Characterization of *Brassica rapa* turnip formation in multiple segregating populations

Peter Vos
830317-908-030
Laboratory of Plant Breeding, Wageningen University
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Supervisors: Ningwen Zhang
Guusje Bonnema

Abstract

In this study eleven F_2 populations were evaluated for turnip formation (TuF) and flowering time (FT). The goal was to select populations which form turnips with a wide range in size for studying the genetic basis of turnip formation. Besides the turnip formation FT is important because in previous studies FT and turnip formation are negatively correlated and QTLs for both traits are mapped in the same genomic region. The major finding on the FT-TuF correlation topic was that the correlation was present when the parents differ greatly in flowering time, on the other hand the FT-TuF correlation was absent when the parents did not differ in flowering time. In the evaluated populations a distinction can be made between the origin of the turnips in the populations. Populations with turnips from the Asian and European centers of variation were represented in this study. The phylogenetic relationship between different turnips suggests that different genes could underlie turnip formation in both centers of variation. This hypothesis is confirmed; in a population between turnips from both centers of variation other genomic regions correlate with turnip size than regions in populations with only the Asian turnips. This indicates that different genes underlie the same trait in different centers of variation. However this is only one population and therefore the conclusion is still fragile. Therefore new crosses have to be made between European turnips with RC-144 and PC-105 for comparison with the best populations in this study (VT-115 x RC-144 and VT-115 x PC-105).

Keywords: *Brassica rapa*, Turnip formation, Flowering time, Segregating populations,

Abbreviations: TuF = Turnip formation, FT = Flowering Time, VT = Vegetable Turnip, RC = rapid cycling, CC = Chinese cabbage, PC = Pakchoi, WO = Winter Oil, OR = Oil seed Rape, YS =Yellow Sarson

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1. Introduction

1.1. Botanical description

Brassica rapa is a species in the angiosperm family Brassicaceae. The Brassicaceae family consists of 338 genera and approximately 3710 species (Beilstein et al. 2008) of which several are agronomically important. In the *Brassica* genus six species are agronomically important. Three of them are diploid: *Brassica rapa (*AA genome, 2n=20), containing three main morphotypes (turnip types, leafy types and oil types); *Brassica nigra* (BB genome 2n=16) (black mustard) and *Brassica oleracea* (CC genome 2n=18) including cabbages, broccoli, cauliflower and kohlrabi (above ground turnip). Three of those six are amphidiploids: *Brassica juncea* (AABB genome, 2n=36) (Indian or brown mustard and several leafy types); *Brassica napus* (AACC genome, 2n=38) including oilseed rape and the turnip forming Swede, and *Brassica carinata* (BBCC genome, 2n=34) (Ethiopian mustard). The relationships between these B*rassica* species and their genomes are summarized in the triangle of U (U 1935) shown in figure 1.

