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Analysis of candidate genes involved in curd initiation for breeding of widely adapted cauliflower (*Brassica oleracea* var. *botrytis*)

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1. Introduction, Knowledge, Objectives

The success of cauliflower production in the field depends to a large extend on the weather conditions. Transition to curd development and flowering requires cold ambient temperature during the vernalization sensitive phase. At temperatures above 20°C the vernalization requirement of most cauliflower cultivars is not met resulting in delayed curd development, prolonged harvest time and periods, and low quality of curds (Fellows et al. 1999). Natural variation of temperature tolerance has been observed and some cultivars have been identified which can be harvested in time even at high temperature. Vernalization requirement, curd induction, and harvest reliability are not expected to be regulated by the same temperature-dependent genes which cause low quality curds (Duclos & Björkman 2008). Thus, it is assumed that genetic markers can be developed to assist cauliflower breeding for a superior combination of quality traits and a wide adaption to different temperature conditions. The project aims to (i) identify candidate genes involved in cauliflower curd development and vernalization, (ii) conduct a sequence analysis of selected candidate genes, and (iii) detect SNP markers associated with curd development by candidate gene association studies.

2. Material and Methods

The plant material investigated comprises a diversity set of 112 nearly homozygous cauliflower lines from Syngenta Seeds B.V., including the parents of a formerly studied DH population (Hasan et al. 2016). The set strongly varies in the temperature response of curd induction, maturing rates, and harvest reliability. Phenotyping of the whole set has been carried out in two independent greenhouse trials under distinct temperature regimes (Matschegewski et al. 2015). Data derived from Syngenta Seeds B.V. on plant development have been used to consider unstressed growing conditions.

Candidate genes involved in flowering time, vernalization, response to temperature, and inflorescence formation of the cauliflower-related model plant *Arabidopsis thaliana* were queried by means of GO annotations from the TAIR database (Lamesch et al. 2012). The respective homologues in *Brassica oleracea* were inferred using Bolbase, a database of an annotated genome of *B. oleracea* var. *capitata* (Yu et al. 2013). Homologues co-occurring within intervals of QTLs for temperature response (Hasan et al. 2016), were selected for detailed sequence analysis, at first on the parents of the respective DH population and homologues with sequence polymorphisms subsequently on the entire diversity set.

Total genomic DNA was isolated following a modified CTAB protocol (Doyle & Doyle

1990). Amplification and sequencing primers were designed using the NCBI primer designing tool based on the *B. oleracea* var. *capitata* genome as available in Bolbase. PCR amplifications were performed using standard protocols at the JKI Quedlinburg. DNA cycle sequencing was conducted by Microsynth AG (Balgach, Switzerland). Sequence analysis was carried out using Sequencher ® v. 5.1 (Gene Codes Corporation). Sequence polymorphisms based on SNPs and insertions/deletions (indels) were taken into account for association genetics studies.
A unified mixed linear model (MLM, Q + K model) as implemented in TASSEL v. 5.2.5 (Bradbury et al. 2007) was applied for association genetics studies. 14385 SNP markers genotyped on the whole diversity set and provided by Syngenta Seeds B.V. have been used to account for genetic structure. The population structure (Q), estimated using a model-based clustering algorithm (STRUCTURE, Pritchard et al. 2000) was included as Q matrix and familial relatedness by a matrix of pairwise co-efficients (K, kinship matrix) calculated as implemented in TASSEL.

3. Results

498 homologues of *Arabidopsis* genes involved in flowering time, vernalization, temperature response, and inflorescence formation were inferred for the *Brassica oleracea* genome. A total of 18 of these candidate gene homologues co-located with QTLs for temperature-dependent curd induction in cauliflower on linkage groups O4, O6, and O9. Six of these candidate genes displayed polymorphisms (72 SNPs and 9 indels) between the parents of the respective DH population.
In the entire diversity set 231 SNPs and indels were identified in these six polymorphic candidate genes from sequences with a total fragment length of 13880 bp. The average rate of polymorphism ranged from 0.023 in the heat shock gene BoHX11 to 0.0013 in the floral meristem gene BoAX14. Two heat shock genes (BoHX11, BoHX13) and a temperature-responsive chromatin remodeling gene (BoHX12) counted for 92.6% of all nucleotide differences identified. Very few polymorphisms were detected in genes directly involved in flowering time and inflorescence meristem regulation (BoSX01, BoAX14, BoEX16).
Analysis of 14385 SNP markers revealed the population structure of four main clusters strongly separating tropical together with subtropical lines from three overlapping clusters of temperate cauliflower lines. Of the initial 231 polymorphisms, 181 with a minor allele frequency of ≥ 0.05 were tested against three temperature-dependent curd initiation traits using the mixed linear model (K+Q). Altogether, 34 marker-trait associations at a significance value $p<0.001$ were identified in three genes (BoHX11, BoHX13, BoEX16), individually explaining between 6.0 and 17.2% of the phenotypic variance.

4. Discussion

Marker-trait associations have been detected for polymorphisms in three candidate genes on two linkage groups depending on the mode of temperature stress. Two of these genes (BoHX11, BoHX13) co-locate with a major temperature responsive QTL on linkage group O6 and encode heat shock proteins. One gene (BoEX16), locating on linkage group O9, encodes a homologue of the PAF1 protein which is required for the enhancement of H3K4

methylation in the FLC chromatin, which in turn is the main flowering repressor integrating the autonomous and the vernalization pathway in *Arabidopsis* (Moon et al. 2005).
Heat shock genes are structurally and functionally conserved (Wang et. al. 2004), but the mutation rate of single heat shock genes in plants has not been investigated on the population level. In comparison to the number of known polymorphisms of the homologues of BoHX11 and BoHX13 in *Arabidopsis*, the high rate of associated polymorphisms in cauliflower is noteworthy. It strongly suggests that allele variation in these heat shock genes contributes to natural phenotypic variation in curd initiation of cauliflower.

5. Conclusions

Our results show the potential of a candidate gene association analysis starting with a broad list of potential candidate genes prioritized by their location within a QTL for the trait of interest. Marker-trait associations between temperature susceptibility and curd development will be of high interest for cauliflower breeders as timely curd development and continuous market delivery are major factors in cauliflower production. This study provides molecular markers for the selection of cauliflower cultivars with adaptation to a wide temperature range. The marker-trait associations are currently under validation and tested for their consistency.

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6. Literature

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