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Full Length Article

Genetic Mapping of QTLs Associated with Resistance against Onion Thrips and Related Morphological Characteristics in White Cabbage

Fruzsina Rőth[1*], William H. Briggs[2], Sjaak van der Ploeg[2] and Zsolt Galli[1]
[1]*Syngenta Kft. Trial Station, Üllői út külterület H-2364 Ócsa, Hungary*
[2]*Syngenta seeds B.V. Westeinde 62. 1601BK Enkhuizen, The Netherlands*
[*]For correspondence: fruzsina.roth@syngenta.com; fruzsi_roth@yahoo.com
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Abstract

Thrips species are well known and dangerous pests in many economically important crops, and plant resistance is in scope of numerous research projects. In the current research, genetic mapping of plant resistance in white cabbage against *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) and related plant traits - amount of wax layer of the leaves and head density was performed on F5 generation of a white cabbage RIL mapping population developed for this purpose. QTL mapping of the damage level caused by thrips revealed two major (Ttq1 and Ttq2) and one minor (Ttq3) QTLs. The highest effect on the phenotypic variance was 28% caused by Ttq1 at the position of 131 cM on chromosome 2, followed by 21.1% of Ttq2 on chromosome 7 at position 27.5 cM and 5.2% of Ttq3 on chromosome 8 at position 33 cM. With the small interaction between Ttq1 and Ttq2 adding 6.1% of variance, all together the identified three QTLs are responsible for 60.5% of phenotypic variance of thrips resistance in the mapping population. Regarding the assumed thrips resistance related morphological plant characteristics, one major and two minor QTLs for amount of wax layer and two major and one minor QTLs for head density were identified. The regions significant for thrips resistance on chromosome 2 and chromosome 7 both had been mapped also as QTLs of wax and density plant traits, supporting the theory that these plant characteristics play a key role in thrips resistant phenotype.

Keywords: *Brassica oleracea var capitata*, Linkage map, *Thrips tabaci*, Marker-assisted selection, Wax, Head density

Introduction

Nowadays, plant protection is a major challenge for growers worldwide when new pests and diseases appear and spread quickly due to human activity and changing climate conditions. The onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) has become one of the most damaging pests of white cabbage (*Brassica oleracea convar. capitata* var. *alba*) worldwide during the past decades (Fox and Detbridge 1977; Shelton *et al*. 1983; Kahrer 1992; Pénzes *et al*. 1996; Shelton *et al*. 1998, 2008). Between the leaf layers of the closing cabbage head, a well-protected habitat is formed for feeding and reproduction of onion thrips. Adults and larvae feeding in the head injure the cells with their sucking-piercing mouth and induce abnormal callus development with brownish discoloration of the leaves (Voorrips *et al*. 2008). Poor quality of the product and increased labor cost of peeling damaged leaves can cause serious economic loss for growers. Regulations of insecticides tend to be stricter (Mouden and Leiss 2020) and available chemicals do not provide effective protection for white cabbage against onion thrips due to the pest's reproductive biology and easily developed insecticide resistance (Fail *et al*. 2013). Beyond some agrotechnical methods (Zindracic *et al*. 2007; Trdan *et al*. 2008b), the only effective growing strategy is the use of thrips resistant cabbage varieties.

Wide varietal differences were already shown by comparison of commercial varieties' thrips resistance (Shelton *et al*. 1988, 1998; Stoner and Shelton 1988; Zindracic *et al*. 2007; Fail *et al*. 2008; Trdan *et al*. 2008a; Voorrips *et al*. 2008; Fail *et al*. 2013) and different studies investigated the connection of certain plant characteristics with thrips resistance of white cabbage. Some of the plant's physiological and morphological traits: epidermal thickness (Bálint *et al*. 2013a), leaf thickness and head compactness (Voorrips and Steenhuis-Broers 2010) do not have a clear correlation, while others, like UV-A and visible reflection of leaves (Bálint *et al*. 2013b), or Brix value (Voorrips *et al*. 2010) were found to be in significant correlation with thrips resistance. Epicuticular wax content of the leaves was also reported in more experiments as a key factor affecting host plant resistance of white cabbage against onion thrips (Trdan *et al*. 2004, 2005, 2008a; Žnidarčič *et al*. 2008),

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however the mentioned plant traits respectively show too low correlation with thrips damage to be useful as an accurate and reliable indicator of its damage (Voorrips *et al.* 2008).

One of the vegetable crops most endangered by thrips species is pepper due to its role in spreading Tomato Spotted Wilt Virus (TSWV). Thrips resistance was found to be affected by one major QTL on Chr6 detected in an F2 mapping population infected by *Frankliniella occidentalis*. According to this study, in the case of pepper, antibiosis seems to play a more decisive role than tolerance and 50% of the phenotypic variability was explained by the QTL identified and was not related to trichome density despite expectations (Maharijaya *et al.* 2015). Contrarily, another QTL on Chr5 was reported previously as associated with resistance against onion thrips and *Bremisia tabaci* (Linders *et al.* 2010). Differing results suggest that this trait is affected by a more complex system. Effect of the QTL identified on Chr6 (Maharijaya *et al.* 2015) was verified on two thrips species (*F. occidentalis* and *T. tabaci*) in four different *Capsicum annuum* backgrounds and with this its general applicability in breeding was proved (Haperen *et al.* 2020). Fine mapping of this chromosome region highlighted role of three genes containing SNP. These diagnostic marker candidates are predicted to led changes in protein structure (Haperen *et al.* 2021).