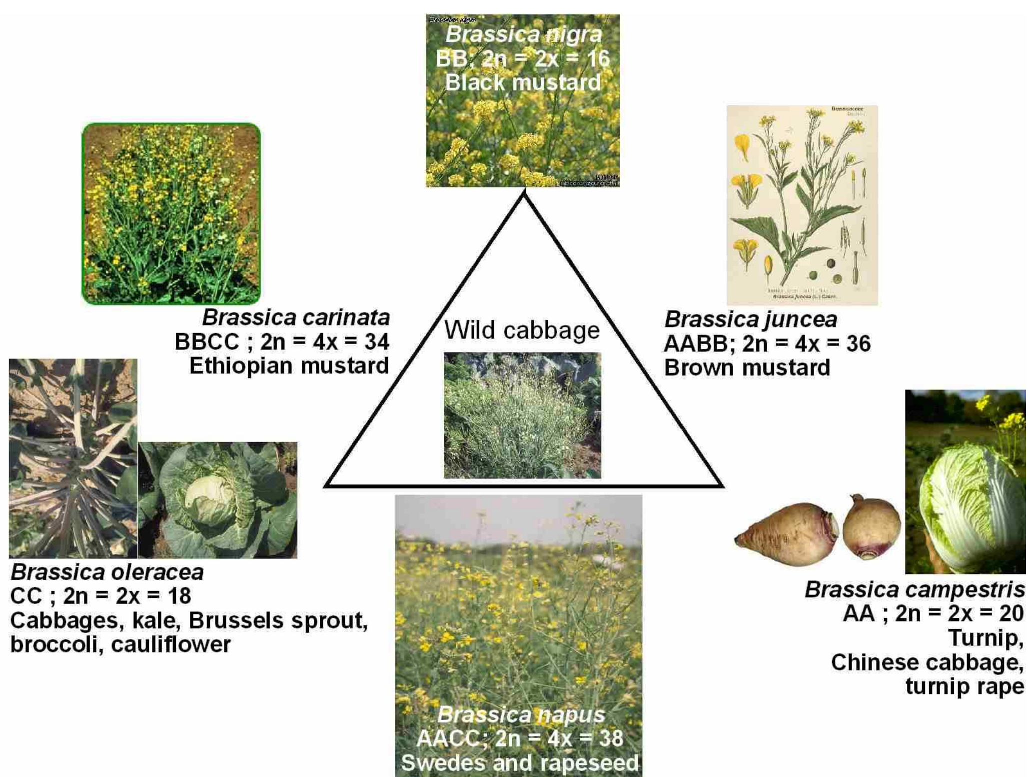

Figure 1: Triangle of U, a schematic overview of the relation between six different Brassica species.

Within those six species *Brassica rapa* is the species that was domesticated first. It has been cultivated for many years from central Asia to Europe and northern America (Gomez Campo

1999). The crop is cultivated for the oil content (Yellow Sarson) and for vegetable purposes (Chinese cabbage, pakchoi, wutacai, turnip and broccoletto).

1.2. Genetic research in Brassica rapa

Since all in the previous section mentioned *B. rapa* morphotypes (oil, leafy and turnip) can be crossed with each other, good genetic analysis of many morphological traits is feasible. Another advantage is genetic studies in *Brassica rapa* is the close relationship to the model plant *Arabidopsis thaliana*. The divergence between the *Brassica* genus and *A. thaliana* occurred around 20 million years ago (Koch et al. 2001). Due to the close relationship between the species sequence information of *A. thaliana* can be used in the *Brassica* genus and also the synteny between the genomes can be very useful. For example the study of (Parkin et al. 2005) in *Brassica napus* showed that 21 conserved genomic units were identified within the *Arabidopsis* genome, which can be duplicated and rearranged to generate the present-day *B. napus* genome. Also colinearity within different *Brassica* species has been demonstrated by Bohuon et al. (1996) showing that the linkage group structure between the C genomes of *B. napus* and *B. oleracea* is conserved. In the study of Schranz et al. (2006) these duplications and rearrangement are also shown in *Brassica rapa.* The observed segmentation in the Brassica genomes suggest that the diploid Brassica genomes (AA and CC) of *B. napus* (AACC) evolved from a hexaploid ancestor (Osborn et al. 1997; Parkin et al. 2005; Parkin et al. 2002).

1.3. Phylogenetics

During the long domestication, breeding and selection at different centers of variation a large range of phenotypic variation within *B. rapa* has been established resulting in three main morphotypes (oil, leafy and turnip types). The most likely explanation of the wide variation within the *B. rapa* species is that these morphotypes arose independently at different

