop a response growth curve for each individual, estimate curve individual-specific parameters, then treat the curve parameters estimates as phenotypes in QTL analysis (Wu et al. 2002), and finally incorporating the estimated effect of QTLs of the model parameters back to the ecophysiological crop model by replacing the model parameters by their QTL effect estimated value.

Several studies have used the complementary aspects of crop modeling and QTL mapping as a tool to assist plant breeding and to predict the phenotype. Yin et al. (2000b) using SYP-BL crop growth model (Yin et al. 2000a) to assess the ability of SYP-BL model with QTL based estimates of physiological input parameters to predict the yield and shoot biomass of recombinant inbred lines of barley (*Hordeum vulgare* L.). The QTL based model predicted the yield and the shoot biomass with accuracy similar to the SYP-BL model. In maize Reymond et al. (2003) established response curves of leaf elongation rate to three key environmental variables meristem temperature, evaporative demand and soil water status. The parameters of these responses were estimated for 100 recombinant inbred lines (RILs) mapping population and identified QTLs for these parameters. The combined model was used

to predict the leaf elongation rate for 11 new (RILs) grown under six regimes and could account for 74 % of the phenotypic variability of leaf elongation rate. Nakagawa et al. (2005) in their attempt to quantify the thermal time and photoperiod responses of flowering time in rice (*Oryza sativa* L.) in back cross inbred lines, They used a three stage beta model which parameterizes the sensitivity to temperature; sensitivity to photoperiod was applied and QTLs for the model parameters were identified. The QTL based model could explain 81 % of the phenotypic variation in flowering time. Recent model developed by Uptmoor et al. (2012) based on earlier model (Uptmoor et al. 2008), used genotype specific parameters and QTL effects as inputs to a model for predicting flowering time in *Brassica oleracea*. The QTL based model explained 66 % and 56 % of the phenotypic variation for time to floral induction and time to flowering, respectively. In bread wheat (*Triticum aestivum* L.) Bogard et al. (2014) using two parameters of an ecophysiological model representing the vernalization requirements and photoperiod sensitivity proposed a QTL-based model which explained 48 to 63 % of the variation in heading date of an independent validation set.

1.8 The problem

Harvest timing of many vegetable crops, including cauliflower, plays a major role in determining produce prices. In temperate zone, cauliflower producers stagger plantings to improve crop continuity to the market. However, if high temperature occurs during the temperature sensitive stage a delay in curding time of some plantings will occur and does not mature in their scheduled expected order which results in either peaks or troughs in product supply which reflects in prices fluctuations.

Understanding the genetic basis of the control of curding time and its related traits associated with temperature effect would help plant breeder to develop cultivars with reliable harvest time, and therefore improve the predictability of harvest time (Dixon, 2007). Developing

cultivars wide adapted to high temperature is a major breeding goal (Sharma et al. 2004; Farnham and Björkmann 2011; Uptmoor et al. 2012)

1.9 Goals of the present dissertation

The first objective of this study was to identify quantitative trait loci (QTL) in *B. oleracea* var. *botrytis* genome affecting curding time and its related traits with respect to temperature sensitivity as primary prerequisite to improve reliability in harvest time of cauliflower and therefore more predictable harvest time.

The second objective was to develop a QTL based phenology model. To achieve this goal, the specific objectives were: First, parameterization of two phase linear phenology model describes the rate of development, from transplanting to curd initiation, in relation to temperature for each DH line of a mapping population. Second, identify the genetic basis of the model parameters. Third, incorporate the estimated QTL based parameters into the phenology model by replacing the model parameters by their estimated QTL effect.

The third objective was to use and test the ability of the QTL based model to predict the curding time in cauliflower under diverse environments.

CHAPTER 2

Quantitative trait loci controlling leaf appearance and curd initiation of cauliflower (*Brassica oleracea* var. *botrytis*) in relation to temperature

Yaser Hasan[1], William Briggs[2], Claudia Matschegewski[4], Frank Ordon[3], Hartmut Stützel[1], Holger Zetzsche[3], Ralf Uptmoor[4]

[1]Institute of Horticultural Production Systems, Leibniz Universität Hannover, Herrenhäuser Str. 2, Hannover 30419, Germany

[2]Syngenta Seeds BV, Westeinde 62, 1601 BK Enkhuizen, The Netherlands

[3]Julius Kuehn-Institute, Federal Research Center for Cultivated Plants, Institute for Resistance Research and Stress Tolerance, Erwin-Baur-Str. 27, 06484 Quedlinburg, Germany

[4]Department of Agronomy, University of Rostock, Justus-von-Liebig-Weg 6, 18059 Rostock, Germany

Correspondence: Ralf Uptmoor, ralf.uptmoor@uni-rostock.de, phone: +49 381 498 3060, fax: +49 381 498 3061

This chapter has been published in Theoretical and Applied Genetics (doi: 10.1007/s00122-016-2702-6)

2.1 Abstract

Temperature is the main environmental factor influencing curding time of cauliflower. Temperatures above 20-22 °C inhibit the development towards curd even in many summer cultivars. To identify quantitative trait loci (QTL) controlling curding time and its related traits in a wide range of different temperature regimes from 12-27 °C, a doubled haploid (DH) mapping population segregating for curding time was developed and days to curd initiation (DCI), leaf appearance rate (LAR), and final leaf number (FLN) were measured. The population was genotyped with 176 single nucleotide polymorphism (SNP) markers. Composite interval mapping (CIM) revealed repeatedly detected QTL for DCI on C06 and C09. The estimated additive effect increased at high temperatures. Significant QTL x environment interactions (Q x E) for FLN and DCI on C06 and C09 suggest that these hotspot regions have major influences on temperature mediated curd induction. 25 % of the DH-lines did not induce curds at temperatures higher than 22 °C. Applying a binary model revealed a QTL with LOD >15 on chromosome C06. Nearly all lines carrying the allele of the early maturing parental line (PL) on that locus induced curds at high temperatures while only half of the DH lines carrying the allele of the unreliable PL reached the generative phase during the experiment. Large variation in LAR was observed. QTL for LAR were detected repeatedly in several environments on C01, C04 and C06. Negative correlations between LAR and DCI and QTL co-localizations for both traits on C04 and C06 suggest that LAR has also effects on development towards curd induction.

2.2 Introduction

Floral transition is a major development switch that is controlled by regulatory pathways, which integrate endogenous and environmental cues (Koornneef et al. 2004). Four main regulatory pathways have been described in *Arabidopsis thaliana*: the photoperiodic pathway,

the autonomous pathway, the vernalization pathway, and the gibberellin pathway (Simpson and Dean 2002).

While *A. thaliana* seeds can be vernalized, *B. oleracea* vegetables undergo two major post-embryonic development transitions, the juvenile–to-adult transition (vegetative phase change) and the adult-to-reproductive transition (floral induction). During the juvenile phase, plants are incompetent to initiate reproductive development even when grown under favorable conditions. Juvenility of cauliflower ends after a specific number of initiated leaves (Hand and Atheron 1987; Wurr et al. 1993). The critical number of initiated leaves is assumed to be genotype dependent, but might be influenced by the environment as well (Wurr et al. 1994).

During the adult vegetative phase, curd induction is sensitive to temperature. High temperatures delay thermal time to curd initiation and increase the total number of initiated leaves (Booij and Struik 1990; Fujime and Okuda 1996; Fellows et al. 1999). While relatively moderate temperature conditions promote curd induction, high temperatures affect the rate of development towards floral induction and subsequently increase thermal time to harvest (Sadik 1967; Salter 1969; Duclos and Björkman 2008).

Growers stagger cauliflower planting-dates to ensure continuous market supply. However, overlapping in maturity time of different plantings occurs if the temperature is high during the temperature sensitive stage of some of the plantings (Wiebe 1980; Wiebe 1990; Booij and Struik 1990; Olesen and Grevsen 2000), which frequently leads to fluctuations in availability and price of the product. The different cultivars of the crop have large variations in optimum temperatures for the development towards curd induction and are adapted to diverse climates from the temperate zone to the tropics. Within the temperate climate winter cultivars with strong vernalization requirement can be distinguished from spring and summer types with shorter vegetation periods and less sensitivity to temperature. It is supposed that a certain

vernalization requirement or at least a facultative response is causative for the temperature sensitivity of spring, summer and even tropical cultivars (Friend et al. 1985).

Temperature response functions for crop development from end of juvenility to floral induction may have four cardinal temperatures: the minimum temperature below which no development occurs, the minimum and maximum optimum temperature between which development rate reaches its maximum and the maximum temperature above which no development occurs. Temperature optimum of most spring and summer cultivars of the temperate region ranges between 10-16°C (Wiebe 1972a, b, c; Wurr et al. 1981; Wurr et al. 1990; Hand and Atherton 1987; Booij and Struik 1990). Nieuwhof (1969) assumed that temperatures above 23 °C are inhibitory for curd initiation.

The regulation of floral induction pathways has been extensively studied in the model plant *A. thaliana*. The *Brassica* genus is closely related to *Arabidopsis.* In both genera, *FLOWERING LOCUS C* (*FLC*) is a primary determinant of vernalization requirement. *FLC* acts as dosage-dependent repressor of flowering (Michaels and Amasino 1999) and is downregulated during vernalization. *FLC* causes a delay in flowering by repressing the expression of the floral integrator *FLOWERING LOCUS T* (*FT*). Lin et al. (2005) could show that a decrease in the *FLC* transcript level was correlated with an increase in *FT* transcript level in the apex. While *A. thaliana* contains only one *FLC* gene, *Brassica* crops have multiple copies of *FLC*. Five copies each were described in *B. rapa* and *B. oleracea* and nine in *B. napus* (Lagercrantz et al.1996; Tadege et al. 2001; Okazaki et al. 2007; Schranz et al. 2002; Yuan et al. 2009; Zhao et al. 2010) Recently, it was shown that vernalization reduced the transcription levels of *BoFLC2* and *BoFLC3* and upregulated expression levels of the flowering integrator *BoFT* in cauliflower (Ridge et al. 2014). *FLC* expression is upregulated by *FRIGIDA* (*FRI*). In *Arabidopsis*, allelic variation at the *FRI* locus is a major determinant of natural variation in flowering time (Clarke and Dean 1994). Dominant alleles of *FRI* confer late flowering, which

is reversed to earliness by vernalization (Johanson et al. 2000). Two *BoFRI* loci have been mapped to regions on C03 and C09, which are syntenic to chromosome 5 of *Arabidopsis* (Irwin et al. 2012). Four *FRI* homologs were described in *B. napus*; at least one gene co-localized to a major flowering time QTL cluster (Wang et al. 2011a).

Temperature effects on meristem identity genes have been identified in *Arabidopsis* (Bowman et al. 1993) and *B. oleracea* (Anthony et al. 1996). Two functional and one non-functional *APETALA1* (*AP1*) orthologs were isolated in the *Brassica* genome: *BoAP1-a* (Anthony et al. 1996), *BoAP1-c* (Smith and King 2000), and *BoAP1-b* (Lowman and Purugganan, 1999). *BoAP1* expression was switched off during vegetative reversion of cauliflower plants grown at temperatures above 25 °C (Anthony et al. 1996). Kop et al. (2003) suggested that *BoAP1-a* plays a role in controlling bract development, which predominantly occurs at high temperatures during curd development.

Quantitative trait loci for flowering time were detected in different *Brassica* mapping populations and some QTL regions overlapped with *FLC* orthologs (Lagercrantz et al. 1996; Osborn et al. 1997; Bohuon et al. 1998; Rae et al. 1999; Sebastian et al. 2002; Schranz et al. 2002; Brown et al. 2007; Long et al. 2007; Zhao et al. 2010; Uptmoor et al. 2012). However, it was also shown that several of the flowering time QTL did not co-segregate with the *FLC* loci in *B. oleracea* (Razi et al. 2008) or that the underlying *FLC* gene was not functional (Okazaki et al. 2007).

Most QTL studies for abiotic stress traits are carried out in only two controlled environments or in multi-environment field trials. Experiments under controlled conditions have the advantage that one environmental factor (e.g., temperature) can be widely varied with precision without influencing other environmental factors (e.g., photoperiod, light intensity, humidity). Fiedler et al. (2014) have shown that finding optimum conditions, reflecting the best compromise between maximum phenotypic variance and error variance as a result of

environmental stress is often difficult. We used multiple controlled environments with different temperatures for the present study and hypothesized that loci controlling temperature sensitivity during cauliflower development should appear as QTL in several high temperature environments and that QTL for earliness *per se* should appear in all environments. The objective of the present study was to identify genomic regions influencing sensitivity to temperature, which are suitable to develop wide adaptation cultivars, i.e., cultivars with a reliable developmental time towards curd induction and harvest time under different temperature regimes.

2.3 Materials and Methods

2.3.1 Plant material and genotyping

A doubled haploid (DH) population of 161 lines was derived from anther culture of a F_1 cross between two homozygous parental lines (PLs). The material is not publicly available. The PLs were differing in reliability in time to curd induction. The unreliable PL (P1) produces high quality curds, but shows increased time to maturity and a broader harvest window (i.e., more harvesting passages due to higher variation in time to maturity) at unfavorable high temperature conditions. Harvest-time delay and variability of the reliable PL (P2) is less pronounced at high temperatures. The population was genotyped with 176 single nucleotide polymorphisms (SNP) markers. The genetic map was constructed using MapMaker 3 and the Haldane function. The total map length was 891.2 cM spanning over nine chromosomes. Average distance between SNP markers was 5.3 cM.

2.3.2 Growing conditions and experimental setup

Two seeds of each DH line and PLs were sown into seedling trays filled with Potgrond P (Klasmann-Deilmann GmbH). Seedlings were raised in a greenhouse at 22°C and a photoperiod of 16 h until all plants had four to five visible leaves. Plants were thinned to one

seedling per line at the two-leaf stage. Seedlings were then transplanted into three-liter pots filled with the same substrate and placed into a greenhouse in a randomized complete block design with four replicates. Plants were grown at a photoperiod of at least 16 h. If natural day length was below 16 h and/or radiation was low, 200 µmol $m^{-2}s^{-1}$ additional photosynthetically active radiation (PAR) at plant height was provided by 400 W Phillips SON-T Agro lamps. Plants were fertigated daily with 0,5 g l^{-1} Scotts Universal solution. Fungicides were applied if necessary.

Air temperature was measured at the level of plant height every 10 minutes using TinyTag View 2 data loggers (Gemini Data Loggers Ltd., West Sussex, UK). The daily mean/min/max temperatures recorded inside the greenhouse for six temperature treatments were 11.8/10.6/15.5, 15.5/13.4/18.9, 17.3/15.5/19.6, 19.0/17.0/21.1, 21.4/16.0/22.8 and 27.0/22.2/28.6 °C. Standard deviations of daily means were 0.2, 1.4, 0.8, 1.1, 0.9 and 1.8 °C. An additional temperature treatment was conducted in growth chambers with two replicates per line. Mean/min/max temperature during this treatment was 26.5/22.5/27.6 °C with 16 h light with an intensity of 550 µmol m^{-2} s^{-1}. All experiments were terminated 120 d after transplanting.

2.3.3 Phenotypic measurements

In every temperature treatment, days to curd initiation (DCI) were recorded twice a week. DCI was considered to have occurred when the diameter of the curd was ≥ 1 cm, which means that true time to floral induction occurred before DCI but DCI is the first visible indication that floral induction already took place. DH lines, which remained vegetative 120 d after transplanting, were considered as missing values. The number of unfolded leaves larger than 1 cm was counted twice a week for the duration of four weeks after transplanting. Leaf appearance rate (LAR) was defined as the slope of the regression of leaf number on growing

degree-days (GDD) with a base temperature of 0°C. Final leaf number (FLN) was counted at the end of each treatment.

2.3.4 Data analysis

Statistical analyses were conducted using SAS 9.3 (SAS Institute 1999). Analysis of variance was conducted using a general linear model. Variance components were used to estimate the broad sense heritability (h^2) as described by Hallauer and Miranda (1988). Pearson's correlation coefficients were computed between all traits in all temperatures.

QTL were identified across environments and in each environment separately by composite interval mapping (Zeng 1994) using the software PLABQTL (Utz and Melchinger 1996). Analyses were based on multiple regressions as described by Haley and Knott (1992). Co-factors were selected by stepwise regressions according to Miller (1990) with a default value for F-to-enter and F-to-delete of 3.5. Permutation tests with 1000 permutations were carried out in order to estimate LOD thresholds to declare the existence of a QTL at the experimental wise error level of $\alpha = 0.05$. QTL were graphically displayed using MapChart 2.2 (Voorrips 2002). Values of non-curding lines were replaced by 120 d for QTL analysis across environments. FLN was calculated as LAR (1/°Cd) × temperature (°C) × 120 d + 4 (number of leaves at transplanting). QTL x environment (Q x E) interactions were computed following the procedure of Knapp (1994). Since not all DH lines developed a curd within the first 120 d after transplanting, a binary model with 0 for "no curd induction during the duration of the experiment" and 1 for "curd induced" was applied for DCI (Broman 2003, Broman and Sen 2009).

2.4 Results

2.4.1 Phenotypic variation

Parental line means, as well as minima, maxima, means and standard deviations for DCI, FLN, and LAR of the DH population in each temperature treatment are presented in Table 1. Significant genetic variation was found for all measured traits (Table 2). Differences between environments were significant as well as were genotype x environment interactions (GEI). Heritability was generally high while being highest for LAR and lowest for FLN.