T. tabaci is also known as a major pest and virus vector of onion, where complex inheritance of thrips resistance was also assumed already in 1999 due to the low values of narrow-sense and broad-sense heritabilities (Hamilton *et al.* 1999). Thrips resistance in onion was associated with the epicuticular wax amount of the leaves and shape of the epicuticular wax crystals (Basri and Ansari 2021). Onion leaf color was also found to correlate with thrips resistance, and light reflections of the leaves are determined by more factors, including waxes (Pobożniak *et al.* 2021). Glossy varieties with decreased wax content are often more resistant to thrips damage (Molenaar 1984). Based on this correlation molecular markers associated with amount of wax could be used in breeding programs as a primary predictor of thrips resistance. Chromosome regions associated with amount and types of wax content of the leaves were already identified on Chr5 and Chr2 in a QTL mapping study (Damon and Havey 2014).

Thrips tabaci Lindeman (Thysanoptera: Thripidae) causes huge losses every year in the white cabbage production in continental climates and plant protection can be effective only by planting resistant varieties. While the genetic background of host plant resistance against thrips is well investigated in other vegetable crops, our knowledge of the genomic information underlying resistance in white cabbage is limited. Until now, there is only one published result of a mapping study targeting the identification of QTLs associated with thrips resistance in white cabbage within the framework of a patent application (Löptien 2013). Thrips resistance was described as a monogenic additive trait based on the inheritance pattern in the mapping population. Altogether seven markers linked to the resistance were mapped and ordered on Chr2 based on the QTL analysis of an F2 population. The two markers mapped closest to the QTL are positioned at 43.1 cM and 47.6 cM on Chr2 (Löptien 2013).

Based on the several plant traits showing correlation with thrips resistance in cabbage, we should not rule out that expression of this trait can be also affected by more genetic factors. Reliable diagnostic markers need to be tightly linked to the target sequences to avoid recombination between them. The aim of the current study was to validate the presence of the QTL on Chr2 previously published with closely linked markers, and possibly identify other candidate chromosome regions playing a role in thrips resistance in white cabbage. Chromosome regions associated with two morphological traits: waxiness of the leaves and density of the head were also aimed to be determined, as traits showing strong correlation with thrips resistance and presumably good candidates for prognosis of resistance level of white cabbages in breeding programs.

Materials and Methods

Plant materials and field experiments

A mapping population of 210 recombinant inbred lines (RILs) was used for genetic mapping of thrips resistance derived form a cross between two white cabbage lines and developed by single seed descent (SSD) method (Snape 1976) continued until F5 generation (Fig. 1). Parent lines were developed in Syngenta Ltd. white cabbage breeding program and used as parental lines of different storage type commercial varieties. Both lines produce firm and dense heads with good internal quality fitting to the requirements of storage; however, differ in resistance to the damage caused by *T. tabaci*: Parent1 line shows strong resistance, while Parent2 line is highly susceptible to this pest.

A field experiment was carried out at the trial station of Syngenta Kft. in Ócsa, Hungary (latitude 47.3026784, longitude 19.2438642) in 2016. After sowing on 19th of April followed by young plant raising, the 210 inbred lines were planted in open field using a Randomized Complete Block (RCB) design with 4 replications in 50 x 60 cm plant spacing on 19th of May. Growing conditions were guaranteed according to the local grower practice until plants reached harvest maturity.

Phenotyping

Three agronomic traits: amount of wax on the leaves around the cabbage head, density of the head and level of damage caused by onion thrips in the head were measured and recorded on 4 replications per plot of the parental and inbred lines before or after harvest.

Phenotypic evaluation of the wax layer on the leaves was carried out on the field before harvest. Amount of wax layer on the leaves around the cabbage head was assessed by visual observation. The wax layer was removed on a 5x5 cm area of the leaf surface by wet sponge to help visual comparison with the same leaf's area covered by wax. Values were recorded on 1 to 9 scale per plot, where values indicate: 1 – no wax on the leaf; 2 – very low amount of wax on the leaf; 3 – low amount of wax on the leaf; 4 – low-middle amount of wax on the leaf; 5 – middle amount of wax on the leaf; 6 – middle-high amount of wax on the leaf; 7 – high amount of wax on the leaf; 8 – very high amount of wax on the leaf; 9 – highest amount of wax on the leaf in the experimental population. The plants with thickest and thinnest wax layer in the mapping population were used as 9 and 1 reference values. In each family, 5 plants were checked and the mean value of the family was estimated. These estimated means were used as a basis for QTL mapping process of wax amount.

Thrips damage and density of the head were evaluated on 4 heads per replication harvested from 15th of September to 18th of October according to the plot's harvest maturity to avoid losing data due to cracking of the early plants. The earlier heads were stored in a cold storage room at 4°C and 85% of air humidity until the last harvest date to ensure an equal time frame for thrip's damage development. After harvest, 25 leaf layers were peeled off from every head (in total 3376 heads) and flattened by making small cuts from the edges of the leaves. The approximate percentage of leaf area damaged by thrips compared to the whole leaf area was estimated by visual assessment. Each leaf's value was recorded. Cumulative thrips damage for each head was calculated by summing the percentage damage of the leaf layers. The means of the RILs and parental lines were used for QTL analysis.