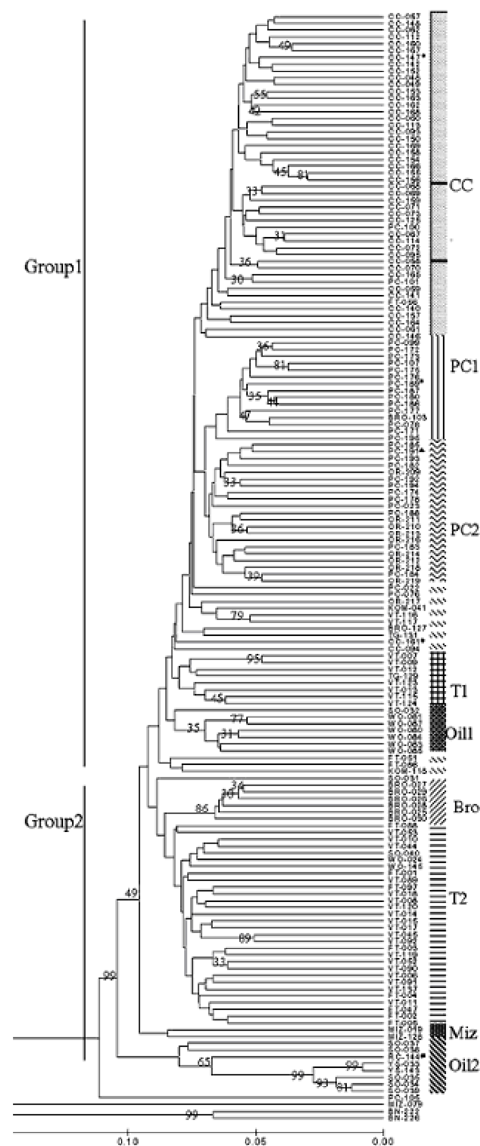

Figure 2: Phylogenetic relationship between many *Brassica rapa* accessions. Group 1 (Asia) and 2 (Europe) represent the two centers of variation. (Zhao et al. 2005)

places from wild *B. rapa* species. Only a few studies have been published about this (He et al. 2003; Osborn et al. 1997; Zhao et al. 2005). Osborn et al. (1997) and He et al. (2003) only used a few accessions and a few RFLP and RAPD markers, however the results of these studies all agree on the same point: two centers of variation. Whether these centers of variation are actually centers of origin or that there is one center of origin from where *B. rapa* dispersed into two centers of variation is not clear yet, therefore the term center of variation instead of center of origin is used.

Europe was proposed as one center of variation for oil and turnip types, East Asia was proposed as the other center of variation where Indian oil types, Chinese leafy types and Chinese turnips types originated. The genetic variation within and between the clusters also suggest that the different morphotypes are caused by a few genes. For example turnips from Asia are more related to other morphotypes from Asia than from turnips from the European center of variation.

Zhao et al. (2005) suggested also these two main groups based on AFLP fingerprints, using the Unweighted Pair Group Method with Arithmic averages (UPGMA), however they added a small group of spring oils originated in the Indian subcontinent (Bangladesh).

In the study of Zhao et al. (2007) a different method was used to look at different accessions. The model-based approach of STRUCTURE was used and four (S1, S2, S3 and S4) instead of three subpopulation are suggested. S1 and S4 mainly originate in oriental areas and together correspond to the Asian group suggested in the previous study, most European accessions were grouped in S2 and the oil types of the Indian subcontinent were grouped in the S3. Both studies show that turnip forming accessions were grouped in two subgroups; turnips are present in both the European subgroup and the Asian subgroup. So turnip formation likely evolved independently in both centers of variation.

1.4. Genetic markers

In the studies of Zhao et al. (2005, 2007) several marker types were used, mainly the multiplex (several markers with one primer pair) marker type AFLP (amplified fragment length polymorphism) was used. Besides AFLP the simplex (one marker with one primer pair) markers type SSR (simple sequence repeats) was used. SSRs are short, tandemly repeated nucleotide motifs (1-6 bp) and abundant in most eukaryotic species. A good property of SSR markers is that they are highly polymorphic, largely due to variation in the number of repeats. SSR markers are analyzed by a PCR based method, it has a co-dominant inheritance and therefore it is a very useful marker technique. Another advantage of SSRs is that they are relatively conserved among genomes and therefore can be used to integrate different maps, which cannot be done with AFLP markers. Many SSR markers have been developed for *B. rapa* (Ling et al. 2007; Suwabe et al. 2002) to construct genetic linkage maps (Choi et al. 2007) and for comparison

between different *Brassica* crop species (Lowe et al. 2004; Suwabe et al. 2004; Suwabe et al. 2008).