The parental lines used to generate the mapping population exhibited large differences in DCI. In all environments, P2 induced the curd earlier than P1. Differences in DCI were higher at temperatures above 15 °C and visible curd initiation was completely inhibited in P1 at temperatures above 20 °C indicating P1's stronger sensitivity to temperature. Curding as well as non-curding leafy phenotypes were observed at high temperatures among the progenies. At 21 °C, 3.7 % of the DH lines did not induce a curd. At 26 °C, the percentage of non-curding lines increased to 22.4 % and was 25.16 % at 27 °C. Large variation in LAR was observed. P2 had higher LAR than P1. Apparent variation in LAR was also observed among DH lines. FLN did not differ much between parental lines when both were curding. DH lines showed large variation in FLN particularly at high temperatures (Table 1). FLN increased with increasing temperatures.

Boxplots for all traits and environments are shown in Fig. 1. Substantial transgressive segregation in one direction was observed for all traits. Apparently, a large proportion of the population initiated the curd much earlier than the two parents in all environments (Fig. 1; Table 1).

Table 1 Parental lines means, DH-population minimum, maximum, means and standard deviation for day to curd initiation (DCI), final leaf number (FLN) and leaf appearance rate (LAR) across environments

Trait	Temp. (°C)	Parental line		DH population			
		Mean (P1)	Mean (P2)	Min	Max	Mean	SD[a]
DCI	12	72.3	66.1	41.0	79.4	63.8	5.5
	16	55.5	47.7	31.0	55.5	44.0	4.3
	17	66.0	42.5	32.0	63.3	44.9	5.1
	19	57.2	43.5	29.3	55.0	41.6	4.6
	21	-	51.7	33.9	82.5	53.7	11.2
	26	-	42.7	26.5	63.0	40.8	7.2
	27	-	58.0	32.4	95.0	58.9	14.1
FLN	12	29.2	28.7	24.8	35.3	28.3	1.7
	16	27.7	27.7	22.0	35.0	27.2	2.2
	17	32.8	30.5	23.0	39.0	29.9	2.6
	19	34.3	30.3	25.0	70.0	31.6	3.0
	21	-	38.0	30.0	64.7	40.4	7.5
	26	-	37.5	29.5	56.0	39.2	6.2
	27	-	43.5	26.8	69.0	41.9	8.1
LAR (1 / °Cd × 1000)	12	16.0	20.5	16.0	27.0	21.2	2.0
	16	16.7	20.0	14.5	27.3	21.8	2.3
	17	15.7	20.7	11.3	32.3	22.0	2.8
	19	15.7	19.2	17.3	30.0	22.8	2.5
	21	16.2	23.0	14.0	31.8	22.3	2.9
	26	16.2	22.2	13.0	30.0	20.4	2.8
	27	16.5	24.2	13.0	33.8	20.6	3.1

[a] standard deviation

Table 2 Variance components and broad sense heritability for days to curd initiation (DCI), final leaf number (FLN) and leaf appearance rate (LAR)

Trait	Variance component				Heritability
	σ^2_G	σ^2_E	σ^2_{GxE}	σ^2_ε	h^2
DCI	24.43*	88.31*	23.84*	22.77	0.85
FLN	5.90*	34.10*	11.44*	8.96	0.75
LAR	4.12*	0.68*	1.33*	5.73	0.91

σ^2_E environmental variance
σ^2_G genotypic variance
σ^2_{GxE} genotype x environment interaction variance
σ^2_ε error variance, h^2 heritability
SD standard deviation
* statistically significant differences at $P < 0.001$

Correlation analysis showed significant negative correlations between DCI and LAR in all temperature treatments. *R* was between -0.33 and -0.73 (Table 3). There was a positive correlation between FLN and DCI at all temperatures. *R* ranged between 0.20 and 0.80 and increased with increasing temperatures. FLN and LAR were significantly correlated particularly at high temperatures, while being highest at 27 °C (*R* = -0.51). *R* between DCI and LAR across environments was -0.78 and 0.51 between DCI and FLN. *R* between LAR and FLN was -0.1.

Fig. 1 Boxplots for thermal time and days to curd induction, final leaf number and leaf appearance rate at seven temperatures. Whiskers give the 0.1 and 0.9 percentile, boxes the 0.25, 0.5, and 0.75 quartile.

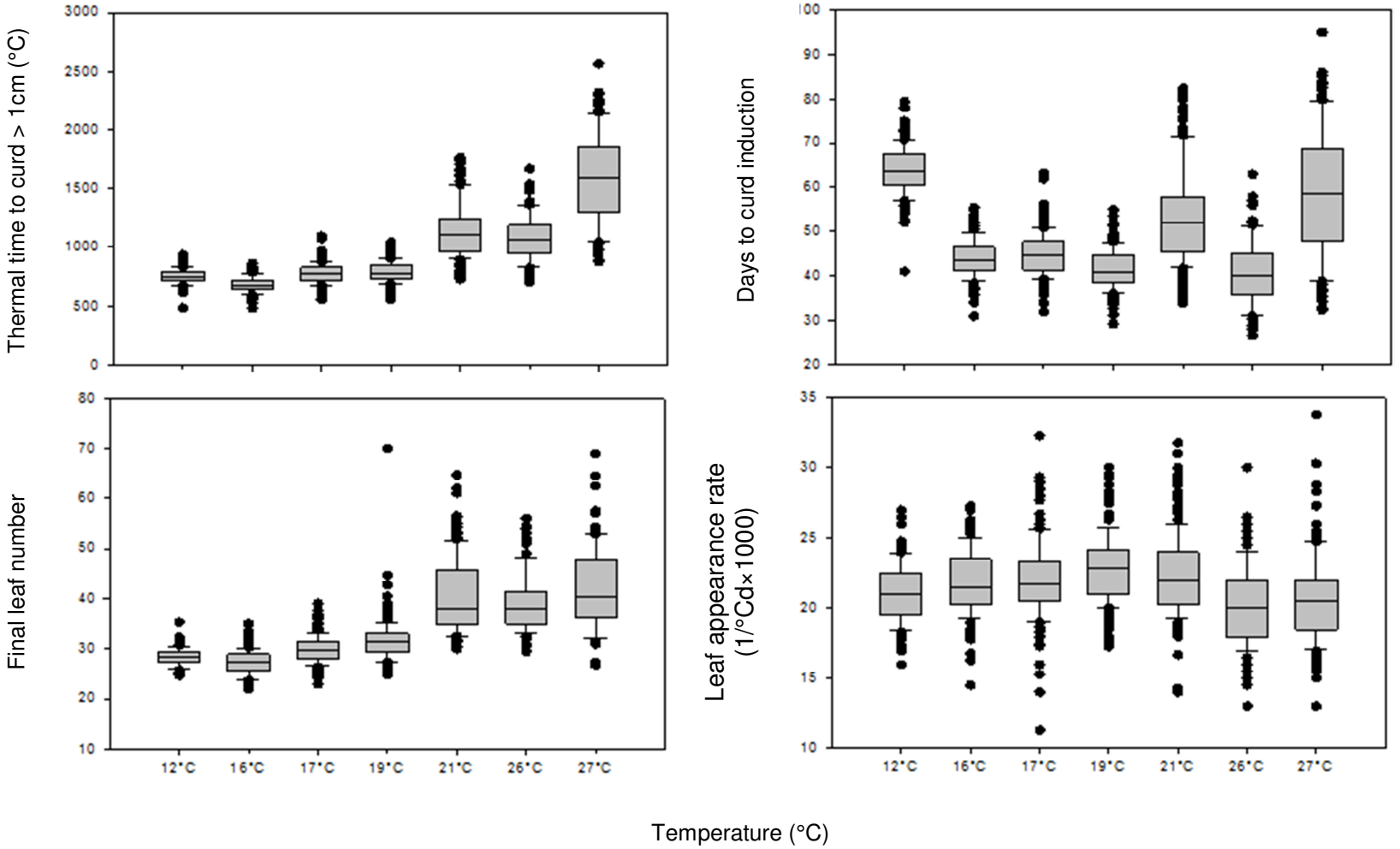

Table 1 Pearson's correlation coefficients among the traits days to curd initiation (DCI), leaf appearance rate (LAR), and final leaf number (FLN) in seven temperatures.

Trait	DCI 12	DCI 16	DCI 17	DCI 19	DCI 21	DCI 26	DCI 27	LAR 12	LAR 16	LAR 17	LAR 19	LAR 21	LAR 26	LAR 27	FLN 12	FLN 16	FLN 17	FLN 19	FLN 21	FLN 26	FLN 27
DCI16	0.74 ***	1																			
DCI17	0.73 ***	0.75 ***	1																		
DCI19	0.65 ***	0.81 ***	0.74 ***	1																	
DCI21	0.53 ***	0.57 ***	0.62 ***	0.69 ***	1																
DCI26	0.56 ***	0.56 ***	0.63 ***	0.55 ***	0.63 ***	1															
DCI27	0.50 ***	0.46 ***	0.62 ***	0.57 ***	0.78 ***	0.69 ***	1														
LAR12	-0.63 ***	-0.54 ***	-0.57 ***	-0.55 ***	-0.45 ***	-0.33 ***	-0.39 ***	1													
LAR16	-0.43 ***	-0.67 ***	-0.53 ***	-0.66 ***	-0.44 ***	-0.42 ***	-0.38 ***	0.58 ***	1												
LAR17	-0.50 ***	-0.57 ***	-0.67 ***	-0.57 ***	-0.48 ***	-0.53 ***	-0.56 ***	0.58 ***	0.59 ***	1											
LAR19	-0.37 ***	-0.57 ***	-0.48 ***	-0.69 ***	-0.42 ***	-0.38 ***	-0.47 ***	0.59 ***	0.83 ***	0.62 ***	1										
LAR21	-0.47 ***	-0.54 ***	-0.59 ***	-0.57 ***	-0.63 ***	-0.54 ***	-0.63 ***	0.58 ***	0.59 ***	0.66 ***	0.66 ***	1									
LAR26	-0.33 ***	-0.38 ***	-0.44 ***	-0.44 ***	-0.43 ***	-0.48 ***	-0.49 ***	0.27 ***	0.42 **	0.42 **	0.44 **	0.59 **	1								
LAR27	-0.51 ***	-0.57 ***	-0.60 ***	-0.63 ***	-0.65 ***	-0.56 ***	-0.73 ***	0.46 ***	0.60 ***	0.58 ***	0.65 ***	0.82 ***	0.68 ***	1							
FLN12	0.36 ***	0.14	0.16	0.01	0.02	0.17	0.10	0.00	0.35 ***	0.08	0.37 ***	0.14	0.06	0.13	1						
FLN16	0.57 ***	0.45 ***	0.43 ***	0.32 ***	0.30 ***	0.35 ***	0.20 *	-0.23 ***	0.09	-0.14	0.12	-0.10	-0.13	-0.11	0.72 ***	1					
FLN17	0.57 ***	0.46 ***	0.57 ***	0.41 ***	0.31 ***	0.34 ***	0.23 **	-0.23 **	0.04	-0.18	0.06	-0.08	-0.10	-0.16	0.66 ***	0.77 ***	1				
FLN19	0.55 ***	0.42 ***	0.53 ***	0.43 ***	0.34 ***	0.32 ***	0.20 *	-0.26 ***	0.06	-0.21 **	0.10	-0.06	-0.12	-0.10	0.61 ***	0.76 ***	0.85 ***	1			
FLN21	0.48 ***	0.40 ***	0.52 ***	0.54 ***	0.79 ***	0.50 ***	0.47 ***	-0.32 ***	-0.12	-0.33 **	-0.14	-0.29 ***	-0.26 **	-0.32 ***	0.34 ***	0.50 ***	0.61 ***	0.69 ***	1		
FLN26	0.49 ***	0.39 ***	0.52 ***	0.42 ***	0.48 ***	0.67 ***	0.39 ***	-0.24 **	-0.02	-0.32 ***	-0.04	-0.23 *	-0.20 *	-0.21 *	0.52 ***	0.60 ***	0.65 ***	0.70 ***	0.80 ***	1	
FLN27	0.54 ***	0.42 ***	0.61 ***	0.50 ***	0.71 ***	0.63 ***	0.80 ***	-0.30 ***	-0.16	-0.45 ***	-0.24 *	-0.43 **	-0.36 ***	-0.51 ***	0.32 ***	0.46 ***	0.52 ***	0.54 ***	0.78 ***	0.71 ***	1

Correlations between traits are statistically significant at $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***)

2.4.2 QTL detection

A total of 90 QTL for the traits DCI, FLN, and LAR were identified in individual environments and 17 QTL were detected across environments. Tables 4, 5, 6, 7 summarize the estimated positions, LOD scores, phenotypic variation explained, and additive effects of significant QTL.

A total of 31 significant QTL for DCI were mapped on eight linkage groups. QTL for DCI were detected repeatedly in several environments on C06 and C09 (Fig. 4; Table 4). Other interesting regions are on C04, C05, and C07. QTL were detected at three temperatures on C04 between markers S0292 and S0815. The phenotypic variation explained ranged from 12.1 to 16.3 % with additive effects from -1.0 to -3.7. QTL for DCI were identified on C05 at 12, 17, and 19 °C. The QTL detected at 12 °C had a LOD score of 16.8 and explained 38 % of phenotypic variation. The twelve QTL for DCI on C06 were detected in three main groups at 0 cM, between 18 and 24 cM, and between 32 and 34 cM. All three regions were supported by across environment QTL, which showed significant Q x E interactions. Individual QTL on C06 explained between 12.2 and 40.7 % of phenotypic variation. Estimated effects of the QTL were large at temperatures higher than 20 °C (-4.4 to -11.3 d). On C07, QTL for DCI were detected in four environments. The region with three QTL was also supported by a across environment QTL with significant Q x E interactions. A QTL hotspot on C09 shows DCI QTL in all temperature regimes apart from 19 and 27 °C. Larger QTL effects (-2.5 to -3.1 d) were observed at temperatures higher than 20 °C. QTL explained between 17.3 and 22.1 % of the phenotypic variation. The allele of P2 accelerated curd induction in all cases except at one QTL locus detected at 12 °C on C03.

Table 4 Positions of putative QTL detected in different temperature regimes, nearest markers, and physical position of the nearest marker (TO1000 genome), LOD scores, additive effects (Add.), and variation explained (R^2) for days to curd initiation.

Temperature (°C)	Linkage group	Position		Nearest marker	LOD	R^2	Add.
		(cM)	(bp)				
12	C03	30	-	S1064	4.8	12.7	1.3
	C05	14	5,586,824	S0546	16.8	38.1	-2.6
	C06	0	2,396,965	S1134	4.9	13.3	-1.3
	C06	32	31,001,053	S0374	8.6	21.8	-1.9
	C07	28	31,643,632	S1081	4.3	11.6	-1.4
	C08	32	35,763,986	S0352	5.5	14.5	-1.8
	C09	54	47,737,604	S0256	8.6	21.8	-1.6
16	C06	0	2,396,965	S1134	4.5	12.2	-1.0
	C06	32	31,001,053	S0374	5.2	13.7	-1.2
	C07	28	31,643,632	S1081	4.6	12.2	-1.2
	C09	58	49,393,351	S0629	6.7	17.3	-1.3
17	C03	70	33,660,097	S1028	4.2	11.3	-1.8
	C05	30	18,578,045	S1071	7.3	18.8	-1.6
	C06	20	24,053,953	S1114	4.8	12.9	-1.7
	C06	34	32,446,947	S0509	4.9	13.1	-4.9
	C09	54	47,737,604	S0256	6.8	17.6	-1.7
19	C04	128	32,446,947	S1137	4.7	12.6	-1.0
	C05	38	38,138,365	S0240	5.8	15.3	-1.1
	C06	0	2,396,965	S1134	10.2	25.7	-1.7
	C06	32	31,001,053	S0374	9.1	23.0	-1.6
21	C01	56	16,017,838	S1098	4.1	11.6	-2.4
	C04	128	32,446,947	S1137	4.3	12.1	-2.4
	C06	22	25,595,952	S0624	5.0	13.9	-4.7
	C07	24	31,643,632	S1081	7.2	19.4	-4.1
	C09	50	46,964,041	S0533	6.7	18.2	-3.1
26	C06	24	31,643,632	S0603	13.7	40.7	-4.4
	C09	46	46,994,768	S0501	6.6	22.1	-2.5
27	C04	112	42,741,416	S0292	4.5	16.3	-3.6
	C06	18	24,053,953	S1114	11.8	37.3	-8.1
	C06	34	32,446,947	S0509	5.4	19.2	-11.3
	C07	8	11,551,682	S1077	4.7	17.9	-3.6

At both high temperatures, 26 and 27 °C, number of curding to non-curding genotypes segregated at a ratio of 3:1 ($\chi^2 = 0.54 < \chi^2_{0.05,1} = 3.84$, p = 0.46, $\chi^2 = 0.002 < \chi^2_{0.05,1} = 3.84$, p = 0.96, respectively). The binary data analysis revealed one major QTL on C06 with LOD > 15 at both temperatures (Fig. 2). LOD peaks were at 12 (15.5, 26 °C) and at 14 cM (17.6, 27 °C). The nearest marker was S1114. While comparing phenotypes of marker allele A genotypes to those carrying marker allele B, it became obvious that the majority of phenotypes with allele B were early curd inducing while less than 50 % of allele A plants visibly induced a curd during the experiment (Fig. 3). Twenty-eight out of 61 allele A carrying lines did not induce a curd at both temperatures. Despite similar mean temperatures, there was a strong difference in DCI between the climate-chamber experiment (26 °C) and the greenhouse experiment (27 °C).