After peeling off the damaged leaves, the rest of the heads were cut in half, and density of the head was scored on a 1 to 5 scale (1 – very loose type; 2 – loose type; 3 – moderately dense type; 4 – dense type; 5 – very dense type). Mean density estimates of the RILs and parental lines were used for further analysis.

Correlation coefficients were calculated by Spearman's Nonparametric Test to discover correlations between severity of the damage caused by thrips and amount of wax on the head surrounding leaves or density of the head.

Genetic linkage map construction and QTL analysis

Genotyping of the parental lines and the mapping population was performed in the Genotyping Laboratory of Syngenta France SAS. Screening the parental lines of RIL population genome with 15.000 genome-wide single nucleotide polymorphism (SNP) based markers in total resulted 248 SNP markers showing polymorphism between the parental lines of the mapping population. A total of 240 markers segregated in co-dominant and 8 markers segregated in dominant way. The RIL population was genotyped with the identified polymorphic marker set on the F4 generation in 2015. Genomic DNA was isolated with standard DNA protocol (Potassium acetate) and resuspend in 100 μL of TE. DNA template was diluted to 10 ng/μL concentration and 4 μL of it was used per cell. Master mix was added (1 μL Sigma Buffer 10x, 1.2 μL $MgCl_2$, 0.8 μL dNTP, 0.132 μL Sigma Taq, 0.125 μL Primers-Probes mix, 0.1 μL ROX, 2.643 μL H_2O QSP) in 6 μL volume per cell. After a spin down briefly, PCR program was executed according to: 2 min 94°C for Sigma Taq Polymerase; 40 times 15 s 94° and 1 min 60°C and finally 5 min 72°C.

Genetic linkage map construction and QTL analysis were executed with the 'qtl' and 'LinkageMapView' packages of 'R' software (version x64 4.0.2) according to the guide of QTL mapping process in R written by Broman and Sen (2009). The "calc.genoprob" R/qtl function with error probability of 0.01 of Kosambi map function was used to order and map the 248 polymorphic markers. Significance thresholds ($P < 0.05$ and $P < 0.01$) for LOD scores were determined by "scanone" R/qtl function with permutations of 1000 replications and Haley Knott regression. Due to the non-normal distribution of variables, the genome was scanned with composite interval mapping ('cim') model for each mapped trait to find candidate QTLs on the identified linkage groups. Genome regions with significant effect on the examined traits and interactions between these loci were considered significant with ANOVA tests. The function 'refineqtl' was used to refine the locations. Effect of the closest markers to the target sequences were shown by 'effectplot' function, and confidence intervals of the identified QTL positions were specified with 'lodint' function.

MapChart software (Voorrips 2002) was used to generate a graphical representation of the physical positions of the QTLs identified in current research and previously published results of thrips resistance of white cabbage projected onto the published TO1000 *B. oleracea* reference genome (Parkin *et al*. 2014).

Results

Phenotypic assessment

Trial field and growing conditions provided consistent circumstances for development of the plants and thereby well usable data for molecular mapping. Within the F5 generation RILs developed for thrips resistance mapping from the cross of the two parental lines, there was already greatly homogenous phenotypic appearance, while between the families, a high degree of phenotypic variability was observed regarding several traits.

The amount of wax on the head surrounding leaves recorded during the growing season was one of the morphological plant traits showing high phenotypic

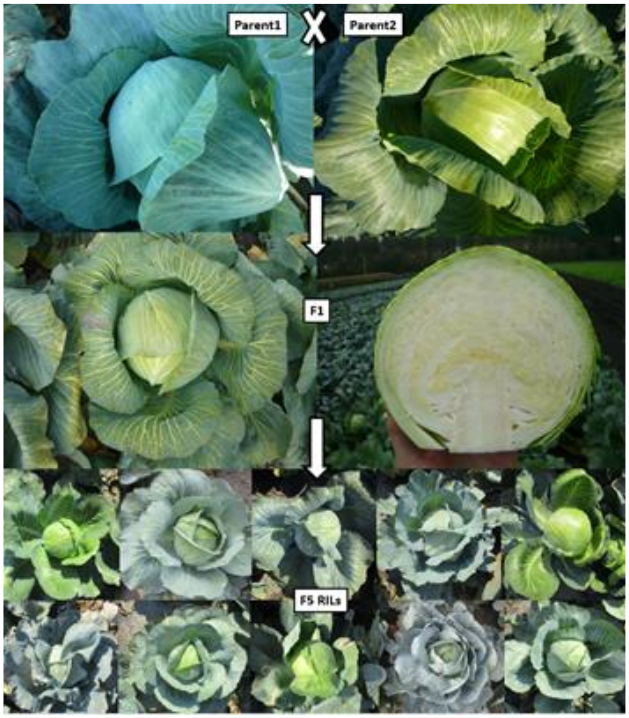

Fig. 1: The resistant Parent1 and sensitive Parent2 lines of the mapping population, F1 plant with the internal structure of the cabbage head and some examples of the F5 generation representing the high phenotypic variability among the RILs