1.5. Maps and Populations

Several maps were generated from different populations at Wageningen University. Maps which are used in the Brassica research at the WUR are maps of DH-30 (VT-115 x YS-143), DH-38 (PC-175 x YS-143) and DH-68 (YS-143 x PC-175). These maps were mainly constructed with SSR and AFLP markers and mainly used for analysis of several traits as different morphological traits (Lou et al 2007), glucosinolate content (Lou et al. 2008) and phytate and phosphate content (Zhao et al. 2008) The most interesting of these maps is the map of the DH-30 population, because in the study on that population also the turnip trait was analyzed (Lou et al. 2007).

1.6. FT-TuF correlation

Lou et al. (2007) have mapped a major turnip QTL on the upper part of linkage group 2. This QTL explained up to 40% of turnip formation in a DH and BC1 of the early flowering Yellow Sarson (YS-143) and a vegetable turnip (VT-115). In the same populations flowering time (FT) and turnip formation (TuF) were negatively correlated. Both a FT-QTL and the TuF-QTL are colocalizing with the FLC2 locus on linkage group R02. In *Arabidopsis* FLC acts in a dosage-dependent manner to delay flowering time (Michaels and Amasino 1999; Sheldon et al. 1999). *B. rapa* contains three copies of the genomic region on the top of *A. thaliana* chromosome 5, where the FLC locus is located. The top of *A. thaliana* chromosome 5 is in synteny with top of linkage group R02, linkage group R03 and the bottom of linkage group R10 (Schranz et al. 2006). Since TuF and FT are negatively correlated and the QTLs colocalize it cannot be determined yet whether the QTLs are caused by the same gene, closely linked genes or whether FT is epistatic over TuF.

Another F_2 map was generated in the study of Lu et al. (2008) of the cross AJH97-2 x QSH97-24. They mainly used AFLP and RAPD (Random Amplification of Polymorphic DNA) markers in order to map QTLs for the turnip trait. In total 18 QTLs of which two major QTLs were mapped. Although the results of this study were very promising, the results cannot be used in other studies because no anchored SSR markers were used to assign chromosome numbers to linkage groups.

1.7. Scope of the thesis

The main goal of this thesis is to select good segregating populations for turnip size. Criteria for a good population are turnip size (the larger the better), the range of the size of the turnips in de F_2 should be wide. Also FT and the relation between FT and TuF are important for selecting good population.

For selecting suitable population also two research questions are important.

1. Are FT and TuF always correlated in different populations?
2. Do the same genes control turnip formation in turnips from the two centers of variation?

For the first research question early and late flowering types are crossed with a turnip type which always is late flowering. Late flowering types are used because in these crosses epistatic interactions may not mask turnip formation. On the other hand a population with the very early flowering RC-144 is used. With the results of those crosses the first research question could be answered.

For the second research question turnips from different centers of variation are used. Different morphotypes are crossed with both Asian and European turnips. Also a population from a cross between two turnips, one from both centers of variation, could be interesting.

During this thesis the phenotypes of all populations were monitored. Besides that a start will be made with a marker analysis on the interesting populations.

Since it is not feasible to generate a complete map within the scope of this thesis, there will be focus on 3 regions; the upper part of linkage group R02, the lower part of linkage group R03 and the lower part of linkage group R10. All regions have colinearity with the upper part of *A. thaliana* chromosome 5 where the FLC locus is located. The regions around the FLC loci are interesting because in previous studies flowering time and turnip formation QTLs were mapped near FLC.