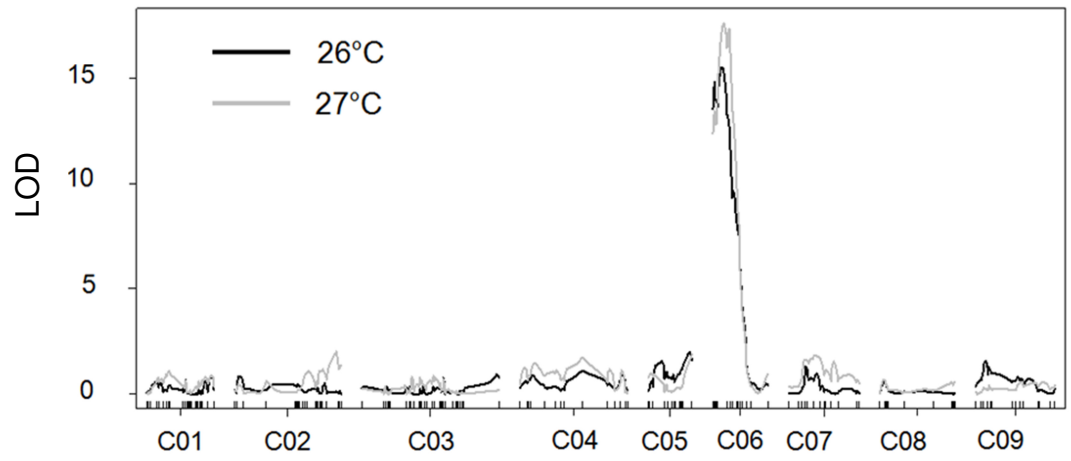

Fig. 2 LOD profile of a binary QTL analysis carried out on 161 *B. oleracea* DH lines with 1 = curd induction within the first 120 d after transplanting and 0 = no curd induced until 120d after transplanting. Experiments were carried out at 26 (black lines) and 27°C (grey lines). Segregation ratio curd induced to no curd was 3:1.

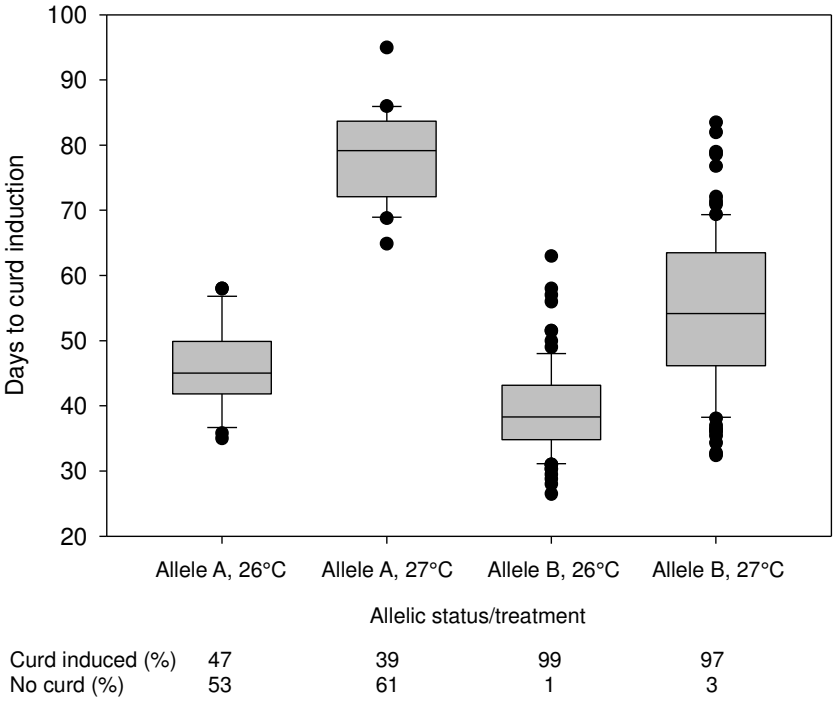

Fig. 3 Boxplots for days to curd induction of DH lines carrying the allele A or B at marker position S1114 on C06 at 26 (climate-chamber experiment) and 27°C (greenhouse experiment). Whiskers give the 0.1 and 0.9 percentile, boxes the 0.25, 0.5 (median), and 0.75 quartile. Values below and above the 0.1 and 0.9 percentiles are marked as black dots. Curding to non-curding phenotype ratios are given below the diagram.

A total of 29 significant QTL for FLN were detected on nine chromosomes (Fig. 4; Table 5). Seven QTL were detected in individual environments on C01, four of them were mapped at a hotspot near marker S0714. The other three QTL were located between 54 and 64 cM. The QTL on C09 explained between 12.5 and 25.1 % of the phenotypic variation. On the top of C04 between 0 and 12 cM, QTL for FLN were detected in six environments and explained between 13.7 and 25.7 % of phenotypic variation. On C06, five QTL for FLN were detected. They explained between 18.2 (16 and 26 °C) and 71.7 % (21 °C) of phenotypic variation. An across environment QTL with significant Q x E interactions was mapped at 12 cM with a LOD score of 42.2. A second across environment QTL with significant Q x E interactions was identified on C09.

Table 5 Positions of putative QTL detected in different temperature regimes, nearest markers, and physical position of the nearest marker (TO1000 genome), LOD scores, additive effects (Add.), and variation explained (R^2) for final leaf number.

Temperature (°C)	Chromosome	Position (cM)	Position (bp)	Nearest marker	LOD	R^2	Add.
12	C01	14	2,993,555	S0714	4.7	12.5	-0.4
	C01	54	16,017,838	S1098	4.3	11.6	0.4
	C03	120	63,658,940	S0568	5.0	13.4	-0.4
	C04	12	2,776,430	S0268	5.5	14.7	-0.5
	C05	2	4,487,966	S1065	9.7	24.2	-0.7
16	C01	14	2,993,555	S0714	6.2	16.3	-1.0
	C01	64	-	S0196	4.5	12.1	1.0
	C03	52	12,722,753	S0510	4.7	12.5	-0-7
	C04	12	2,776,430	S0268	10.1	25.3	-0.9
	C06	4	7,191,194	S0697	7.0	18.2	-0.7
	C09	56	47,737,604	S0256	4.5	12.1	-1.3
17	C01	14	2,993,555	S1076	4.9	13.0	-1.2
	C04	12	2,776,430	S0268	7.0	18.2	-0.9
	C07	78	44,290,525	S0580	4.4	11.7	0.6
	C08	10	27,764,158	S1099	4.9	13.0	-2.5
19	C04	12	2,776,430	S0268	5.1	13.7	-1.3
	C07	72	44,290,525	S0580	4.4	11.8	1.2
21	C01	8	1,974,096	S0550	4.5	12.6	-1.4
	C04	6	2,330,145	S0932	9.2	25.7	-2.4
	C06	10	8,249,736	S1058	42.2	71.7	-6.3
26	C05	24	13,976,621	S0254	5.2	18.1	-1.5
	C06	24	31,643,632	S0603	5.2	18.2	-2.9
	C09	52	46,964,041	S0533	7.5	25.1	-1.9
27	C01	66	39,540,366	S0306	5.1	18.4	-3.1
	C02	8	4,713,842	S0567	6.2	21.8	3.4
	C04	0	2,330,145	S0932	5.9	22.8	-2.4
	C06	0	2,396,965	S1134	5.8	20.8	-3.1
	C06	18	24,053,953	S1114	8.4	28.3	-4.3
	C07	14	23,522,707	S0976	5.4	19.3	-2.1

A total of 30 significant QTL for LAR were mapped in individual environments on seven chromosomes (Fig. 4; Table 6). While seven QTL for LAR on C01 co-localized to FLN QTL, the five QTL on C04 and seven QTL for LAR on C06 were co-localized to QTL for DCI. The LAR QTL on C01 were mapped in two main regions between 14 and 24 cM and between 54 and 58 cM. Across environment QTL were detected in both hotspot regions on C01 and at 32 cM on C06 (Fig. 4; Table 7).

Confidence intervals of QTL for the same trait were overlapping at many genomic regions and suggest a high reproducibility of results. However, at several other regions significant QTL were detected only at one temperature regime. For DCI, five QTL with no overlapping confidence intervals to QTL detected in other temperature regimes or to across environment QTL were identified on four chromosomes (Fig. 4). There was no region in which QTL were identified repeatedly only at the upper temperature extreme (26 and 27 °C).

Table 6 Positions of putative QTL detected in different temperature regimes, nearest markers, and physical position of the nearest marker (TO1000 genome), LOD scores, additive effects (Add.), and variation explained (R^2) for leaf appearance rate.

Temperature (°C)	Linkage group	Position (cM)	Position (bp)	Nearest marker	LOD	(R^2)	Add.
12	1	22	4,137,327	S0464	4.1	11.0	-0.6
	3	92	-	S1047	4.1	11.1	0.7
16	1	24	5,962,896	S1066	6.0	15.8	-0.7
	1	56	16,017,838	S1098	8.4	21.3	0.9
	3	32	11,335,498	S0623	5.6	14.8	-0.7
	4	128	32,446,947	S1137	6.2	16.2	0.7
	6	34	32,446,947	S0509	7.1	18.4	0.8
	8	52	35,763,986	S0352	4.8	12.9	0.9
17	4	116	42,805,083	S0354	5.7	15.2	1.0
	6	18	24,053,953	S1114	8.5	21.7	1.2
19	1	22	4,137,327	S0464	5.2	13.8	-0.7
	1	58	16,017,838	S1098	4.2	11.4	0.7
	3	62	23,340,859	S1031	4.1	11.1	-1.1
	3	122	64,912,969	S0395	4.6	13.0	-0.6
	4	118	42,805,083	S0354	12.3	29.1	1.1
	6	32	31,001,053	S0374	5.4	14.3	0.8
21	4	114	42,486,882	S1115	8.3	21.2	0.9
	6	20	24,053,953	S1114	4.2	11.4	0.9
	8	10	27,764,158	S1099	9.2	23.1	-1.1
	9	46	46,994,768	S0501	4.2	11.4	0.6
26	1	54	16,017,838	S1098	4.2	11.3	0.8
	6	6	8,249,736	S1058	4.1	11.0	0.8
	6	30	29,739,413	S0588	7.6	19.5	1.2
27	1	14	2,993,555	S0714	6.1	16.1	-0.7
	1	58	16,017,838	S1098	7.2	18.7	0.7
	2	96	50,748,828	S0992	8.9	22.4	-1.3
	4	128	32,446,947	S1137	4.6	12.3	0.5
	6	2	2,874,638	S0136	4.1	11.0	0.7
	6	32	31,001,053	S0374	4.6	12.4	0.9
	8	8	24,986,532	S1105	9.8	24.4	-1.0

Table 1 Across environment QTL and QTL x environment interactions (QxE) for days to curd induction (DCI), final leaf number (FLN), and leaf appearance rate (LAR). Additive effects are shown for means across environments and for individual environments.

Trait	Chromo-some	Position		Nearest Marker	LOD	R^2	Additive effects at different temperatures (°C)								Mean squares (QxE)	QxE
		(cM)	(bp)				All	12	16	17	19	21	26	27		
DCI (d)	C04	80	10,726,862	S0655	5.6	14.7	-3.0	-1.1	-1.1	-1.1	-0.9	-4.3	-9.2	-6.7	605.2	*
	C05	20	11,521,440	S0975	5.5	14.5	-2.7	-2.0	-1.1	-1.3	-0.8	-1.2	-0.4	-2.9	318.3	*
	C06	2	6,360,269	S1106	8.4	21.2	-4.3	-2.0	-1.7	-1.0	-1.6	-3.1	-12.6	-5.4	13635.4	*
	C06	14	24,053,953	S1114	5.6	14.9	-5.3	0.8	1.0	-0.7	0.2	-5.9	-9.1	-16.5	16946.1	*
	C06	32	31,001,053	S0509	6.1	15.9	-3.3	-1.7	-1.5	-1.2	-1.5	-4.1	-8.5	-7.0	8128.6	*
	C07	26	-	S0289	6.8	17.8	-3.2	-0.5	-0.6	-0.6	-1.4	-4.7	-0.5	-5.1	1487.2	*
	C09	54	47,737,604	S0256	8.9	22.5	-3.1	-2.2	-1.7	-1.6	-1.3	-3.9	-5.6	-3.2	729.2	*
FLN	C01	14	-	S1076	6.3	16.5	-1.0	-0.3	-0.3	-0.4	-0.8	-1.4	-0.4	-1.1	8.0	
	C05	18	11,521,440	S0975	6.1	16.0	-1.0	-0.7	-0.8	-0.8	-1.0	-0.2	-0.9	-1.1	10.6	
	C06	12	24,053,953	S1114	42.2	70.1	-3.7	-0.2	-0.9	-1.2	-1.8	-6.1	-7.7	-9.4	1822.2	*
	C07	82	45,623,104	S0728	8.1	20.8	1.2	0.0	0.3	0.6	0.9	1.1	1.7	1.5	5.0	
	C09	52	47,746,541	S0408	4.6	12.2	-0.9	-0.2	-0.5	-0.7	-0.6	-1.8	-2.4	-1.6	138.5	*
LAR (1 / °Cd x 1000)	C01	24	5,962,896	S1066	5.5	14.5	-0.6	-0.5	-0.8	-0.6	-0.8	-0.7	0	-0.5	12.6	*
	C01	48	12,537,551	S0729	6.3	16.4	0.7	-0.3	0.8	0.2	0.4	0.2	0.5	0.4	13.5	*
	C02	98	40,453,338	S1035	5.9	15.5	-0.9	-0.2	-0.1	-0.4	-0.4	-0.6	-0.3	-1.2	18.8	*
	C06	32	32,446,947	S0509	4.2	11.4	0.7	0.6	0.8	0.8	0.9	1.4	1.5	1.9	24.5	*
	C07	0	7,312,194	S1101	5.6	15.2	0.5	0.3	0.4	0.7	0.5	0.6	0.2	0.5	4.8	

* significant QTL x environment interactions ($P < 0.05$)

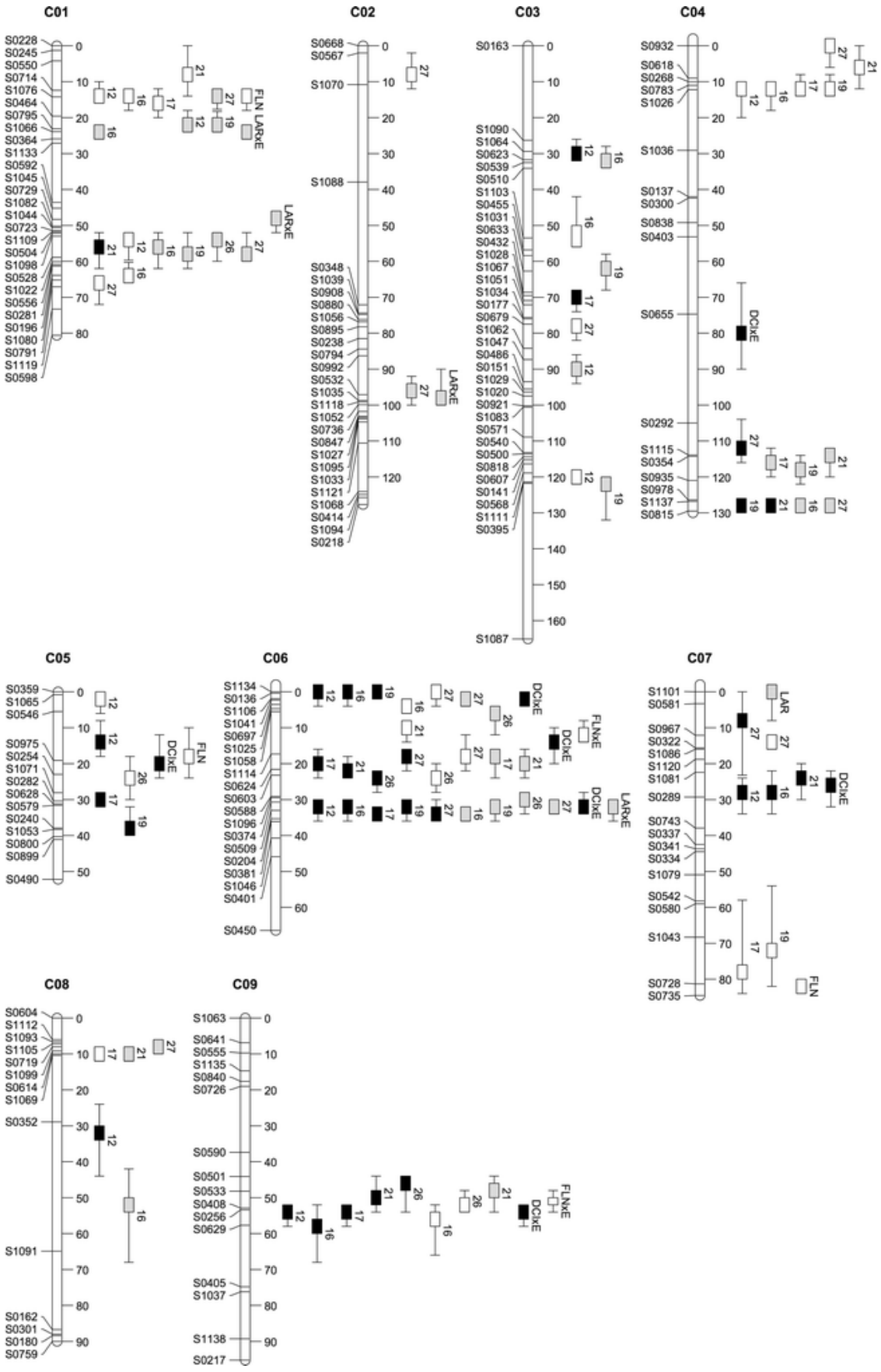

Fig. 4 Linkage map and QTL for days to curd initiation (DCI, *filled*), leaf apperence rate (*grey*), final leaf number (FLN, *unfilled*). Arabic numbers at QTL boxes indicate the temperature treatments between 12 and 27°C. Across environment QTL carry the abbreviation of the trait and xE if QTL x environment interactions were significant. Boxes indicate positions of LOD peaks ± 2 cM, whiskers the support intervals (LOD > 1).