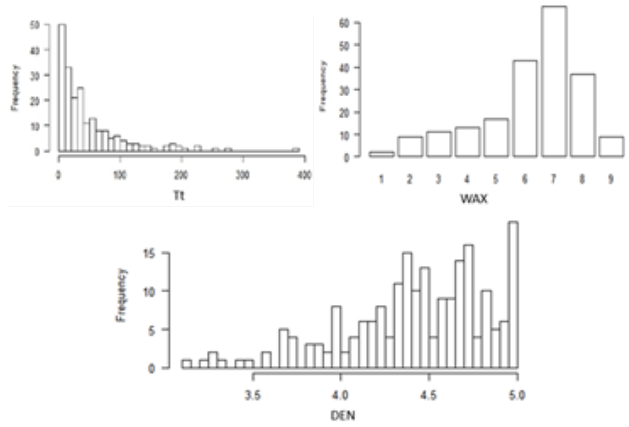

Fig. 2: Phenotypic variation of mean cumulative thrips damage (Tt), mean amount of wax on the leaves (WAX) and mean head density values (DEN). Histograms represent the frequency of data collected in RILs

variability. Values were noticed from 1, where the glossy leaves were covered with thin or even no wax layer, to 9, where the thickest wax layer was found within the mapping population. The estimated mean data of the families did not show a normal distribution (Fig. 2).

During the growing period, the level of natural onion thrips infection reached a high level as usual during summertime in continental climate, and significant

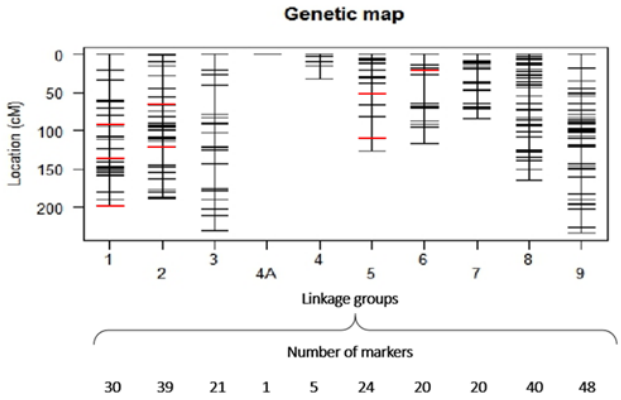

Fig. 3: Genetic linkage map constructed from the 240 codominant (black bars) and 8 dominant (red bars) SNP markers found to be polymorphic between the two parental lines of the RIL population, corresponding to the 9 chromosomes of *B. oleracea*

differences were shown between the cumulative thrips damage of mapping population families (Chi square = 628.9, p = 1,24E-43, df = 209). The mean cumulative damage varied on the scale from the lowest 0.13 to the highest 389.56 value, more than five times overexpressing the result of 69.25 recorded in the susceptible parent line of the RILs. The dataset of thrips damage also deviated from a normal distribution (Fig. 2).

Unsurprisingly, density of the cabbage heads scored after peeling and scoring thrips damage, did not represent extremely wide variability. Both parental lines belong to storage type white cabbage breeding program, where dense structure and good quality of the cabbage head is a key factor for long term storage. Significant difference had been found between head density of RILs (Chi square = 309, p = 4,38E-08, df = 209), main values per families moved from 3.1 to 5, also did not follow normal distribution (Fig. 2).

In comparison of the datasets of the three traits negative correlation was found between thrips damage and waxiness of the leaves with correlation coefficient -0.49 and very strong positive correlation was shown between thrips damage and head density with 0.97 correlation coefficient.

Genetic linkage map construction

The genetic linkage map was constructed based on the 240 codominant and 8 dominant SNP markers showing polymorphism within the RIL population. Markers used for QTL analysis give mostly a good level of coverage across the cabbage genome, and linkage groups correspond to the 9 chromosomes of *B. oleracea*, with the exception of Chr4, where distance between the markers were so high that two parts of the chromosome were identified by the software as separated linkage groups, and named 4 and 4A (Fig. 3). Total length of the genetic linkage map corresponds to physical length 446.22 Mbp. Distribution and genetic distances of the SNP markers on the linkage groups are represented on Fig. 3. Poor coverage of some regions of the

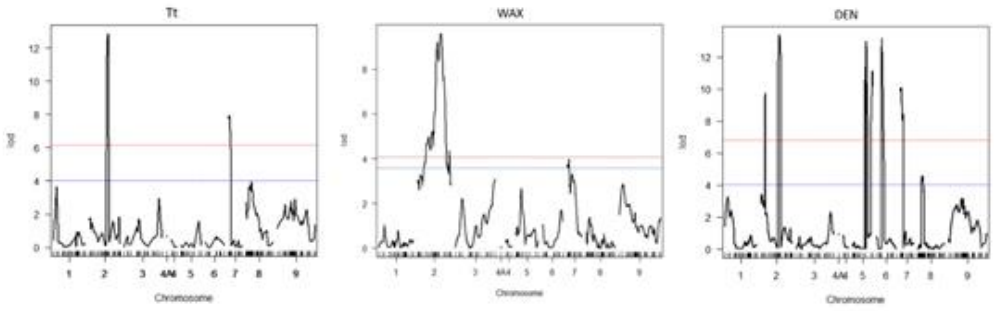

Fig. 4: LOD scores of thrips damage, WAX and DEN in the mapping population. The significance thresholds of LOD scores are represented by blue ($P < 0.05$) and red ($P < 0.01$) lines