2. Material & Methods

2.1. Plant Material

For this experiment eleven F_2 populations of crosses with one turnip forming parent and one non-turnip forming parent were used. The crosses can be distinguished by the origin of the turnip forming parent. Crosses 118 (VT-44 x WO-81) & 119 (WO-81 x VT-44) were with a turnip forming parent from the European centre of variation. Cross 49 (VT-52 X VT-12) was with two turnip forming parents, one from both centers of variation, Europe and Asia. Eight crosses (27 (VT-115 x OR-219), 29 (VT-115 x PC105), 30 (VT-115 x YS-143), 32 (VT-115 x CC-146), 34 (PC-175 x VT-115), 36 (VT-115 x RC-144), 45 (CC-74 x VT-9), and 48 (CC-168 x VT-9) were with the vegetable turnip (VT) from the Asian centre of variation. For an overview see table 1.
Another way of distinguishing the population is by taking the flowering time of the parents in account. The turnips are all late flowering and all other types differ in flowering time. RC-144 is for example a very early flowering type and the PC-105 is a late flowering type. In figure 3 the phylogenetic relationship between the different parental lines used in this thesis is shown. This figure is extracted from the phylogenetic tree on page 5 (Zhao et al. 2005)

Table 1 Populations used in this thesis. Origin of parents and # plants used per population are also shown.

Cross	Origin ♀	Mother ♀		Father ♂	Origin ♂	# Plants
27	Japan	VT-115	x	OR-219	China	52
29	Japan	VT-115	x	PC-105	China	52
30	Japan	VT-115	x	YS-143	USA	52
32	Japan	VT-115	x	CC-146	China	52
34	China	PC-175	x	VT-115	Japan	52
36	Japan	VT-115	x	RC-144	USA	52
45	China	CC-74	x	VT-9	Japan	52
48	China	CC-168	x	VT-9	Japan	52
49	NL	VT-52	x	VT-12	Japan	104
118	Russia	VT-44	x	WO-81	Pakistan	80
119	Pakistan	WO-81	x	VT-44	Russia	80

Fifty-two plants per cross were evaluated during the experiment, with the exception of populations 118 (VT-44 x WO-81) & 119 (WO-81 x VT44) (table 1) with 80 plants. These reciprocal crosses were derived from the same parental plants, and therefore a combined population of 160 plants could possibly be used later to perform a QTL analysis. Also from cross 49 (VT-52 x VT-12) more plants (104) were evaluated, this is because in this population it is expected that 52 plants is not enough to visualize segregation of the turnip trait. For example if

the TuF QTLs of the two centers of variation are different QTLs, theoretically only $^{1}/_{16}$ of the F_2 will have no TuF QTL and no turnip phenotype. And this is only accounting for the simplest, one major gene inheritance. It is even more likely that more genes underlie the turnip trait, so an even larger population is needed to have a good segregation of the turnip trait in this cross. Therefore a population 104 of plants is used.

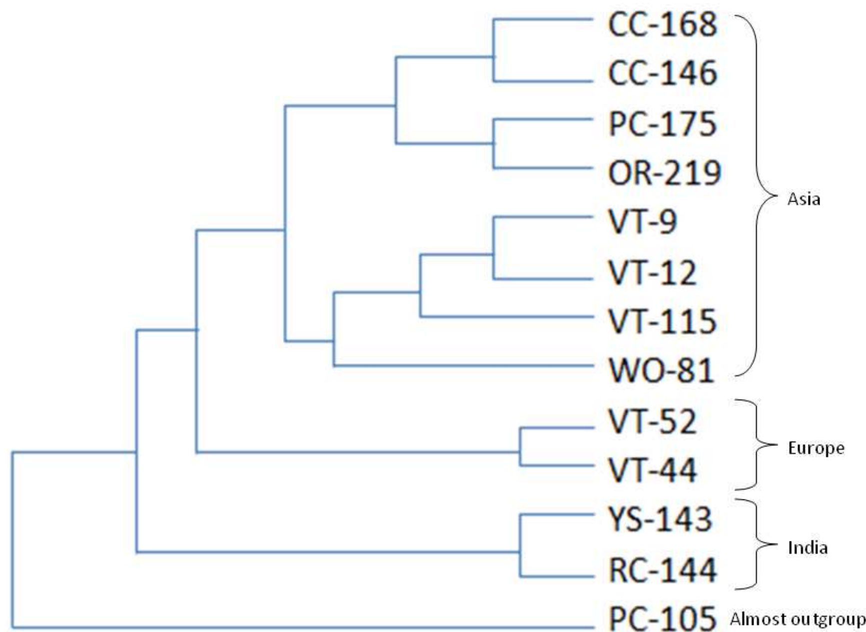

Figure 3: Schematic overview of the phylogenetic relationship within accessions used in this thesis. Extracted from Zhao et al. (2005). Also the origin of the accessions is given. CC-74 is missing because no phylogenetic information available.