2.5 Discussion

The present study identified 90 QTL, which individually explained between 11 and 72 % of the additive genetic variation of the three measured traits DCI, FLN, and LAR in single environments. The mean number of QTL detected per trait and environment was 4.3 on average. Although the 90 QTL were distributed over all nine linkage groups, a higher number of QTL was observed on C01, C04, and C06.

The study revealed promising QTL regions for DCI on C04, C05, C06, C07 and C09. Many QTL studies on flowering time were already performed in *B. oleracea*. Bohuon et al. (1998) detected QTL for flowering time on C02, C03, C05 and C09 in a *B. oleracea* var. *albogabra* × var. *italica* cross. Eleven flowering time QTL were identified on C01, C02, C03, C05 and C09 in backcross substitution lines of the same cross (Rae et al. 1999).

QTL alleles of P1 were in the present study almost always associated with increasing effects on DCI and FLN. However, each PL inherited alleles with both positive and negative effects for all traits. In accordance to that, transgression was observed for all traits. Bohuon et al. (1998) found that the early flowering PL of a *B. oleracea* cross segregating for flowering time inherited at least one late flowering QTL. Sebastian et al. (2002) found that an annual *B. oleracea* var. *botrytis* line carried an allele for vernalization while the biennial *B. oleracea* var. *gemmifera* line carried the opposite allele at the same position.

Composite interval mapping revealed a major QTL region between S1058 and S0588 on C06 associated with DCI QTL showing large additive effects especially at the three high temperatures 21, 26, and 27 °C (Table 4). DCI QTL did co-localize to large effect FLN QTL at 21 and 27 °C (Table 5). Okazaki et al. (2007) detected a QTL for flowering time on C06 in a F_2 population derived from a broccoli (annual) x cabbage (biennial) cross and suggested that the QTL is equivalent to a QTL identified in F_3 families also derived from a cabbage x

broccoli cross (Camargo and Osborn 1996). Percentage of progenies with annual vs. biennial habit as well as days to flowering revealed QTL on C06 in the latter study. Uptmoor et al. (2008) identified in a *B. oleracea* var. *albogabra* × var. *italica* cross a temperature response QTL on C06 and suggested facultative vernalization effects to be causally related to flowering time variation. Binary analysis on curding to non-curding phenotypes at high temperatures revealed a major QTL in the same region as analysis of DCI data showed. We mapped the binary trait as a QTL. in a DH population derived from an F1, the segregation ratio of a monogenic trait would be 1:1. Since the segregation ratio of the trait was 3:1, at least two genes must be involved, which shows the complex nature of the trait. Such binary traits are jointly controlled by several genes (Yi and Xu 1999) and may be influenced by environmental effects. Nearly all DH lines carrying the B allele induced a curd while nearly 50 % of DH lines carrying the A allele did not induce curds, which may give hint for an interaction with a second locus but no significant QTL x QTL interactions were found (data not shown).

However, results suggest that at least one major locus on C06 affects curding time through variation in sensitivity to high temperatures. Ryder et al. (2001) identified two segments on C06 showing co-linearity with *Arabidopsis* chromosome 1, which harbors key genes for flowering time. *BoAP1-a* and *BoAP1-c* were mapped in these two regions. The physical position of *Boi2AP1* (Carr and Irish 1997), which is *BoAP1-a*, is C06: 35,676,652 according to the BolBase A12 sequence (Liu et al. 2014). The physical position of *Boi1AP1* (*BoAP1-c*) is C06: 7,705,861. The *BoAP1-a* locus was suggested to be associated with curding phenotypes and the stage of arrest in *B. oleracea* (Smith and King, 2000; Gao et al. 2007). However, Labate et al. (2006) concluded that the fraction of phenotypic variation explained by *BoAP1-a* is low, but the locus interacts with temperature: The expression of *BoAP1* in the shoot apex of cauliflower is switched off during vegetative reversion at high temperatures

(Anthony et al. 1996). High temperatures also promote formation of bracts (Booij and Struik 1990; Grevsen et al. 2003). Kop et al. (2003) found evidence for correlations between the allelic state of *BoAP1-a* and the severity of bracting. They suggested that *BoAP1-a* or closely linked genes play a major role in controlling bract development. If involved in vegetative reversion and bract development, *BoAP1* may have repressed curd induction and led to the development of leaf primordia during high temperature treatments of the present study. The physical position of *BoAP1-c* is between BLAST-hits for S1134 and S1114 and, accordingly, close to the QTL for binary analysis of DCI data at 26 and 27 °C. *BoAP1-a* maps less than 2 Mbp apart from S0588, which is a flanking marker of the third QTL region on C06.

Previous studies carried out on *B. oleracea* suggested that QTL for flowering time mapped on C09 may result from the variation in vernalization requirement (Bohuon et al. 1998; Rae et al. 1999). The higher QTL effects at high temperatures observed in the present study may give hint that the region on C09 has an influence on variation in sensitivity to temperature. Several studies have identified flowering time QTL in *B. napus*, *B. oleracea*, and *B. rapa* chromosomal regions syntenic to the telomeric region of the short arm of *A. thaliana* chromosome 5. This region carries several flowering time genes including the flowering repressor *FLC* (Lagecrantz et al. 1996; Osborn et al. 1997; Bohuon et al. 1998; Schranz et al. 2002; Okazaki et al. 2007). Synteny between the region on chromosome 5 in *A. thaliana* and C09 in *B. oleracea* has already been shown and the *FLC* paralog *BoFLC1* was mapped on C09 (Salatheia 2003; Pires et al. 2004). *FLC* paralogs were considered as candidate genes for variation in vernalization and flowering time in *B. napus*, *B oleracea*, and *B. rapa* (Osborn et al. 1997; Schranz et al. 2002; Okazaki et al. 2007). The position of the QTL region on C09 overlaps with the *FLC* paralog. In the TO1000 *B. oleracea* genome sequence (Parkin et al. 2014; Wang et al. 2011b) the position of S0629 is C09: 49,393,351 and the *FLC* position is C09: 51,033,935. A *FRIGIDA-LIKE* (*FRL*) ortholog is located close to S0533. However, it

was also already suggested that *FLC* independent pathways could be responsible for flowering time variation (Uptmoor et al. 2012; Ridge et al. 2014).

QTL for DCI were detected on C06 at 16 and 19°C but not at 17°C and at 19 and 27°C but not at 21°C and 26°C. LOD scores were high at 17, 21 and 26°C but below the threshold. A similar situation was observed at C09. We conclude that failure of QTL detection at specific temperatures is due to sample size and random errors rather than due to biological interactions. However, as mentioned above, large variations in QTL effects at different temperatures give hint that these regions are not simply temperature insensitive QTL. The suggestion is supported by the DCIxE QTL positions on C06 and C09. Significant QTLxE interactions will be observed if variance of QTL effects is large across environments. Both, QTLxE interactions and increasing QTL effects at higher temperatures support the assumption that differences in vernalization response are the main reason for differences in time to curd initiation. The most promising genomic region for breeding towards wide-adaptation cauliflower is between S1058 and S1096 on C06.

Interestingly, C04 was not described as a QTL region for floral/curd induction in *B. oleracea* before. Probably, floral induction pathways differ between different *Brassica* vegetables since the different crops differ in vernalization requirements (Wurr et al. 1995). According to Matschegewski et al. (2015) orthologs of *FRL1* and *SPL3* are located on C04. Nearest BLAST hits to the gene positions were the three markers on bottom C04. However, the QTL for DCI at 27°C mapped more than 10 cM apart from S0978 and the QTL for DCI across environments more than 20 cM. Another ortholog of an *FRIGIDA-LIKE* gene maps according to Bolbase on C07: 34,770,026 close to the putative position of S0782. The region on C07 is syntenic to *Arabidopsis* chromosome 5. Irwin et al. (2012) suggested a *FRIGIDA* ortholog to be involved in temperature-driven floral transition. The QTL hotspot at that region did not

include DCI QTL but QTL for FLN at 17 and 19 °C and across environments. FLN is highly correlated to DCI.

Flowering time is highly correlated to FLN in many plant species. In cauliflower, early curding and low FLN indicate summer annual types, while late curding and high FLN indicate winter annual types (Wurr and Fellows 2000). The main difference between winter and summer types is that winter types have low optimum temperatures for vernalization and low daily vernalization rates, i.e., winter conditions are required for curd induction. The delay in curd initiation due to high temperatures during the adult vegetative development stage is correlated with an increase in FLN (Wiebe 1972b; Booij and Struik 1990; Hand and Atherton 1987). Before floral induction takes place, the apical meristem is developing leaf primordia. Since leaf primordia are assumed to be developed at a constant rate during later growth stages (Kage and Stützel 1999), the more leaves are produced, the longer the duration of the vegetative-growth phase. As a result, QTL for DCI and FLN often map to the same genomic regions. QTL co-localization hotspots for DCI and FLN with higher effects at high temperatures (> 20°C) in comparison to low temperatures were observed on C06 and C09. As mentioned before, increasing QTL effects with increasing temperature give hint for the regulation of the extent of temperature sensitivity and QxE interactions were significant in both regions. However, it remains unclear if high temperatures lead to a delay in thermal time to floral induction or if curd development is repressed independently from floral induction pathways.

Although, our results showed significant correlations between DCI and FLN, several QTL for FLN were detected independent of DCI QTL. FLN depends on the duration of the adult development stage, when plants are sensitive for environmental signals like temperature, and on the length of the juvenile phase. The durations of both development stages influence time to floral induction. However, FLN is also directly related to LAR and, thus, variation in FLN

may occur without variation in time to floral induction. A QTL hotspot for FLN and LAR was detected on C01. The hotspot is close to the position of a copy of miR156; a miRNA that controls juvenile transition (Wu et al. 2009). The position of S0714 is C01: 2,993,555; the position of miR156 is C01: 3,777,428.

DCI and LAR were highly correlated and QTL for both traits were overlapping in several genomic regions. In all the coincidences of QTL for DCI and LAR, the additive effect had opposite directions. Our results suggest that the genetic architecture underlying DCI is strongly correlated to the genetic control of LAR. Méndez-Vigo et al. (2010) found that variation in *A. thaliana* flowering time depends also on the rate of leaf production and most QTL for flowering time co-localized with QTL for rate of leaf production. Wurr et al. (1981) found that leaf initiation rate is higher in early curding cauliflower types than in late curding types. The same was found in the present study.

Since it is assumed that a certain number of leaves must be initiated before juvenile-to-adult phase transition, LAR may have a direct impact on the duration of juvenility. In concordance with our results, Thomas (1980) found that differences in the duration of the juvenile phase in Brussels sprouts (*B. oleracea* var. *gemmifera* L.) were exclusively due to differences in LAR with early cultivars having higher LARs. However, it is well known that there is high variability in the length of juvenile phase among different cauliflower genotypes (Hand and Atherton 1987; Wurr et al. 1998; Wurr et al. 1994).

Previous studies found a change in LAR during the vegetative growth phase and hypothesized that the rate change may occur at juvenile-to-adult phase change (Hand and Atherton 1987; Booij and Struik 1990). We were not able to estimate changes in LAR precisely since the leaf number was counted only eight times during the first 27 d after transplanting. The confounding effects of both juvenile phase and vernalization requirement on curding time in cauliflower makes the interpretation of flowering-time QTL difficult, since variation in

flowering time may result from variation in length of juvenility and/or sensitivity to temperature.

Evaluating the same mapping population in different environments allows the distinction between constitutive and adaptive QTL. A constitutive QTL is consistently detected across environments, while an adaptive QTL is detected only in specific environments. The highest probability to identify QTL for sensitivity to temperature would therefore be under sub-optimal high temperature conditions. However, QTL on C06 and C09 were identified under both optimal and sub-optimal high temperature conditions. The hint for effects related to temperature sensitivity is the extremely high additive effect of DCI and FLN QTL at high temperatures. In fact it may be less straightforward to distinguish between constitutive and adaptive QTL (c.f. Collins et al 2008). We found several QTL regions in which QTL for at least one trait appeared in nearly every environment, indicating its constitutive nature. At the same time, QTL effects, e.g., for DCI on C06 increased with increasing temperatures, indicating the adaptive nature; QxE interactions turned out to be significant in that region. Thus, QTL effects change with changing levels of the environmental factor (Vargas et al 2006).

2.6 Conclusion

A major breeding goal for summer cultivars is to establish genotypes, which develop rapidly also under high temperatures, i.e., which do not show a delay in harvest time at unfavorable weather conditions during the summer. We assumed that deep phenotyping in controlled environments with different temperature regimes results in the detection of QTL for temperature sensitivity. However, we conclude from the present study that the identification of such QTL is less simple, since the most interesting QTL regions showed QTL under both optimum and sub-optimal high temperature conditions and across environment QTL for DCI showed always significant Q x E interactions. The identification of QTL regulating

development during the adult stage probably became more complicated due to variation in the length of juvenility within the population. Further studies are needed to precisely estimate the length of the adult vegetative developmental stage starting with juvenile-to-adult phase change and ending with floral transition. However, a large number of DH lines showed curd development even at highest temperatures, suggesting that new cultivars, well adapted to high temperatures during the adult vegetative stage, can be established. In addition, detected QTL will support the development of stable genetic markers that benefit marker-assisted breeding strategies towards the breeding of elite and thermo-tolerant cultivars. Most promising regions are located on C06 and C09. Both regions showed significant QxE interactions for FLN and DCI QTL and increasing additive effects with increasing temperatures. Importance of one hotspot on C06 was supported by binary analysis of curding vs. non-curding data. Candidate genes in the hotspot regions need to be sequenced and sequence variations have to be correlated to phenotypic variation.

Acknowledgments

We acknowledge financial support from the German Federal Ministry of Education and Research (BMBF). The project is part of the AgroCluster WeGa Kompetenznetz Gartenbau (project no. 0315542A).

CHAPTER 3

Predictions of curding date in cauliflower (*Brassica oleraceae* var. *botrytis*) using QTL-based parameters of an ecophysiological model

Yaser Hasan[1], Bill Briggs[2], Frank Ordon[3], Hartmut Stützel[1], Ralf Uptmoor[4]

[1] Institute of Horticultural Production Systems, Leibniz Universität Hannover, Herrenhäuser Str. 2, Hannover 30419, Germany

[2]Syngenta Seeds BV, Westeinde 62, Enkhuizen, The Netherlands

[3]Julius Kuehn-Institute, Federal Research Center for Cultivated Plants, Institute for Resistance Research and Stress Tolerance, Erwin-Baur-Str. 27, 06484 Quedlinburg, Germany

[4]Department of Agronomy, University of Rostock, Justus-von-Liebig-Weg 6, 18059 Rostock, Germany

3.1 Abstract

Coupling of crop modeling and QTL mapping assists in prediction of a phenotypic trait of a new genotype under various environmental conditions. In cauliflower (*Brassica oleracea* var. *botrytis*), temperature is the main environmental factor affecting the rate of development towards curding. The sensitivity to temperature varies among genotypes, allowing the developmental rates and genotype specific parameters to be estimated. Using the curding per se data for 151 DH lines which were previously evaluated under six different temperature regimes in the greenhouse, a two phase linear phenology model, describing curding rate to temperature was parameterized. QTL analysis by composite interval mapping was carried out on model parameters: SL which parameterizes the sensitivity to temperature below optimum, SR parameterizes the sensitivity to temperature above optimum, BP describe the point of rate change and also the computed R_{max} describe the maximum development rate at optimum temperature. A total of twenty QTLs were detected for all parameters. A major QTL was identified on C06 accounted for 6.3 %, 6.1 % and 28.5 % of the phenotypic variation in BP, SL and SR, respectively. Although this QTL was detected for both SL and SR, the SR QTL has five times the additive effect of SL QTL with very strong lod score (LOD=11) which indicate that high temperature enhance the role of this genomic region. At this genomic region no QTL for R_{max} was detected which further support the central role in suboptimal temperature effect. Six QTLs were specific for R_{max} indicating earliness per se. A QTL based model was developed by estimating the marker-based value of each phenology model parameters from the additive allele effects of QTLs detected for each parameter, and later incorporate the marker based parameter effect values to the crop growth model to predict the curding time in cauliflower. The QTL based model explained 46 % of the phenotypic variation in curding time of the parameterization set. Prediction quality of the model was validated in field trials on independent validation data set of DH lines derived from the same parental lines at four locations in six experiments. In the validation set, the QTL-based model

was able to predict the curding time with a root mean square error of prediction of 3 to 8 days, explaining 28 to 65 % of the variation in curding time. The QTL based model was further used to predict the performance of a test cross of each DH per se to one parent at two locations in four experiments. The QTL-based model could predict the curding time with RMSE of 3 to 9 days and explain 24 to 41 % of the genetic variation in curding time. This suggests that, the QTL based model is a promising tool for plant breeders to accelerate their breeding program, test different plant breeding strategies and to design ideotypes for contrasting target environments.