nine cabbage chromosomes was experienced and caused by the high heterozygosity level of the resistant Parent1 line on the top of Chr5 and in the large gaps on the top of the Chr7 and Chr4. Reason for the missing map information on the top quarter of Chr8 is the fully monomorphic region between the parents. The 240 codominant markers of the RILs' genotyping segregate A/H/B and the 8 dominant markers segregate A/B, where A means the homozygous allele coming from the thrips resistant parent, B is homozygous for the allele coming from the susceptible parent, and H represents the heterozygous genotype.

QTL identification

Multiple QTL analysis of thrips resistance of white cabbage revealed three loci associated with level of damage caused by onion thrips in the current mapping population. Composite interval mapping model was used to determine the positions of putative QTLs reaching the LOD (logarithm of the odds) threshold 4.04 calculated from test of 1000 permutations, due to that data did not show normal distribution. Peaks values of LOD have exceeded the threshold and reached a peak point with 12.85 LOD on Chr2 in position 113.8 cM and 7.92 LOD on Chr7 in position 7 cM (Fig. 4). An additional putative QTL was detected by the software almost reaching the threshold with peak of 3.8 LOD score on Chr8. Refined positions of the QTLs were shifted to 131 cM on Chr2, 27.5 cM on C7 and 33 cM Chr8 with the SO1546, SO566 and SO1554 markers located closest to the object regions, respectively. All detected QTLs were found to be significant. Based on general characterization of QTLs by their effect on phenotype (Collard *et al.* 2005) two of them (Ttq1 on Chr2 and Ttq2 on Chr7) have major and one of them (Ttq3 on Chr8) has minor effect on thrips resistance of white cabbage and explained phenotypic variance of 28.04, 21.1 and 5.23% respectively in this mapping population. Small interaction identified between the Ttq1 and Ttq2 explaining 6.12% of phenotypic variance. According to the model including all the three QTLs and this interaction they are together responsible for 60.49% of phenotypic variance in the mapping population (Table 1).

The closest marker to the peak of Ttq1 is SO1546, where allele 'A' is originated from the resistant parent in homozygous form provides higher level of thrips resistance than allele 'B' (Fig. 5.). The large standard deviation in case of heterozygous genotype can be explained by the absence of heterozygotes for this marker in the current population. Marker SO566 was found to be located the closest to the peak value of Ttq2. Unexpectedly, the higher level of thrips resistance was provided by the allele 'B' for this marker, contributed by the susceptible parent. The third marker located the closest to the peak point of Ttq3 is SO1554. The allele 'A' originated from the resistant parent in this marker position provides higher level of thrips resistance than allele coming from susceptible parent line. (Fig. 3) Interaction between allelic configuration of SO1546 and SO566 markers are visualized on Fig. 6.

Waxiness of the leaves were analyzed and mapped because strong negative correlation between epicuticular wax content and the level of thrips damage on white cabbage was already reported in literature (Voorrips *et al.* 2008). Three QTLs were detected in with the maximum LOD scores of 10,01 on Chr2 in position 133 cM and 3,83 on Chr7 in position 9,5 cM above threshold 3.7 LOD (Fig. 4). One more putative QTL was identified and proved to have significant effect on waxiness of the leaves at position 224 of Chr3 linkage group with 3.3 LOD value. After using refine QTL function of the software the supposed positions of the QTLs slightly moved to 132 cM on Chr2 (Waxq1), 225 on Chr3 (Waxq2) and 34 on Chr7 (Waxq3). The final model represented 30.9% total effect on the trait analyzed, including Waxq1 proved to be major QTL explaining 18.01% of phenotypic variance, and Waxq2 and Waxq3 showed a minor effect with 5.31 and 7.58% on phenotypic variance of waxiness of the leaves (Table 1). Both markers identified as closest to the peak positions of QTLs on Chr2 (SO1546) and Chr7 (SO566) were detected also with significant effect in mapping process of thrips damage, in addition with SO409 marker found to be closest to Waxq2. In the case of markers SO1546 and SO409 allele 'A' derived from the thrips resistant parent gives the higher WAX content compared to the allele 'B', while for SO566 allele 'B' coming from the susceptible parent gives higher

Table 1: Identification of the major and minor QTLs and interactions between these QTLs associated with thrips resistance (Thrips), amount of wax layer on the leaves (WAX) and density of the head (DEN) in white cabbage mapping population, representing the assumed position of the QTLs with the closest markers and confidential intervals and percentage of the genotypic variance explained by the QTLs