2.2. Experimental details

2.2.1. Germination

The seeds were sown in petridishes on four layers of wet filter paper. Approximately 20-25 seeds per petridish were sown. The germination was induced at a climate chamber at temperature of 25 degrees (in the dark). After three days the seedlings were transferred to the lab where the seedlings could develop green tissue. After $4^{1}/_{2}$ day the seedlings were big enough for the transfer to the greenhouse into small jiffy pots. After three weeks the seedling were transferred to bigger pots of 17 cm diameter.

2.2.2. Block design

A randomized block design was used. In total two blocks of 368 (340 F_2 & 28 Parental) plants each were planted. Every block contains 26, 52 or 40 plants per cross (depending on the cross) and two of every parent.

Table 2: A scheme for the used randomized block design. All populations were splited into two parts and allocated on separate tables (blocks) in the greenhouse.

Block 1

118	VT-44	x	WO-81
36	VT-115	x	RC-144
45	CC-74	x	VT-9
32	VT-115	x	CC-146
29	VT-115	x	PC-105
34	PC-175	x	VT-115
27	VT-115	x	OR-219
48	CC-168	x	VT-9
49	VT-52	x	VT-12
30	VT-115	x	YS-143
119	WO-81	x	VT-44

Block 2

49	VT-52	x	VT-12
118	WO-81	x	VT-44
30	VT-115	x	YS-143
34	PC-175	x	VT-115
27	VT-115	x	OR-219
48	CC-168	x	VT-9
29	VT-115	x	PC-105
119	VT-44	x	WO-81
45	CC-74	x	VT-9
32	VT-115	x	CC-146
36	VT-115	x	RC-144

2.3. DNA Isolation

For the DNA isolation the RETCH 1.3 protocol was used (Gert van Arkel & Maarten Nijenhuis) (Appendix 5). Of the DNA stock, 2 working solutions were made, both with a concentration of 50 ng μl^{-1}, one for the marker selection phase and one for the population screening phase. For the marker selection phase the stock has a lay-out as shown in table 3, which has to be read horizontally. With a lay-out like this it was easy to load the DNA samples on the PCR plates and in this way 12 markers could be possibly tested for polymorphisms per PCR plate. Unfortunately there was only time for 8 markers per population.

Table 3 Lay-Out DNA stock for marker selection phase. P1 & P2 are pooled parental lines and F2 = randomly picked F2 plant.

P1	F2	F2	F2
F2	F2	**P2**	**P1**
F2	F2	F2	F2
F2	**P2**	**P1**	F2
F2	F2	F2	F2
P2	**P1**	F2	F2
F2	F2	F2	**P2**

The characters in bold (P1 & P2) are parental lines. In this case it is a pooled sample of an equal amount of DNA of all four parents of the same accession in the greenhouse. The concentration was the same as the concentration of the F_2. This is done because of the heterogeneity and heterozygosity of the parental lines and all alleles present

in one parental line can be shown in one lane on the Li-Cor.

2.4. Molecular marker analysis

After DNA isolation a selections of 46 SSR possible markers (appendix 1) (with known map positions in DH-38, DH-68 and some in DH-30) were tested for polymorphisms between the parental lines.

First the markers on linkage group R02 and R10 were evaluated (group A, appendix 1), because they were most likely to be interesting. After testing those markers, the markers which are mapped on other linkage groups in DH-30, DH-38 and DH-68 are tested (group B). Third, the markers located on chromosome 2 and 10 where one primer pair amplifies multiple loci were evaluated, because these could be more difficult to score.