3.2 Introduction

Temperature response is a major determinant of the rate of many plant processes including the rate of plant development. In cauliflower, the rate of development towards curding is largely determined by different responses to temperature along different development phases. Typically, these responses are described in terms of cardinal temperatures minimum, optimum and maximum. Most of the models that describe curding time in cauliflower are temperature driven with major focus on vernalization phase. Grevsen and Olesen (1994a) used symmetrical linear responses of the rate of curd induction to temperatures below and above optimum with base, optimum and maximum temperatures of 0, 12.5 and 25.6 °C respectively. Similarly, Wurr and Fellows (2000) have used piecewise linear model describing the rate of progress towards induction in relation to temperature using three cardinal temperatures of 5, 13 and 27 °C. Wurr et al. (1993) in cultivar White Fox examined different segmented temperature response function forms to predict the time of curd initiation and indicated that a three-stage linear temperature function with four cardinal temperatures of 9, 9, 9.5 and 21 °C was appropriate for this variety. Kage and Stützel (1999) developed cauliflower harvest prediction model by a combination of empirical relationships derived from field data of two varieties Fremont and Linday. The vernalization process started after

end of juvenile and was simulated according to Wiebe (1972b) using a three segmented linear model with for cardinal temperatures -2, 10, 13, and 28 °C. These models were conventionally used to predict the curding time of a given genotype under different environmental conditions on the bases of its specific parameters using climatic data to better schedule the production of cauliflower and also to assist in design of high performance ideotypes.

The advent of molecular markers has enabled the dissection of the variation of a phenotypic trait and to identify quantitative trait loci (QTL) linked to markers on a molecular map. Normally QTL analyses which conducted at each single environment, individually, to identify QTL that affect the phenotype are restricted to the environment and genotype tested (Bogard et al. 2014). These separate analyses do not consider the trait dynamic because it fails to capture the change in phenotype with changing environment. Genotype by environment interaction (GEI) is a common phenomenon in complex agronomic trait where changes in the relative performance of a genotype across different environments can be observed. Despite the identification and the potential of numerous quantitative trait loci involved in the control of different plant traits in different plant species, the implementation in plant breeding is not straightforward and greatly hinders by GEI. To overcome this difficulty, a growing interest in the use of ecophysiological models (Yin et al. 2003). These models describe the multi phenotypic responses of a given genotype to diverse environment by a set of parameters known as genetic coefficients (White and Hoogenboom 1996; Baenziger et al. 2004). Since physiological models have the potential to predict the performance of a genotype in diverse of environments, it can be powerful tool to resolve genotype-by-environment interactions (Yin et al. 2003; Hammer et al. 2005). On the other hand, the quantitative trait locus (QTL) models determine the contribution of genomic region to trait variation under a limited number of environments (Reymond et al., 2003). In the view of added value of crop modeling to

classical quantitative genetics, combining ecophysiological model and quantitative trait loci has been suggested as an approach to understand and predict complex traits variation across environments (Reymond et al. 2003; Yin et al. 2004; Hammer et al. 2006; Collins et al. 2008). The principle of this approach is to develop a response growth curve for each individual and estimate curve individual-specific parameters, and then treat the curve parameters estimates as phenotypes in QTL analysis (Wu et al. 2002). Several studies have used the complementary aspects of crop modeling and QTL mapping as a tool to assist plant breeding. The first study coupled crop modeling and QTL mapping was conducted in barley using the SYP-BL crop model (Yin et al. 2000a). QTLs were detected for each of the model input traits used. The identified QTLs were coupled to the SYP-BL model by replacing the original model input parameters with those calculated from the QTL effects. Reymond et al. (2003) established response curves of leaf elongation rate to temperature, evaporative demand and soil water status. The parameters of these responses were estimated for lines of maize RILs mapping population and identified QTLs for this parameters. Yin et al. (2005a) developed a model for spring barley using ecophysiological phenology model combining crop models for predicting flowering time and QTL mapping have been developed. Nakagawa et al. (2005) used a three stage beta model to quantify the thermal and photoperiod response of rice (*Oryza sativa* L.) flowering time. Recent model developed by Uptmoor et al. (2012), based on earlier model (Uptmoor et al. 2008), used genotype specific parameters and QTL effects as inputs to a model for predicting flowering time in *Brassica oleracea* . Bogard et al. (2014) using two parameters of an ecophysiological model representing the vernalization requirements and photoperiod sensitivity proposed a QTL-based model to predict heading date in bread wheat (*Triticum aestivum* L.). Gu et al. (2014) linked seven parameters of the ecophysiological GECROS model with quantitative genetics to support marker-assisted crop design to improve yields of rice (*Oryza sativa*) under drought stress.

The objective of this study were (1) the genotype-specific parameterization of two phase linear model describing the rate towards curding as a function of temperature, (2) conduct QTL analysis on the model input parameters and (3) incorporate the marker based parameter effect values to the crop growth model to predict the curding time in cauliflower.

3.3 Material and methods

3.3.1 Plant material

A doubled haploid (DH) population of 151 lines was derived from anther culture of a F_1 cross between two contrasted homozygous inbred parental lines (PLs). The PLs were segregating for reliability in time to curd induction associated with temperature sensitivity. The unreliable parent produces high quality curds. The population was genotyped with 176 single nucleotide polymorphisms (SNP) markers. The genetic map was constructed using the Haldane function. The total map length was 891.2 cM spanning over nine chromosomes. Average distance between SNP markers was 5.3 cM.

3.3.2 Growing conditions and experimental setup

3.3.2.1 Greenhouse (Parameterization set)

Two seeds of each DH line and PLs were sown into seedling trays filled with Potgrond P (Klasmann-Deilmann GmbH). Seedlings were raised in a greenhouse at 22 °C and a photoperiod of 16 h until all plants had four or five visible leaves. Plants were thinned at the two-leaf stage. Seedlings were then transplanted into 3 liter pots filled with the same substrate and placed into a greenhouse in a randomized complete block design with four replicates. Plants were grown at a photoperiod of at least 16 h. If natural day length was below 16 h and/or radiation was low, 200 µmol $m^{-2}s^{-1}$ additional photosynthetically active radiation (PAR) at plant height was provided by 400 W Phillips SON-T Agro lamps. Plants were fertigated daily with 0,5 g l^{-1} Scotts Universal solutions. Fungicides were applied if necessary.

Air temperature was measured at the level of plant height every 10 minutes using TinyTag View 2 data loggers (Gemini Data Loggers Ltd., West Sussex, UK). The daily mean/min/max temperatures recorded inside the greenhouse for six temperature treatments were 11.8/10.6/15.5, 15.5/13.4/18.9, 17.3/15.5/19.6, 19.0/17.0/21.1, 21.4/16.0/22.8 and 27.0/22.2/28.6 °C. Standard deviations of daily means were 0.2, 1.4, 0.8, 1.1, 0.9 and 1.8 °C. All treatments were terminated after 120 days. In every temperature treatment, time (d) to curd initiation (DCI) DCI was considered to have occurred when the diameter of the curd was ≥ 1 cm.

3.3.2.2 Field trials

Germany (validation set)

The QTL based model was validated on a set of independent DH lines derived from the same parental lines grown in field trials. Numbers of DH lines in each field experiment are shown in Table 10. Two field trials were conducted one in Ruthe (52°11`N, 9°50`E), and the other one in Rostock (54°10`N, 12°4`E). Twelve seeds of each DH line were sown into seedling trays and raised in the greenhouse for one month. Seedlings of each DH line were transplanted into the field on 6th of May 2013 in Rostock (54°10`N, 12°4`E) and 13th of June 2013 in Ruthe (52°11`N, 9°50`E). Each DH line plot have eight plants in four rows of 1.2 m per plot, 0.40 m between rows resulting in plant density of 3.4 plants m^{-2} . In Rostock there were three plots per line while in Ruthe only one plot per line. A border row was planted at the margin. Day to curd initiation was recorded twice a week from transplanting until the curd is visible. In these two experiments we used the same criteria used in the greenhouse for determining the curding time (diameter of the curd was ≥ 1 cm). Both experiments were terminated after 120 days.

Netherlands and Hungaria (**Parameterization, Validation and Test cross sets**)

In all field trials in Netherlands and Hungaria , the plot size was 3.0×3.0 m^2 and inter-and

intra row spacing was 45cm. Plots were replicated three times in a randomized block design and plot value harvest time was recorded (plant breeder harvest time criteria).

3.3.2.3 Data set, locations and years

a. *Parameterization set*

The DH per se of the parameterization set which was used to parameterize the model in greenhouse was also grown and evaluated in two field experiments according to plant breeder harvest time criteria in Zeewolde (52°11`N, 9°50`E)), Netherlands in 2011 and 2012.

b. *Validation set*

The same validation set of lines which were grown in field trials in Germany were also grown in four experiments, two in Zeewolde (52°11`N, 9°50`E) in Netherlands and tow in Osca (52°11`N, 9°50`E) in Hungaria.

c. *Test cross*

The predictive ability of the QTL based model was further tested on a set of test cross of each DH per se to one single tester were grown and phenotyped in field trials for day to harvest in Zeewolde (Netherlands) in 2012 and 2013 and in Osca (Hungaria) in 2011 and 2012. Genotyping data of DH per se training set serves for the test cross. The hybrids were tested in two locations for two years. Plot values for days from transplanting to maturity were used.

The mean temperature and standard deviation, number of genotypes, year and transplanting date for each for each trial location year are presented in Table 10.

In all experiments conducted in Netherlands or Hungaria the criteria of defining the curding time is the plant breeder commercial harvest time trait. This resulted in a systematic over estimation curding time, as compared to the criteria of visible curd used in greenhouse which is much less than the harvest time. Therefore the observed data was corrected by subtracting the same value from all lines for each data set.

3.3.3 Model description and parameter estimation

3.3.3.1 The crop model, Model inputs

Using two segment linear phase model, curding rate to temperature was dissected into three parameters slope left (SL), slope right (SR) and the breakpoint (Bp). Additional parameter represented the maximum developmental rate at optimum temperature was computed. The model parameters were estimated for each DH line. These parameters were identified for 151 DH lines were previously grown and evaluated at six different temperatures in the green house. For a given set of model parameters and environmental conditions, the model predicts the curding rate. The only weather input is the mean temperature. There was no observation in 7 lines at 21 °C and 45 lines at 27 °C were available, and the model could not be parameterized for that lines. For that reason the rate of development at these temperatures was assumed to be zero as default. This assumption is quite relevant to the physiology of cauliflower (Wurr and Fellows 2000).

The model was fitted using the nls function located in the standard nls library in R program (Bates and Chambers, 1992). The model can be written as follows

$DR = a_1 + SL \times T$ for $T \leq t_{BP}$

$DR = a_2 + SR \times T$ for $T > t_{BP}$

Where

DR is the rate of development (1/days to curd initiation).

a_1, a_2 are the intercepts for the first and second segment respectively.

SL, SR are the slope of first and second segment respectively.

T is the temperature and $t_{BP}$is the temperature at brekpoint

The maximum rate of development at the breakpoint was computed as follows

$$R_{max} = SL \times t_{Bp} + a_1$$

when $T = t_{BP}$

$$a_1 + SL \times T = a_2 + SR \times T$$

Solve for one of the parameters in terms of the others by rearranging the equation above:

$$a_2 = a_1 + (SL - SR) \times T$$

By replacing a_2 with the equation above, the result is a piecewise regression model that is continuous at $T = t_{BP}$

$$DR = a_1 + SL \times T \text{ for } T \leq t_{BP}$$

$$DR = \{a_1 + T(SL - SR)\} + SR \times T \text{ for T} > t_{BP}$$

Incorporating the R_{max} the maximum development rate at optimum temperature into the final model rewritten as

$$DR = R_{max} - \left(SL \times \left(t_{Bp} - T\right)\right) \text{ for } T \leq t_{BP}$$

$$DR = R_{max} + \left(SR \times (T - t_{BP})\right) \text{ for } T > t_{BP}$$

Where DR the rate of development is, R_{max} is the maximum development rate at optimum temperature, SL is the slope left, SR is the slope right, T is the actual temperature, t_{BP} temperature at break point.

3.3.4 QTL analysis on model parameters

QTLs were identified for each parameter by composite interval mapping (Zeng 1994) using the software PLABQTL (Utz and Melchinger 1996). Analyses were based on multiple

regressions as described by Haley and Knott (1992). Cofactors were selected by stepwise regressions according to Miller (1990) with a default value for F-to-enter and F-to-delete of 3.5. A LOD threshold of 2.0 was used to declare the putative existence of a QTL. QTL were graphically displayed using Map chart 2.2 (Voorrips 2002).

The QTL based parameters estimates were calculated for each DH-line as follows:

$$y_{j=m+\sum e_i g_{ij}} \qquad (1)$$

Where y_i is the predicted value of the jth DH-line, m is the estimated population mean, e_i is the additive effect of the ith QTL in the jth DH-line, defined as 1 for P1 and -1 for the P2 allele. For each genotype, the model parameters Bp, SL, SR and R_{max} values were obtained from the equation (1) and then the ecophysiological model was used to predict the curding time using parameters which estimated by genetic markers.

3.3.5 Validation of the QTL based model

The QTL based model was validated on a set of independent data set, simulated curding rate were compared to measured lines and the root mean square error (RMSE) was used as measure of accuracy, also the coefficient of determination R^2 of linear regression between simulated and observed values was used to indicate the percentage of phenotypic variation accounted for by the model.

3. 4 Results

Phenotypic variation in phenology model parameters (parameterization set)

The DH population exhibited considerable phenotypic variation in model parameters BP, SL, SR and also the computed R_{max} (Fig. 5 and Table 8) showing transgressive segregation. The SL and R_{max} showed a unimodal distribution. For parameters BP and SR bimodal distribution was observed suggesting a major gene with large effect segregating in this population (Fig. 5

and Table 8). The bimodal distribution of SR showed more transgressive segregants with less negative values than the one more negative. The P2 allele contributes for less negative value of SR which might explain the presence of phenotypic transgressive segregation. Few lines which showed a slope right of nearly zero (Fig. 5).

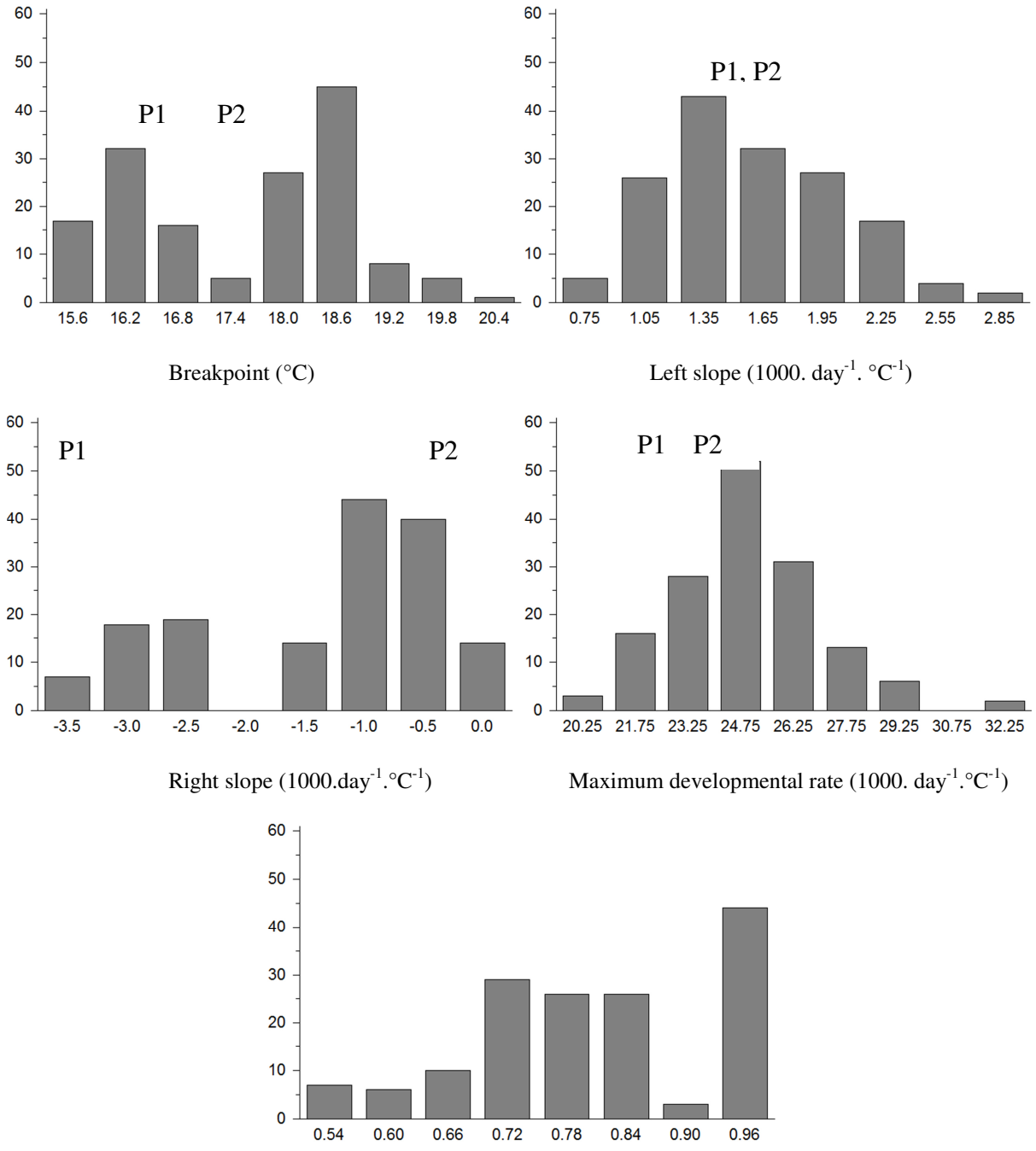

Fig. 1 Frequency distributions of model parameters breakpoint (Bp), slope left (SL), slope right (SR) and maximum developmental rate (R_{max}) and R-squared for model fit.