Trait	chr	pos	QTL	closest marker	Lodint start		Lodint end		LOD (scanone)	LOD tresh. 0.05	LOD ANOVA	%var	df	F value	*P* value (Chi2)	*P* value (F)	sig.	Remark	Model var % calc.
					pos	marker	pos	marker											
Thrips	Chr2	131	Ttq1	SO1546	113.8	SO1679	145.2	SO560	12.85	4.04	19.09	28.04	6	17.27	0	4.44E-16	***	major	60.49
	Chr7	27.5	Ttq2	SO566	0	SO577	37.2	SO1877	7.92		15.06	21.10	6	13.00	0	2.30E-12	***	major	
	Chr8	33	Ttq3	SO1554	13.6	SO1884	71.8	SO522	3.93		4.23	5.23	2	9.66	0	0.0000994	***	minor	
	Int Chr2:Chr7	–			–	–	–	–	–	–	4.91	6.12	4	5.65	0	0.00025	***	interaction	
WAX	Chr2	132	Waxq1	SO1546	99.7	SO3133	145.2	SO560	10.01	3.70	10.43	18.01	2	26.10	0	8.38E-11	***	major	30.90
	Chr3	225	Waxq2	SO409	26.3	SO1453	231.3	SO600	3.10		34.39	5.31	2	7.69	0	6.04E-04	***	minor	
	Chr7	34	Waxq3	SO566	0	SO577	46	SO561	3.93		49.08	7.58	2	10.98	0	2.99E-05	***	minor	
DEN	Chr2	131.5	Denq1	SO1546	113.8	SO1679	145,2	SO560	13.38	4.04	22.40	30.91	6	21.10	0	<2E-16	***	major	68.72
	Chr7	28.5	Denq2	SO566	0	SO577	37.2	SO1877	10.13		17.92	23.42	6	15.99	0	6.00E-15	***	major	
	Chr8	26.1	Denq3	SO1618	13.6	SO1884	63.6	SO886	4.00		5.92	6.74	2	13.80	0	2.46E-06	***	minor	
	Int Chr2:Chr7	-		–	–	–	–	–	–	–	6.70	7.65	4	7.83	0	7.07E-06	***	interaction	

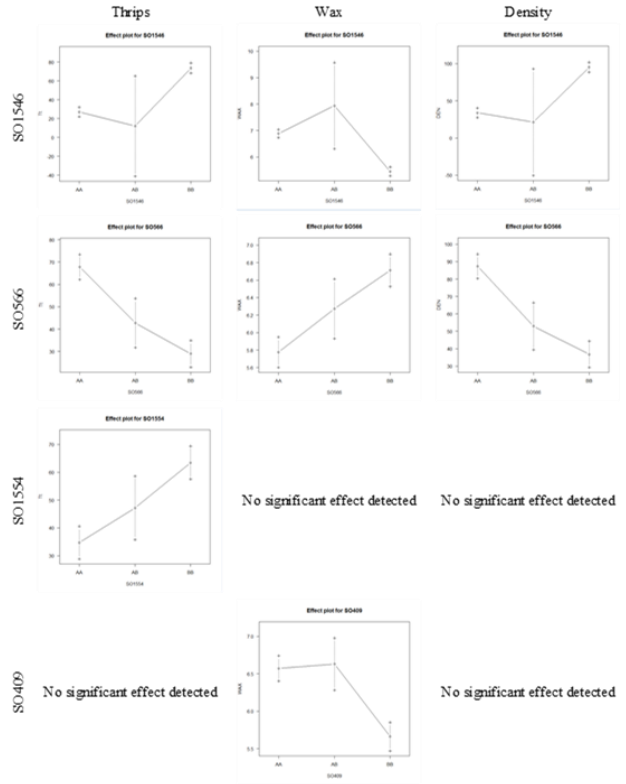

Fig. 5: Effect of the closest markers to the identified QTLs for the thrips damage, wax amount on the leaves and cabbage head density in the mapping population

wax content than allele 'A' coming from the resistant parent (Fig. 5). The QTL mapping of 'density of heads' revealed five loci reaching the threshold of 4.04 LOD score, on Chr2 with 13.38 LOD at 112.5 cM, on Chr5 with 13 LOD at 83.5 cM, on Chr6 with 13.17 LOD at 29.5 cM, on Chr7 with 10.13 at 7.3 cM and on Chr8 with 4.59 LOD at 31 cM positions (Fig. 4). ANOVA tests proved the significant effect on density in case of three from these candidates and refine QTL function helped to define the positions of the QTL with higher probability. Positions and closest identified markers to the QTLs are 131.5 with SO1546 marker on Chr2 (Denq1), 28.5 and SO566 on Chr7 (Denq2) and 26.1 and SO1618 marker on Chr8 (Denq3), explaining 30.91, 23.42 and 6.74% of the phenotypic variance, respectively. Including the interaction between the two QTL's on Chr2 and Chr7 with an explained phenotypic variance of 7.65%, 'density of the head 'can be determined in 68.72% by the two major and one minor QTL's in the current population (Table 1). Similarly, to thrips damage and wax amount analysis, SO1546 was also identified as the closest marker to the QTL on Chr2 effecting head density, providing little more dense head structure by allele 'B' deriving from the susceptible line, compared to allele 'A'. Effect of alleles of SO1618, the closest marker to the QTL on Chr8 shows the same direction. It is in contrast with the effect of the marker SO566 mapped to QTL on Chr7, where presence of allele 'A' contributed by resistant parent shows correlation with higher density values (Fig. 5). Effect of the allelic interaction between SO1546 and SO566 markers on density is visualized in Fig. 6.