Since most of the parental lines in *B. rapa* are self-incompatible it could happen that not all plants within an accession are homozygous and also that some plants slightly differ from others. This so called heterogeneity of the parental lines caused some problems because the genotype of the parents of which the cross was made is not available anymore.

This means that a marker tested as polymorphic on the parental lines but wasn't polymorphic in the F_2 progeny and vice versa. Therefore the selected markers had to be validated first on five F_2 plant together with a pooled parental sample (phase two marker selection) before all F_2 plants were tested. Markers were only used when polymorphisms were present in the tested samples. For each population 16 markers of the 46 were selected for the 2nd phase of the markers selection. If polymorphisms were present the whole population was genotyped in the population screening phase.

2.4.1. PCR program for SSR markers

PCR reactions for the Li-Cor were performed in 96-well plates in a volume of 10µl. The PCR mixture consisted of 0.1 µl SuperTaq Polymerase (5U µl^{-1}), 1 µl 10x SuperTaq buffer, 0.4 µl dNTPs, 1.0 µl of both forward (labeled, 1 µM) and reverse (unlabeled, 5 µM) primers, 5,5 µl MilliQ and 1.0 µl DNA stock (50 ng µl^{-1}) The PCR was performed in a GeneAmp PCR system 9700 (Applied Bio-System) with the following program, first a denaturation for 2 minutes at 94 °C, then a touchdown of 10 cycles with denaturation at 94°C for 1 minute, annealing at 65°C for 1 minute and elongation at 72°C for 1,5 minute, with a 1 °C decrease in annealing temperature every cycle, then 30 cycles with denaturation at 94°C , annealing at 55°C and elongation at 72°C, 1 minute for each step, then the final step was the elongation at 72°C for 5 more minutes before cooling down to 10°C. The touchdown PCR program was used because it stimulates amplification of the correct product over any non-specific product.

After the PCR, the reaction products were mixed with an equal volume of formamide-loading buffer (98% formamide, 10 mM EDTA pH 8.0 and 0.1% bromo phenol blue) and denaturated at 94 °C. After denaturation the samples were cooled and 0.5 µl were loaded on a 5.5%

polyacrylamide gel and analyzed with Global Edition IR^2 DNA analyzer (Li-Cor Biosciences, Lincoln, NE).

2.4.2. Lightscanner

After testing markers on the Li-Cor some markers were used to compare the data with the lightscanner (HR I 96 Idaho technology inc.) data. The lightscanner measures melting curves of DNA fragments. The main advantage of the lightscanner is that it is a much faster procedure than genotyping with the Li-Cor method. A PCR for the light scanner only takes 1.5 hour and measuring and analyzing also only a half hour. Compared to the Li-Cor procedure (PCR = 3 hours and Li-Cor genotyping = 3 hours) is much faster.

PCR reactions for the Light scanner were performed in 96-well plates in a volume of 10µl. The PCR mixture consisted of 0.1 µl Phire^{TM} Hot start DNA polymerase, 2 µl 5x Phire^{TM} reaction buffer buffer, 0.4 µl dNTPs, 0.025 µl of both forward and reverse primers from the stock, 1µl LCGreen. 5,45 µl MilliQ and 1.0 µl DNA stock (12.5 ng µl^{-1}) The PCR program for the light scanner was as follows: 30s at 98 °C, then 40 cycles of 5s at 98°C, 5s at 60°C, 20s at 72°C and a final elongation step of 1 minute at 72°C.