Table 1 Parental lines means, DH-population minimum, maximum, means and standard errors for three estimated parameters of the phenology model Breakpoint (BP), slope left (SL), and slope right (SR) and computed maximum developmental rate (R_{max}). Pearson's correlation coefficients between the parameters are shown. An asterisk indicates a significant correlation at $P \leq 0.05$.

Parameter	Parental lines		DH population				Pearson's correlation coefficient		
	(P1)	(P2)	Min	Max	Mean	SD[a]	SL	SR	R_{max}
BP (°C)	16.33	17.15	15.50	20.20	17.50	1.51	-0.74**	-0.56**	0.059
SL*1000 (day $^{-1}$. °C $^{-1}$)	1.49	1.49	0.70	2.90	1.55	0.19		0.42**	0.47**
SR*1000 (day $^{-1}$. °C $^{-1}$)	-1.73	-0.6	-3.70	0.20	-1.35	1.04			0.070
R_{max}*1000 (day^{-1})	23.0	23.5	19.9	32.6	24.87	4.51			

Correlation coefficients between BP were significantly negatively highly correlated with SL and SR with (*R*= - 0.74) and (*R*= - 0.56) respectively. SL was significantly positively correlated with SR and R_{max} with (*R*= 0.42) and (*R*= 0.47) respectively. There was no correlation between R_{max} and SR. Correlation coefficient between each model parameters and the curding per se for individual environments showed that R_{max} is strongly negatively correlated with curding per se at 16, 17 and 19 °C with r in the range of - 0.80 and - 0.86 and reduced to - 0.61, - 0.58, - 0.46 at 12, 21, and 27 °C, respectively. The SR is negatively correlated with curding per se and this correlation increase with the increase of temperature ranged between - 0.21 at 12 °C to - 0.65 at 27 °C. SL is negatively correlated with curding per se at 16, 17 and 19 °C ranged between - 0.42 to - 0.58 and less correlated with 12, 21 and 27 °C ranged between - 0.12 at 12 °C and - 0.33 at 27 °C. In all cases, weak positive correlation between the BP and the curding per se was observed.

3.4.1 QTL detected

A total of 20 QTLs for all parameters BP, SL, SR and the computed R_{max} were identified with one, five, four and ten QTLs respectively. Table 9 summarizes the estimated positions, LOD scores, phenotypic variation explained, and additive effects of significant QTL. Single BPQTL was mapped on C06 with negative additive effect. This QTL explain 6 % of PV. Five QTLs were detected for SLQTL, three on C01, and one each on C06 and C09 explained 6 % - 14.8 % of PV. Four SRQTL were detected on C04, C06, C08 and C09 explained 8 % to 28.5 % of the PV with positive additive effect except at C08. The major SRQTL on C06 explained 28.5 % of the phenotypic variance with LOD=11.24 and large additive effect. At this QTL the P2 allele contributes for higher (less negative) slope. Only in one case the SR has negative additive effect on top of C08 indicating rapid development at high temperature in P1 compared to P2. Ten R_{max}QTLs were identified. Two of them at C01 co localized with SLQTLs. On top of C08 and C09 R_{max}QTL explained 15.1 % and 9.5 % of PV with LOD score of 5.15 and 3.24 respectively. The R_{max}QTL on C08 colcalized with SRQTL and on C09 colocalized with SLQTL .The remaining six R_{max}QTLs were detected independent of SL or SR, one on C05 explained 15 % of the PV with high LOD score of 5.27, two on C06, two on C07, one at C08 and two at top and bottom of C09 explained 15.1 % and 9 % of PV with LOD score of 5.15 and 3.04 respectively. The additive effect of all R_{max}QTLs was positive with two exceptions at C01 at marker S0464 and on C07 at S0580.

Table 2 Linkage group, position, nearest marker, LOD scores, the percentage of phenotypic variation explained (R^2) and the additive effect for QTLs detected for phenological model parameters in *Brassica oleracea* DH population

Parameter	Linkage group	Position (cM)	Nearest marker	LOD	R^2	Add.
BP	6	20	1114	2.16	6.3	-0.306
SL	1	24	1066	3.62	10.3	-0.146
	1	52	1109	5.37	14.8	0.225
	1	78	1119	2.77	8.1	-0.15
	6	18	1114	2.09	6.1	0.099
	9	8	641	3.3	9.5	0.168
SR	4	56	403	2.78	8	0.209
	6	18	1114	11.24	28.5	0.57
	8	10	1099	3.02	8.6	-0.236
	9	78	1037	2.8	8.1	0.194
R_{max}	1	22	464	2.5	7.4	-0.455
	1	58	1098	2.15	6.4	0.407
	5	30	1071	5.27	15	0.619
	6	2	136	3.25	9.6	0.495
	6	32	374	2.75	8.1	0.494
	7	0	1077	3.24	9.8	0.565
	7	72	580	3.47	10.2	-0.746
	8	18	1069	3.24	9.5	0.674
	9	2	1063	5.15	15.1	0.964
	9	56	256	3.04	9	0.52

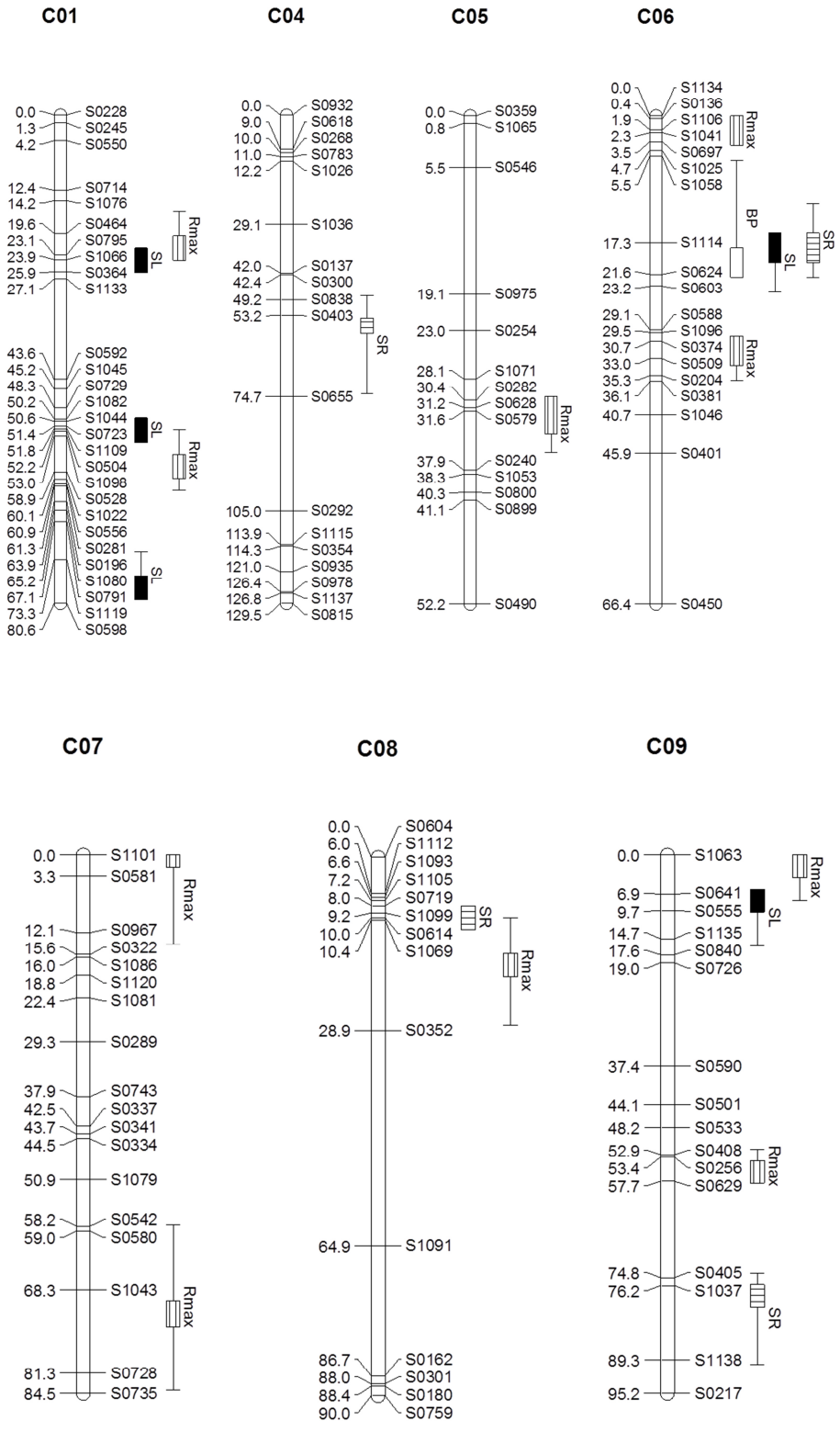

Fig. 2 Linkage map and twenty QTLs for model parameters BP (*unfilled*), SL (*filled*), SR (*horizontal stripe*) and R_{max} (*vertical stripe*).

The observed curding time across environments was compared to curding time predicted by physiological model as well as by QTL based model. The phenology model explained 95 % of the variation (Fig. 7A) while predictions with QTL based parameters model explained 46 % of the variation (Fig. 7 B).

The model was validated on a set of independent genotypes (validation set) at six experiments. Mean temperature and standard deviation, minimum, maximum, mean and standard deviation of curding time and the characteristics of the linear regression of the relation between simulated curding time QTL based and observed data are presented in Table 10. In all the validation data sets, curding time predicted with QTL based parameters explained 27 % to 65 % of the variation with RMSE of 3 to 10 days (Table 10, Fig. 7). The QTL based model performed well in ranking genotypes for curding time in all field trials of the validation set, with Sperman correlation coefficient between simulated QTL based and observed data ranged between 0.51 to 0.78 (Table 10). The predictive quality of the QTL based parameters model on test cross set in four experiments explained 0.24 % to 0.41 % of the variation with RMSE ranged between 3 to 9 days. In test cross data set, the ability of ranking genotypes determined by Sperman correlation coefficient varied between 0.49 and 0.63.

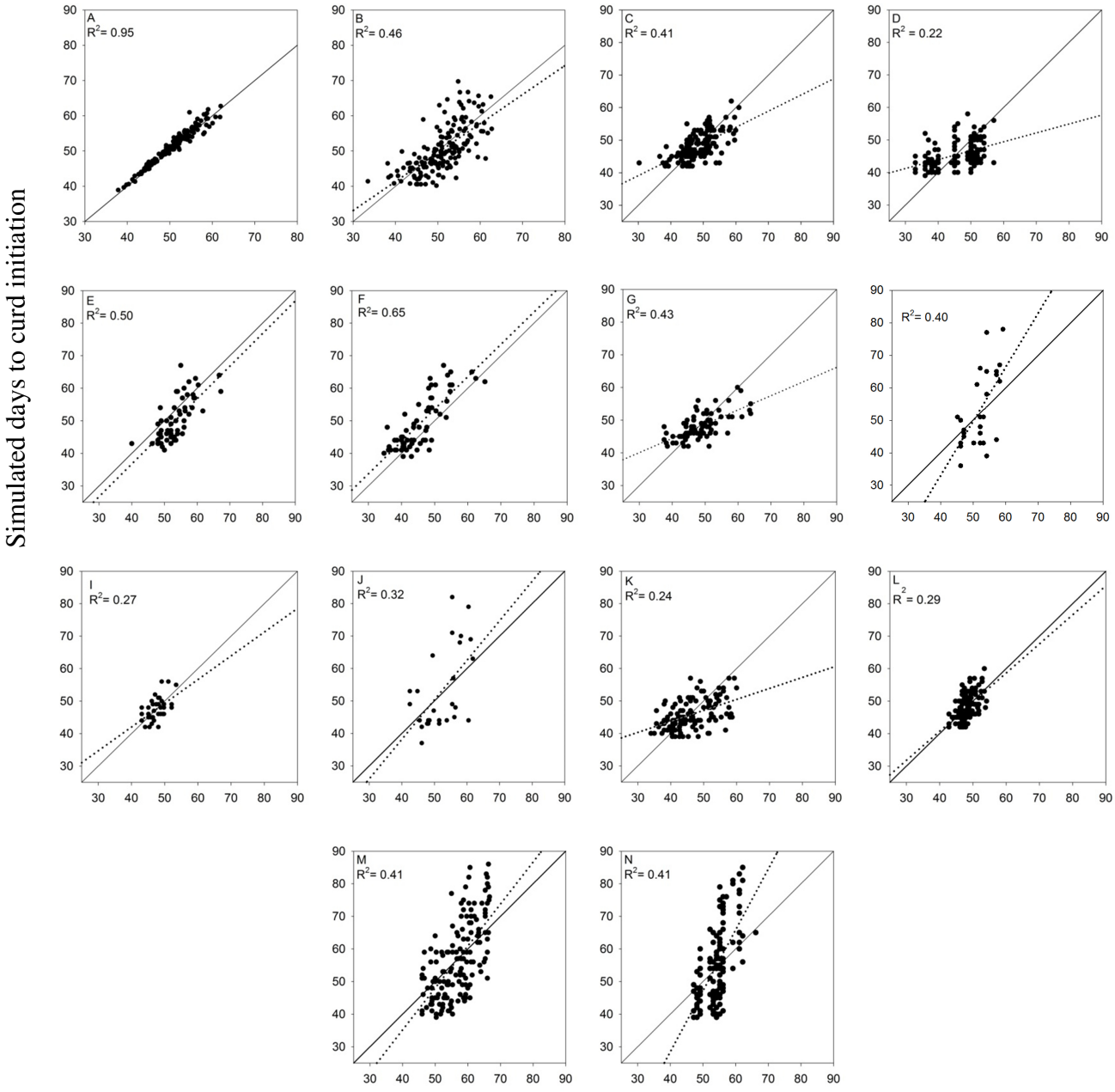

Fig. 3 Comparison between observed days to curding and corresponding simulated days to curding by the model with original parameters (A) and by QTL based parameters (B) for the parameterization set across all environments in greenhouse. Comparison of observed days to curding and simulated days to curding in field trials using the QTL based model parameters for parameterization set, in Zeewolde 2012 and 2011 (C) and (D) respectively, and validation set, in Rostock 2013 (E), in Ruthe 2012 (F), in Zeewolde 2012a, 2012b (G) and (H) respectively, in Osca 2012 and 2011 (I) and (J) respectively, and for the test cross set in Zeewolde 2013, 2012 (K) and (L) respectively, in Osca 2012, 2011 (M) and (N) respectively. The solid line represents the linear regression of predicted value to the observed value. The dotted line is the 1:1 line. In the upper part of each plot R^2 value is given.