The order of the markers used and positions of the QTLs with confidence intervals identified on Chr2, Chr3, Chr7 and Chr8 linkage groups of white cabbage, in association with phenotypic appearance of thrips damage, waxiness of the leaves and cabbage head density are represented in Fig. 7.

Discussion

In the current study, RILs of the mapping population developed for mapping thrips resistance in white cabbage showed high phenotypic variance regarding thrips damage and allowed useful data for successful identification of chromosome regions with significant effect on resistance and related plant traits. The chromosome regions identified with higher effect for thrips resistance on Chr2 has been mapped also as major QTL concerning wax and density traits. The second major QTL of thrips resistance located on Chr7 was also detected with significant major and minor effects regarding density and wax phenotypes. Position of the third, minor thrips resistance QTL on Chr8 is positioned very close to a minor QTL of density (Fig. 7).

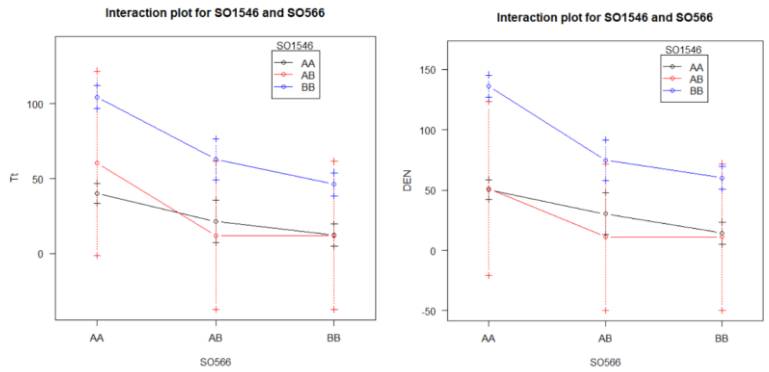

Fig. 6: Interaction between the different alleles of SO1546 and SO566 markers in correlation with thrips damage (Tt) and head density (DEN) in the mapping population

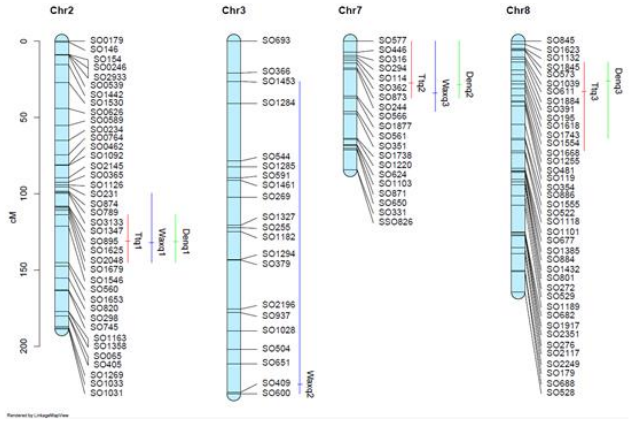

Fig. 7: Identified QTLs and confidence intervals associated with thrips damage (Ttq1, Ttq2 and Ttq3), waxiness of the leaves (Waxq1, Waxq2 and Waxq3) and density of the head (Denq1, Denq2 and Denq3) in white cabbage mapping population

Analyzing effect of the markers mapped closest to the target sequences concerning the single characteristics (Fig. 5) demonstrates that alleles of markers on Chr2 and Chr8 (SO1546 and SO1554) derived from the resistant parental line causes less thrips damage according to the expectations. Surprisingly, in case of the QTL located on Chr7 (SO566), the allele originating from the sensitive parental line provides a higher resistance level. In breeding practice, the ideal allele combination can be easily followed and built in the elite lines by diagnostic marker development and application in backcrossing, giving possibility to reach even higher resistance levels compared to the resistant parent used to develop the mapping population. Regarding the effect of the markers SO1546 and SO0566 on all mapped plant characteristics in current population, the level of thrips damage shows a negative correlation with wax and positive correlation with density, meaning RILs with higher thrips resistance level have more waxiness on the leaves and a less compact, more loose head structure. Interaction between these two markers was identified in mapping results of thrips resistance and head density (Fig. 6). Beyond the additive effect of the individual QTLs, interaction in presence of the appropriate allele combinations results in an additional 6.12 and 7.56% increase in thrips resistance level and head density values. Correlations demonstrated in this study are in line with the former published results and our observations, that antixenosis caused by higher wax layer of the leaves can cause decreased amount of immigrating adult thrips and decreased thrips damage, and the loose internal head structure can inhibit feeding and reproduction of thrips due to the remaining water between the leaf layers. Density, firmness, or compactness of the white cabbage head had been investigated in a few experiments, but it is mainly used to describe earliness of the variety or the plant's development stage (Trdan *et al*. 2004; Voorrips and Steenhuis 2010). Opposite effect of the common QTLs' allele variants on thrips damage rate compared to wax obviously reflects the negative correlation identified between the two traits (Voorrips and Steenhuis 2010). In one of the very few available publications reporting mapping results of white cabbage agronomic traits, one robust QTL from 7 QTLs identified over the genome was located on Chr2 and a greater amount of wax powder had been found in strong correlation with darker leaf color (Huanghao *et al*. 2016). High amount of wax can also affect and hamper movement of such small insects as thrips on the surface of the leaves, but antixenosis unambiguously plays an important role in thrips resistance of white cabbage. Additionally, not only the color and light reflectance of the leaves but also compounds of the wax layer can influence behavior and host plant selection of pests (Eigenbrode and Espelie 1995; Fail *et al.* 2008). Three compounds of epicuticular wax: triterpenoids of α-amyrin, β-amyrin, and lupeol had already proved to be correlated with thrips damage in white cabbage (Trdan *et al*. 2008a). Our results representing polygenic inheritance of all the three mapped traits with the prior published results also reflect that researchers must take the complexity of plant-based pest resistances in account together with numerous influential plant morphological characteristics.

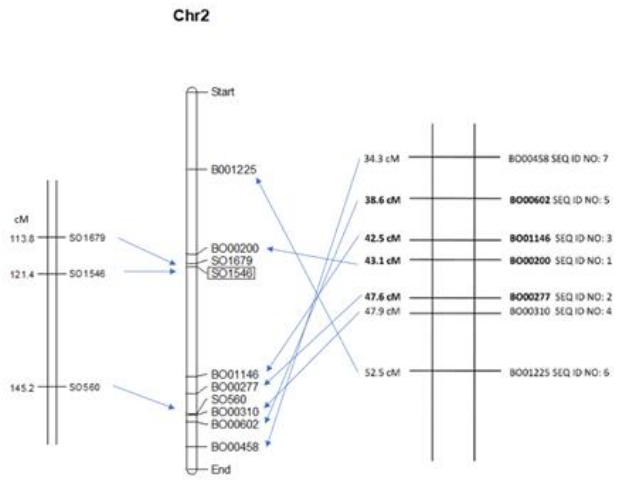

Fig. 8: Physical map representing the positions (bp) and order of markers previously identified and associated with thrips resistance by Löptien (2013) and markers identified in current study on Chr2 of TO1000 reference *B. oleracea* genome (Parkin *et al*. 2014), in comparison with the genetic linkage map of the closest and flanking markers of Ttq1 QTL and the published schematic overview of a part of the chromosome (genetic distance in cM) indicating markers linked to the genetic determinant conferring thrips resistance and flanking markers (Löptien 2013)

Studies constructing high resolution genetic maps are curiously useful as a basis of QTL analysis and MAS. The TO1000 reference genome representing 75% of the predicted *B. oleracea* genome published by Parkin *et al.* (2014) provides an excellent tool for further evolutional studies and for genetic improvement of the cultivated crops of the species (Parkin *et al*. 2014). The TO1000 reference map was used to identify the positions and construct a physical map of Chr2 representing SNP markers closely linked to the thrips resistance QTL and the markers of the confidence interval introduced based on our study, together with the six markers published by Löptien, including the closest linked, BO01146 and BO0200 markers (Fig. 8). Based on this comparison we can state that the SNP marker (SO1546) found is closely related to the region previously described. In comparison of the physical map and the genetic linkage map published in the 'Thrips resistant cabbage' patent application (Löptien 2013) there appear to be relatively large distances (4.5 cM) between the two closest markers on the genetic linkage map, additionally in reality the distance can reveal an even wider chromosome region due to the order of the six markers shows many deviations with the TO1000 physical map positions. The differences between the findings and conclusions of these studies also highlights the importance of screening a high number of entries in mapping programs and marker validation in different and wide genetic background for molecular mapping and marker development of complex plant traits.

To date, the only study aimed to discover genetic background of thrips resistance in white cabbage identified a QTL on Chr2 and described the thrips resistance in the study population as monogenic additive (Löptien 2013). Presence of a QTL in association with thrips resistance on Chr2 is also identified in this study but is only responsible for 28,04% of the phenotypic variance. The further two QTLs identified on Chr7 and Chr8 shows that thrips resistance is most likely under polygenic inheritance and raises the possibility that in different mapping populations even more QTLs could be identified.

Conclusion

Current experiment and analysis proved the former published presence of a region on white cabbage Chr2 in significant association with resistance against onion thrips. However, the monogenic dominant characteristic of this resistance was not confirmed in this mapping population due to other two significant QTLs had been detected on Chr7 and Chr8, together explaining almost the same effect size than the QTL on Chr2. Chromosome regions associated with two morphological traits, waxiness of the leaves and density of the head were also identified in strong correlation with thrips resistance and more QTLs of these plant characteristics were mapped in the same or close positions to thrips resistance related regions. The three thrips resistance associated QTLs mapped in our study can be the base for diagnostic molecular marker development with fine mapping. Collective effect of the three QTLs responsible more than 60% variation in phenotype is very promising result in a multigenic inherited plant trait. Even using molecular markers only for following the two major QTLs means that already 55% variation can be screened. After validation in more populations with different genetic background these molecular markers will provide an effective tool for plant breeders to accelerate breeding of new thrips resistant varieties.

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Author Contributions

The authors ZSG, WHB and SP contributed to design the mapping population. FR and ZSG executed the phenotyping process, WHB contributed to the genotyping work. WHB, ZSG and FR worked on QTL analysis and writing the article.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Ethics Approval

Not applicable.

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