Table 3 RMSE and percentages of variance explained of the relationship between simulated QTL based and observed curding time of parameterization set, validation set and test cross set grown in field trials

Data set	Location	Year	Planting date	N*	Curding time (days)			QTL based *vs*. Obs				Mean temperature±SD	Correlation Sperman rank
					Mean	Min	Max	RMSE	R^2	Slope	Intercept		
DH per se	Zeewolde	2012	24/05/2012	161	48	30	61	4	0.41	0.49	24	15.7±2.8	0.62
	Zeewolde	2011	14/06/2011	147	45	33	57	6	0.22	0.27	33	16.2±2.3	0.48
Validation	Rostock	2013	06/05/2013	62	50	41	67	5.5	0.50	1.0	-3.2	17.5±4.2	0.72
	Ruthe	2012	13/06/2013	64	46	35	65	6	0.65	0.99	3.8	20.0±3.4	0.78
	Zeewolde_a	2012	24/05/2012	76	48.5	37.5	64	5	0.44	0.43	27	15.7±2.8	0.65
	Zeewolde_b	2012	24/05/2012	39	47	43	53	3	0.27	0.73	12.76	15.7±2.8	0.51
	Osca	2012	6/07/2012	29	53	42.5	62	10	0.32	1.21	-10.5	21.8±3.9	0.54
	Osca	2011	7/07/2011	28	52	45	59	9	0.40	1.66	-33.6	22.2±3.1	0.58
Test cross	Zeewolde	2013	26/06/2013	125	46	34	60	6	0.24	0.33	30.2	17.1±3.01	0.49
	Zeewolde	2012	13/06/2012	154	48	43	54	3	0.28	0.89	-4.89	16.2±2.84	0.53
	Osca	2012	6/07/2012	147	57	46	67	9	0.41	1.28	-16.3	21.7±3.9	0.59
	Osca	2011	7/07/2011	136	54	47	66	9	0.41	1.86	-46.29	21.6±3.3	0.63

* N is the number of lines

3.5 Discussion

Studying quantitative trait loci is complex due to genotype by environment interactions. One strategy to resolve this complexity is to develop a response curve for the trait of interest. This approach assumes that the network of genes coordinated in such a way that plant react for environmental conditions in a predictable way (Collins et al. 2008). In this study, two phase linear model describing the curding rate response to temperature was established for each DH line. The slope left parameter characterizes the genotype performance under suboptimal low and the slope right characterize the genotype performance under suboptimal high temperatures. R_{max} parameter characterizes the genotype performance under optimum condition. The parameters of the response represent key characteristics of each DH line per se and QTL analysis carried out on these parameters. Comparing QTLs detected for the model input parameters SL, SR, and R_{max} with hot spot QTLs for curding per se in single environment (Hasan et al. 2016) revealed that most QTLs detected for curding per se were also detected for one of the model parameters on C05, C06, top of C07, and bottom of C09. Uptmoor et al. (2008) parameterize a model describes the time to flowering as a function of temperature in *B.oleracea* DH-population and conduct QTL analysis on the model input parameters. Their results showed also common QTL regions using individual environment analysis and response curves. Similarly, Reymond et al. (2003) established response curves of maize leaf elongation rate to temperature, evaporative demand and soil water status and conducted QTL analysis on the parameter of the response curves. Most of the QTLs detected for the response curve were also detected in single environment analysis.

In all genomic regions, when a co localization of SLQTLs , SRQTLs and curding per se QTLs had occurred, the effect was consistent, indicating that the favorable allele for curding per se in single environment analysis were also favorable allele for curding rate to temperature. Several studies using model input parameters in QTL analysis did not identify

new chromosomal regions which were not identified in separate environment analysis (Reymond et al. 2003, Quilot et al 2005). In this study, we have identified three additional chromosomal regions which were specific for model parameters. Two QTLs for SL and R_{max} on top of C01 and also for SL and R_{max} on top of C09 and for R_{max} only at bottom of C07.

A major QTL on C06 was detected for all three parameters SL, SR and Bp colocalized with hot spot regions for curding per se. The SRQTL explained 28.5 % of the phenotypic variation with a strong evidence (LOD>11) in compare to SLQTL explained 6% of the phenotypic variation with (LOD=2.09). This might indicate that, this genomic region is involved in temperature sensitivity. Furthermore, the SRQTL effect was five times the SLQTL indicating that, the suboptimal high temperature tended to enhance the role of this QTL. Interestingly, at this genomic region no R_{max}QTL was detected, which further support the proposed suboptimal temperature sensitivity mentioned above. Using curding per se data of the same population, a QTL hotspot region for day to curd initiation with large additive effect increasing with the increase of temperature in the same region was detected. Uptmoor et al. (2008) identified a temperature response QTL on C06 and suggested vernalization effect to be casually related to flowering time variation. Previous study in *Brassica oleracea* on cabbage x broccoli cross (Camargo and Osbron, 1996) identified three regions on linkage groups C02, C06 and C08 were associated with flowering time. The locus on C06 explained relatively large portion of the phenotypic variation (30 %) in flowering time and has been considered as a major locus. Temperature plays a significant role in curd development. Curd phenotype in cauliflower is associated with temperature effect. Gao et al. (2007) conducted QTL analysis in *Brassica oleracea* mapping population based on visual scoring of the inflorescence phenotype in the greenhouse. Three QTL segments were detected. Two were on C01 and one on C06. The bottom of C01 of Arabidopsis harbor key genes for flowering time such as *FT*, *AP1*. The homologe region was inverted and duplicated in C06 of *B. oleracea* in which

BoAP1-a (floral regulatory gene) was associated with cauliflower curd phenotype (Smith and King 2000). *BoAP1* is the *Brassica oleracea* orthologue to the *Arabidopsis* AP1 gene, and was found in two copies in *Brassica* genomes. These two copies were referred to as *BoAP1-a* and *BoAP1-b*. In cauliflower heat causes a decline in the expression of BoAP1 in the shoot apex (Anthony et al. 1996). The development of bracts in cauliflower curds appears to be influenced by temperature, with plants more susceptible to bracting under temperatures higher than optimal (Nieuwhof, 1969; Fujime and Okuda, 1996). Kop et al. (2003) found that high temperatures increased bracting with the same genotype and suggested that *BoAP1-a* plays a major role in the genetic regulatory pathway controlling bract development. In F3 families derived from a cabbage by broccoli cross (Camargo and Osbron, 1996) PF as a qualitative evaluation of the annual/biennial habit measuring the proportion of annual plants (PF) and flowering time index (FT), QTLs for both traits were detected on the top of C06 explained 29 % of flowering time variation and have been considered as major locus.

The R_{max} value represents the maximum developmental rate at optimum temperature condition. In all ten R_{max}QTLs, the P2 allele increases the R_{max} at all loci with two exceptions at top of C01 and bottom of C07. Interestingly, four R_{max}QTLs were colocalized with four hot spot regions in single environment analyses, one each at middle of C05, top of C07 and at two genomic regions at C06. These QTLs were detected independent of two other model parameters (SL, SR) indicating that the modeling approach was able to separate the two individual physiological process linked to temperature sensitivity and earliness itself and to distinguish between QTLs that affect curding through suboptimal temperature sensitivity pathway and that affect curding for other aspects such as earliness per se. This would be not possible based on separate environment analyses. Sebastian et al. (2002) identified QTLs for vernalization, on C07 at (18 cM) in a cross of cauliflower and brussels sprout, and reported

that the QTL on C07 can account for vernalization requirement. Several studies reported QTL on C05 (Bohuon et al. 1998; Uptmoor et al. 2008).

Colocalization of R_{max}QTL and SLQTL occur at three genomic regions; two on C01 and one at top of C09. The two SLQTLs at marker S1066 (24cM) and S1109 (52 cM) co localized with two R_{max}QTLs at S0464 (22cM) and S1098 (58cM) on C01. In both genomic regions the additive effect was consistent between two parameters. However, the two genomic regions have opposite additive effect direction while P2 allele on the top of C01 at marker S1066 causes late curding; it causes early curding at the other genomic region at marker S1109. The additive effect of SLQTL at S1109 is greater than SLQTL at S1066 whereas the R_{max}QTL showed exactly the same absolute additive effect but different direction. Rae et al. (1999) in a backcross of substitution lines from a cross between *Brassica oleracea* var. *italica* and *Brassica oleracea* var. *alboglabra* detected two adjacent flowering time QTLs on chromosome 1; one late flowering between 0.0 and 30.3 cM and one early flowering between 30.3 and 38.1 cM. In similar pattern, in previous study on the same mapping population we detected four LAR QTLs at each of both genomic regions with opposing direction of the additive effect. The hot spot region on top of C01 is close to the position of a copy of miRNA 156. miRNA control the juvenile to adult transition (Wu et al. 2009).

Combining both single environment and response curve parameters analyses allow the distinction between QTLs for particular environment from that QTLs interact with wider range of environments. Since the left and the right slope characterized the curding rate to temperature in winter-spring like environments and summer like environments respectively, selection of QTL alleles affecting the rate of development response curve parameters should be more effective than selection of alleles for QTLs detected in single environment analysis when the aim is to develop lines for winter-spring cropped or summer-cropped environments.

Coupling of crop model and quantitative genetics assists in prediction of genotype performance and improve the breeding efficiency (Reymond et al. 2003). In this study, the ecophysiological model was more efficient in simulating the curding time across environments (R^2= 95%) compared to the QTL based model parameters (R^2=0.46). Uptmoor et al. (2008) investigating the flowering time of *Brasica oleracea* segregating mapping population, the crop model showed a high precision (R^2=0.86) while it reduced using the combined QTL and crop model (R^2= 0.56). Whether the predictive capability of the QTL-based model can be applied on independent data set which was not included in QTL analysis of the model input parameters, validation set was employed to investigate the prediction accuracy. Using the criteria of visible curd to determine the days to curding in Ruthe and Rostock the QTL-based model explained 0.65 % and 0.50 % with RMSE of 6 and 5.5 days respectively. However, using the usual plant breeder commercial harvest time trait in four other experiments, the QTL based model still show good capability to predict the day to curding explained 0.27 % to 0.43 % and RMSE of 3 to 10 days. Bogard et al. 2014 using QTL based parameters of an ecophysiological model were able to predict the heading date in wheat of an independent data set with a root mean square error of 5 to 8.5 days, explaining 48 to 63 % of. The correlation between observed and simulated days to curding of validation set ranged between 0.51 to 0.78 in all validation set experiments indicating that the QTL information can replace measured parameters. The above genetic panels consist of DH per se. In hybrid breeding program, huge number of cross combinations between inbreds lines and testers need to be evaluated in expensive field trials to identify the superior hybrids in the target environment. Thus, it is of great importance for plant breeder to identify in advance the most promising test cross for field experiments to reduce the phenotyping cost. In this study we test the prediction ability of the QTL based model using testcross data set which was evaluated in four field trials according to plant breeder commercial harvest time trait. The results showed that the QTL based model could predict the days to curding explained 0.41 %

of phenotypic variation with RMSE of 9 days at two high temperature environments and 0.24 % to 0.28 % with RMSE of 3 to 6 at two low temperature environments. This indicates that the transferability of the marker effect estimates of previous breeding cycle used in the QTL based model to predict the next breeding cycle seems promising.

3.6 Conclusion

Describing the development rate towards curding in cauliflower using only few parameters which individually describe the development rate towards curding under sub optimal (SL and SR) as well as under optimal conditions (R_{max}) appears reasonable. Coupling crop modeling and quantitative genetics is a useful tool allows the estimation of curding time in a new environment as long as temperature data and genotypic data are available. The prediction can be extended for new DH lines derived from the same parental lines and still the predictive ability of the QTL based model on high level using the test cross data set. The QTL based model can assist plant breeder to accelerate and orientate their breeding program, test different breeding hypotheses and simulation of curding time for different allelic combinations under different environments.

CHAPTER 4

4.1 General discussion and future aspects

In cauliflower, stagger planting is used to improve the crop continuity to the market, but these do not always mature in the same expected order and overlapping between different plantings can occur which results in peak and toughs in availability of the product and price fluctuations. Temperature effect on growth and development of cauliflower considered as a major reason influencing the curding time. Identifying the genes involved in the regulation of curding time and its related traits in response to temperature would help plant breeder to develop cultivars with reliable harvest time, and therefore more predictable orders of maturity (Dixon, 2007).

The present study identified 31 significant QTL for DCI. Individual QTL explained between 11% and 41% of the phenotypic variation (Chapter 2, Table4). Promising QTL hot spot regions were mapped on C04, C05, C06, C07 and C09. QTL hotspots identified in this study shared similar genome location with previous studies. Both Bohuon et al. (1998) in a *B. oleracea* var. *albogabra* × *B. oleracea* var. *italica* cross and Rae et al. (1999) in backcross substitution lines of the same cross detected QTL for flowering time on C05 and C09. Okazaki et al. (2007) detected a QTL for flowering time on C06 in a F_2 population derived from a broccoli (annual) × cabbage (biennial) cross and suggested that the QTL is equivalent to a QTL identified in F_3 families which were derived from a cabbage × broccoli cross (Camargo and Osborn 1996). Uptmoor et al. (2008) identified in a *B. oleracea* var. *albogabra* × *B. oleracea* var. *italica* cross a temperature response QTL on C06 and suggested facultative vernalization effects to be causally related to flowering time variation. Sebastian et al. (2002) identified QTL on C07 in a cross of cauliflower and brussels sprout, and reported that the QTL at C07 can account for vernalization requirement. By contrast, the QTL hotspot region on C04 has not been reported previously.

On C04, only at three high temperatures three DCIQTLs were mapped at 19 °C, 21 °C on the same position and at 27 °C 16 cM apart, but there were no detected QTLs in other environments indicating its neutral effect at low temperatures range. In contrast, two DCIQTLs were detected on C05 at 17 °C and 19 °C colocalized with R_{max}QTL and in proximity a strong DCIQTL at 12 °C explained 38 % of phenotypic variation with lod score of (LOD>16) indicating their association with variation in DCI at low-optimum temperature and its neutral effect at high temperatures. Although constitutive QTLs are the target of plant breeder, these QTLs which conditionally expressed are also useful to incorporate in breeding program as the favorable alleles for specific environments will have no negative effect on other environments.

In three independent approaches a common major QTL on C06 strongly associated with high temperature effect was mapped. First, using curding per se data, the QTL on C06 was detected in three high temperatures 21 °C, 26 °C, and 27 °C explained 11 % to 40 % of the genetic variation with large additive effects (Chapter2, Table 4) co localized with large effect of FLNQTLs at 21 °C and 27 °C (Chapter 2, Table 5) and significant Q × E for both traits DCI and FLN (Chapter 2, Table 7). Second, in binary analysis on curding to non-curding phenotypes revealed a major QTL on chromosome C06 in the same genomic region mentioned above with high lod score (LOD>15) involved in the curding response. At this QTL, nearly all DH lines carrying the B allele induced a curd at temperatures higher than 21 °C while nearly 50 % of DH lines carrying the A allele failed to induce curds (Chapter 2, Fig 2 and 3). Third, QTL analysis on the model parameters reconfirmed the same genomic region on C06 for all three parameters of the phenology model SR, SL and Bp. The SRQTL explained 28.5 % of the phenotypic variation with strong evidence (LOD>11). The additive effect of SRQTL was five times the SLQTL suggesting that sub optimal high temperature enhances the role of this QTL. Furthermore, no R_{max} QTL was detected which further indicate

that this QTL involved in suboptimal high temperature sensitivity (Chapter 3, Table 9; Fig 6). These findings indicate that the QTL on C06 is a strong candidate for high temperature sensitivity in cauliflower. Ryder et al. (2001) identified two segments on C06 showing co-linearity with *Arabidopsis* chromosome 1. Both *BoAP1-a* and *BoAP1-c* genes were mapped in these two regions. The *BoAP1-a* locus was suggested to be associated with curding phenotypes and the stage of arrest in *B. oleracea* (Smith and King, 2000; Gao et al. 2007). However, Labate et al. (2006) concluded that the fraction of phenotypic variation explained by *BoAP1-a* is low, but the locus interacts with temperature. The expression of *BoAP1* in the shoot apex of cauliflower is switched off during vegetative reversion at high temperatures (Anthony et al. 1996). High temperatures also promote formation of bracts (Booij and Struik 1990; Grevsen et al. 2003). Kop et al. (2003) found evidence for correlations between the allelic state of *BoAP1-a* and the severity of bracting. The previous study suggested that *BoAP1-a* or closely linked genes play a major role in controlling bract development. The physical position of *Boi2AP1* (Carr and Irish 1997), which is *BoAP1-a*, is C06: 35,676,652 according to the BolBase A12 sequence (Liu et al. 2014). The physical position of *Boi1AP1* (*BoAP1-c*) is C06: 7,705,861.

Sebastian et al. (2008) based on qualitative measure indicate a QTL on C07 at 17 cM to be associated with vernalization requirement. Similarly, in this study the hotspot QTLs on C07 located at 30 cM showed higher additive effect at higher temperatures and DCIQTL×E suggested sensitivity to temperature.

On chromosome 9 the hot spot region for curding per se and the higher QTL effects at high temperatures (Chapter 2, Table 4) co localized with R_{max}QTL (Chapter 3, Table 9) and significant Q × E interaction for both traits DCI and FLN (Chapter 2, Table 7) indicate that the hotspot region on C09 has an influence on variation in sensitivity to temperature. Previous studies carried out on *B. oleracea* suggested that QTL for flowering time mapped on C09 may

result from the variation in vernalization requirement (Bohuon et al. 1998; Rae et al. 1999). Synteny between the region on chromosome 5 in *A. thaliana* and C09 in *B. oleracea* has already been shown and the *FLC* paralog *BoFLC1* was mapped on C09 (Salatheia 2003; Pires et al. 2004). *FLC* paralogs were considered as candidate genes for variation in vernalization and flowering time in *B. napus*, *B oleracea*, and *B. rapa* (Osborn et al. 1997; Schranz et al. 2002; Okazaki et al. 2007). The position of the QTL hotspot on C09 overlaps with the *FLC* paralog. In the TO1000 *B. oleracea* genome sequence (Parkin et al. 2014; Wang et al. 2011b) the position of S0629 is C09: 49,393,351 and the *FLC* position is C09: 51,033,935. A *FRIGIDA-LIKE* (*FRL*) ortholog is located close to S0533. However, it was also suggested that *FLC* independent pathways could be responsible for flowering time variation (Uptmoor et al. 2012; Ridge et al. 2014).

Detected QTL will assist the development of stable genetic markers that help marker-assisted breeding strategies towards thermo-tolerant cultivars. Most promising regions are located on C06 and C09. Both regions showed significant Q x E interactions for FLN and DCI QTLs and increasing additive effects with increasing temperatures. Importance of one hotspot on C06 was supported by binary analysis of curding vs. non-curding data.

High heritability was estimated for LAR (0.91). All 30 significant QTLs identified for LAR are novel, with no previous QTL study reported for this trait in this crop. In all environments, individual LARQTL showed a lod score greater than 4 and explained 11 and 29 % of the phenotypic variation. Main hot spot regions were detected on C01 at two genomic regions, C04 and C06 (Chapter 2, Table 6). In all the coincidences of QTL for DCI and LAR, the additive effect had opposite directions. This result suggests that the genetic architecture underlying DCI is strongly correlated to the genetic control of LAR. Méndez-Vigo et al. (2010) found that variation in *A. thaliana* flowering time depends also on the rate of leaf production and most QTL for flowering time co-localized with QTL for rate of leaf

production. The strong genetic correlation between DCI and LAR may suggest potential use to manipulate crop duration and crop maturity time simultaneously.

Since it is assumed that a certain number of leaves must be initiated before juvenile-to-adult phase transition, LAR may have a direct impact on the duration of juvenility and subsequently on flowering time. Thomas (1980) found that differences in the duration of the juvenile phase in brussels sprouts (*B. oleracea* var. *gemmifera* L.) were exclusively due to differences in LAR with early cultivars having higher LARs. In this context, the three QTL hot spot regions for LAR may be considered as potential candidate for aspects involved in juvenile duration. Interestingly, the hotspot on top of C01 is close to the position of a copy of miR156; a miRNA that controls juvenile transition (Wu et al. 2009). The position of S0714 is C01: 2,993,555; the position of miR156 is C01: 3,777,428.

In agreement with the significant genotype by environment interaction for LAR (Chapter 1, Table 2), three LARQTL×E were mapped on the tow hot spot regions on C01 and one on C06 (Chapter 1, Table 7) indicating their environmental dependency. Given the potentially important role of LAR in the juvenility end mentioned above, the environmental dependency of LARQTLs might support previous studies report that the end of juvenility may vary with the environmental conditions (Wurr et al., 1994; Fellows et al., 1999).

At both genomic regions on C01, in addition to LARQTLs and LARQTL×E, two R_{max}QTLs and two SLQTLs were also mapped in both regions indicating their sensitivity to low-optimum conditions (non-heat stress environments). In similar genomic regions on C01, Gao et al. (2007) in a cross of broccoli and cauliflower based on visual scoring of inflorescence type found two QTLs involved in the stage of arrest of reproductive development explained 21 and 6 % of the variation. The stage of arrest is highly sensitive to temperature effect (Douclas and Bjöorkman, 2007). Rae et al. (1999) in a backcross of substitution lines from a cross between *Brassica oleracea* var. *italica* and *Brassica oleracea* var. *alboglabra* detected

two adjacent flowering time QTLs on chromosome 1; one late flowering between 0.0 cM and 30.3 cM and one early flowering between 30.3 and 38.1 cM. In this study, at both genomic regions on C01 the additive effect of the same allele also had antagonistic QTL effect for all three traits LAR, R_{max} and SL. Although the study of Gao et al. (2007) mapped two QTLs associated with curd phenotype indicating sensitivity to temperature, it is not clear whether the QTL effect at both genomic regions have opposite additive effect. However, the findings of Rae et al. (1999) and from this study might suggest that both genomic regions may act in antagonistic manner. R_{max}QTLs at both genomic regions have similar absolute additive effect value indicating that at optimum temperature the effect of both QTLs on curding rate cancelled each other. In contrast, the SLQTL effect size at top of C01 is almost half of the effect size at the other genomic region indicating that in the range of low temperatures both QTLs are probably differentially expressed in antagonistic manner to regulate the rate of curding in response to low temperatures.

Flowering time is highly correlated to FLN in many plant species. In cauliflower, the delay in curd initiation due to high temperatures during the adult vegetative development stage is correlated with an increase in FLN (Wiebe 1972b; Booij and Struik 1990; Hand and Atherton 1987). In this study, in addition to DCI as measure of curding time, the FLN produced was determined as complementary measure of curding time. A total of 29 significant QTL for FLN were detected. At the two main hot spot regions for DCI on C06 and C09, co localization between DCI and FLN QTLs was observed and a significant FLNQ×E was mapped which highlights the value of this measure in cauliflower to support the genetic variation detected in curding time.

Progress in molecular plant breeding particularly for complex traits is constrained by the ability to predict plant phenotype based on its genotype in relevant target environments to underpin crop trait improvement (Hammer et al. 2010). Crop development models have the

potential to bridge this predictability gap (Hammer et al. 2010). The improvement of phenotypic predictions through crop modeling result from its potential to deal with complex interactions among plant growth and development processes, environmental effects and genetic control (Hammer, 2012). This study showed how a simple phenology model, which quantifies the causality between rate of development towards curding and temperature, can enhance our understanding of the phenotype complexity and assist the identification of quantitative trait loci (QTLs) for relevant new phenotypic traits (model parameters). Unlike curding per se measured at certain time point in single environment, phenology model described changes in the phenotypic response with the environment change in a set of parameters. It integrates the plant development and the interaction between plants and environment in a casualty interconnected way which is difficult to obtain by conventional phenotyping process. The model parameters represent a new phenotype with new biological meaning. Weather a phenotype measured as a standard agronomic trait or as a model parameter, plant breeder is mainly interested in the genetic basis of the phenotypic variation (Baenziger et al. 2004). In this study, the genetic basis underlying of the phenology model parameters was identified. A total of 20 QTLs were detected for all four parameters explain 6 % to 28.5 % of the phenotypic variation (Chapter 2, Table 9). Although most of the markers detected for model parameters were detected for curding per se on C05, C06, top of C07, and bottom of C09, new conclusions have been emerged. In particular, comparing QTLs detected for the model parameters SL, SR, and R_{max} with hot spot QTLs for curding per se in single environment revealed that in all genomic regions, when a co localization of SLQTLs, SRQTLs and curding per se QTLs had occurred, the effect was consistent, indicating that the favorable allele for curding per se in single environment analysis were also favorable allele for curding rate to temperature. Unlike QTLs detected for curding per se which are restricted to a particular environment, the QTLs for SL and SR are a result of wider range of environments. Thus, selection of QTL alleles affecting the rate of development response

curve parameters should be more effective than selection of alleles for QTLs detected in single environment analysis. Another interesting added value from modeling approach is that, the three R_{max}QTLs were colocalized with three hot spot regions in single environment analysis, one each at middle of C05, and at two genomic regions at C06. These QTLs were detected independent from other suboptimal temperature sensitive model parameters (SL, SR) indicating that the modeling approach was able to distinguish between QTLs that affect curding through suboptimal temperature sensitivity pathway and that affect curding for other aspects such as earliness per se**.** Breeding for early maturing cultivar might be a desirable trait in cauliflower. However, earliness is strongly influenced by temperature. Differences among the DH lines in R_{max} indicate the differences in the net durations of developmental transitions under optimum temperature for each DH line. Regardless of whether these differences associated with the duration of vegetative or reproductive phase, it is relevant to suggest the three R_{max} QTLs occurred independent from the SL and SR as potential in breeding for earliness (early maturity). Furthermore, QTLs detected for R_{max} and SR are largely uncorrelated. This implies that sensitivity to sub optimal high temperature and maximum development rate at optimum temperature seem amenable to independent genetic improvement.

To develop QTL based model, the QTL effect of the identified QTLs of the model parameters was incorporated into the phenology model. Several recent papers illustrated the potential of applying genomic research to modeling (White and Hoogenboom 2003; Baenziger et al. 2004; Yin et al. 2004; Uptmoor et al. 2008). In this study, in the parametrization set, the observed curding time across environments was compared to curding time predicted by the phenology model and by the QTL based model. The phenology model accounted for 95% of the phenotypic variation while the QTL based model accounted for 46% of the phenotypic variation (Chapter 3, Fig. 7, and Table 10). In several studies which used this approach, the

QTL based model has reduced ability to predict the phenotype compared to the phenology model. Although the use of such approach has a considerable potential in predicting the phenotype through the genotype for application in plant breeding, it is still in its infancy (Hammer and Jordan, 2007; Parent and Tardiue, 2014) and the capability of current crop models remains questionable due to inadequate understanding or incomplete quantification of key parameters (White, 2006). Yin et al. (2005b) indicate the weakness of current crop models in predicting the differences in complex traits within segregating populations as a major constrain for applying QTL based model. In addition to the weakness of current crop models in simulating observed variation, the QTL based model approach shares challenges of QTL analyses such as population size recommended for reliable QTL detection, considering epistasis, and detection of false positive (White, 2006). On the other hand, the accumulation of different types of errors such as the experimental error, the model error, and the error of QTL statistics are other sources of uncertainty in such approach (Uptmoor et al. 2008). In this study, in addition to the simplicity of the phenology model considering only the temperature as driving force of curding time and model errors, the reduced accuracy in QTL based model may attribute to the limited number of QTLs which individually account for only 6 to 28.5 % of the phenotypic variation of model parameters (Chapter 3, Table 9). Generally, limited number of QTLs might be due to small sample size or insufficient coverage of the markers.

For predicting the curding time within an independent data set of DH lines derived from the same parents, the QTL based model account for 0.27 to 0.65 of the phenotypic variation with RMSE of 3 to 10 days (Chapter 3, Fig. 7,Table 10). Bogard et al. (2014) using QTL based parameters of ecophysiological model were able to predict the heading date in wheat in an independent data set with RMSE of 5 to 8.5 days and explaining 48 to 63 % of the phenotypic variation.

Most of studies used the coupling of crop modeling and QTL mapping were restricted to the parameterization set, however few were extended to validation set but none of them test the QTL based model on hybrid lines. Prediction of hybrid performance is an attractive alternative to expensive field testing for identification of superior hybrids and can greatly accelerate hybrid breeding program (Shrag et al. 2009). In this study, the QTL based model could provide a satisfactory prediction of the test cross performance with low to moderate R^2 values ranged between 0.24 under relatively low temperature and 0.41 under relatively high temperature (Chapter 3 Table 10). Depending on the environment (field trials), the difference between the earliest and latest curding time varied from 11 to 26 days, reflecting the large genotypic variability among the test cross data set. However, the correlation between observed and predicted curding time was moderate and ranged between 0.49 to 0.63 (Chapter 3 Table 10). This showed that genetic effects which were estimated from the DH per se (parameterization set) may potentially used for prediction of test cross. Hofheinz et al. (2012) found that, the performance of hybrid can be predicted either with effects estimated from the same breeding cycle or with effects estimated in a previous breeding cycle.

Model selection can be challenging. Simple models facilitate the interpretation of the model coefficient and give better understanding of the physiological process. Model selection should be constrained on the basis of research objectives and on the understanding of the biological process. In this context, the bilinear model used in this study appears reasonable. The most important point in model selection in such kind of studies is to be able to simulate the genetic variability (Parent and Tardieu 2014). In this study, the bilinear model was able to simulate the genetic variability of curding time responses to temperature of large number of genotypes grown in common ranges of temperature by set of genotype specific parameters. Thus, identify the genetic basis of temperature response parameters. The model structure and model parameters characterize the phenotypic responses across environments describing the

maximum amount of genotype by environment interaction by differential sensitivity to environmental conditions in three domains: suboptimal low temperature; optimal temperature and high suboptimal temperature. This is in agreement with current understanding of cauliflower crop modelers of the rate towards curding as a function of temperature with minimum, optimum and maximum temperatures (Wurr et al. 1993; Grevsen and Olsen 1994; Kage and Stützel 1999).

Almost all crop models used two types of inputs: environmental and physiological inputs (Yin et al., 2005). In this study, the only environmental input is the temperature and the only physiological input is the time from transplanting to curd initiation. In this context the model is rather simple. In several studies which use the QTL based parameters approach, the physiological model was restricted to only one function such as flowering time (Yin et al. 2005; Uptmoor et al. 2008), leaf elongation rate (Reymond et al. 2003) and peach quality (Quilot et al. 2005). Such simple model can be easily implemented. Since the physiological trait used is relatively easy to measure, and it can be used by plant breeder in standard crop performance in practical cauliflower breeding program.

4.2 Future research

Mapped QTLs represent an essential step to further use in marker assisted selection. Some QTLs were repeatedly mapped across a wide range of environments. However, further studies are needed to validate suggestive QTLs and their effect in different genetic background before MAS is implemented.

The physical position of some potential candidate genes involved in flowering time, temperature sensitivity, inflorescence merstiem identity and juvenile to adult transition was inferred from *Brassica* reference genome and were found to be close to QTL hot spot regions on top of C01, bottom of C04, middle of C06, C07 and C09. Candidate genes in the hot spot regions need to be sequenced and sequence variation could be correlated to phenotypic variation.

Juvenility can act as a confounding factor in curding time. Usually researchers use the reproductive competence to distinguish between adult and juvenile phase by conducting reciprocal transfer experiments from warm-less inducing conditions to cool-more inducing conditions at different plant ages (Matsoukas et al., 2013). Implementing such procedure for a mapping population is challenging. Although the major focus of this study is temperature effect on curding time and not juvenility, an attempt to explore the juvenile to adult transition was examined based on previous studies which indicated a change in leaf appearance rate during the vegetative growth phase and hypothesized that the rate change may occur at transition from juvenile to adult stage (Hand and Atherton 1987; Booij and Struik 1990). In this study, the hypothesized change in LAR could not be precisely estimated. The most probable reason is that, the leaf number was counted only eight times during the first 27 days after transplanting. Future studies may further investigate this hypothesis by including more data points starting from early seedling stage.

Considering the complexity of maturity time in cauliflower, the phenology model used in this study is still at early stage with respect to integration of other physiological process and their genetic basis. For instance, determining the length and the genetic basis of timing of juvenility and then incorporate these aspects by a sub model to the current model might will improve the model performance.

The phenology model used in this study has some strengths points such as simplicity, low number of parameters, reproduces the genetic variability and describes the rate of curding to temperature in quite relevant way of cauliflower physiology. However, it has some weak points such as lack of ability to describe the range of optimum temperature due to the abrupt change from increasing part to decreasing part (no plateu); overestimate the minimum and maximum temperature in case of extrapolation beyond the data range. However, this model can be used as a platform for further improvement using more complex models such as three stage linear model or beta function.

In this study, identification of the marker and marker effect of model parameters was done by using traditional QTL mapping. QTL analysis does not capture genes below the significant level and therefore could not be incorporated to the phenology model. Alternatively, using other approach to trace all marker effect instead of only significant QTL may be used. Mapping QTL using genomic selection can offer these properties.

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Curriculum Vitae

Personal data

Name: Yaser Hasan

Date of birth: 27.01.1974

Marital status: Married

Place of Birth: Hama – Syria.

Nationality: Syrian

Academic background

Ph.D. student (2011- 2016)

Project title: Genetic dissection and QTL based modeling of vernalization and curd development in cauliflower (*Brassica oleracea* var. *botrytis*)

Leibniz Universität Hannover, Faculty of Natural Sciences, Institute of Horticultural Production Systems

Master of Science in International Horticulture (2008 - 2010)

Thesis title: Modeling vernalization-induced bolting of sugar beet (*Beta vulgaris* L.).

(Grade: Summa cum laude)

Leibniz Universität Hannover, Faculty of Natural Sciences, Institute of Biological Production Systems

Diploma in Plant Protection (2000 - 2001)

Graduation project: Powdery mildew (*Erisiphe betae*) in sugar beet (*Beta vulgaris* L.).

(Grade: very good)

Damascus University, Faculty of Agriculture, Plant Protection Department.

Bachelor in Agricultural Engineering-Plant protection (1993 - 1999)

Graduation project: Efficiency of different light-activated pesticides against Diptera.

(Grade: good)

Tishreen University, Faculty of Agriculture, Plant Protection Department.

List of publications

Hasan Y, Briggs W, Matschgewski C, Ordon F, Stützel H, Zetzsche H, Uptmoor R (2016). Quantitative trait loci controlling leaf appearance and curd initiation of cauliflower (*Brassica oleracea* var. *botrytis*) in relation to temperature. Theoritical and Aplied Gentics, DOI: 10.1007/s00122-016-2702-6

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Acknowledgments

I am greatly indebted to **Prof. Dr. Ralf Uptmoor** for his valuable contribution, advice and support as first supervisor. Particularly I thank him for generously dedicating his valuable time even though he was no longer at LUH.

I would like to express my deepest gratitude to **Prof. Dr. Hartmut Stützel** for offering me the opportunity to do my Ph.D and also for his guidance, feedbacks, and encouragement as a second supervisor.

I am grateful to all members at the Institute of Horticultural Production Systems in the Department of Vegetable Systems Modeling for their great assistance in all the diverse ways throughout my research work.

I wish to express my heartfelt gratitude to my parents for their role in my life. I also thank my brothers and sisters and their respective families for their encouragement and support.

Finally, I am grateful to my ever supporting wife, Shaza Nabhan, for her understanding and patience, and also to my lovely children, Ahmad and Susanne.