2.5. Phenotypic trait scoring

During the growth of the plants in the greenhouse, the following traits were measured:

- Turnip width (mm) in several growing stages. With a caliper Turnip width was measured four times with two week intervals, starting at 50 days after sowing.
- Turnip weight (g), when a whole population had flowered the turnip was harvested, and the swollen part was weighted.
- Turnip color (7 classes), ordinal, 1 = white, 2 = green/white, 5 = light purple/pink, 6 = purple, 7 = dark purple, 8 = red, 9 = dark red. Parental lines were Dark red (VT-9), Dark red (VT-12), White (VT-52, VT-115), Yellowish (VT-44)

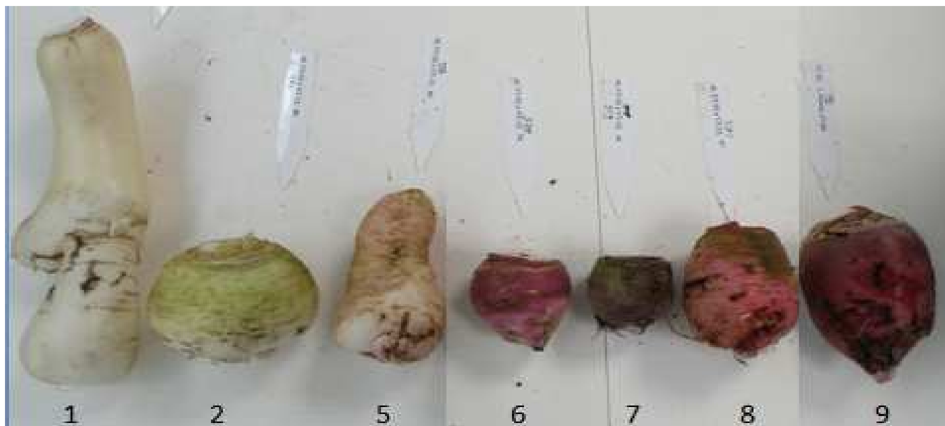

Figure 4: Different classes in turnip color

- Turnip shape (1 = round, 2 = straight, 4 = flat, 5 = oval, 6 = long). As shown in figure 4. Class 3 is missing because it was fused with another class after scoring.

Figure 5: Different classes in turnip shape

- Swelling onset date. (#days after sowing when the width of the turnip exceeds approximately 5 mm)
- Flowering Time (FT). (#days after sowing of appearance of the first flower)
- Area Under Turnip Growth Curve (AUTGC) is the area calculated under the curve of the four TuWidth data points.

2.6. Statistical analysis

For the statistical analysis the SPSS 17.0 software package is used.
The correlation between traits analysis was done with the bivariate correlation option.
To check the skewness of the inheritance of the markers a χ^2 goodness-of-fit was done. All markers with a segregation as expected (13(a):26(h):13(b) were analyzed with a one-way ANOVA (ANalysis Of VAriance) to check the phenotypic differences for the different genotypes (a, b, h) per marker and if these groups differ significantly.
In population where a few outliers in turnip weight were present the non-parametric test of Kruskal-Wallis was done to analyze the differences in phenotype among the different genotypes.

3. Results

3.1. Phenotypic data

During the growth of the eleven populations several measurement were done regarding turnip size. Because the calculated AUTGC and the first three data points of TuWidth showed a very high positive correlation with TuWidth at 90 days and TuWeight these data are not shown in table 4. These data have no added value compared to the TuWidth at 90 days and TuWeight and are therefore also not used in the further analysis. So when referred to TuWidth that is the TuWidth measured at 90 days after sowing. Also the Turnip color and turnip shape are not included in the results and discussion of this thesis. The traits were scored in different classes. During scoring it was very difficult to put phenotypes in certain classes therefore the information obtained from those traits is not reliable en therefore not taken in account in this thesis.

In table 4 an overview is given of the important traits in this thesis. The average of TuDays, FT, TuWidth and TuWeight is given and also the range of the FT, TuWidth and TuWeigth. Both populations 29 (VT-115 x PC-105) and 36 (VT-115 x RC-144) were classified as interesting and therefore kept for F_3 seed production (pop 36) and DH production (pop 29) and therefore no TuWeigth data could be obtained for these populations.

Table 4: Overview of phenotypic data. Averages and ranges of important traits. [1]TuWidth measured after approximately 90 days.

			FT Range			TuWidth Range			TuWeight Range	
	TuDays	FT	Upper	Lower	TuWidth (mm)[1]	Lower	Upper	TuWeight (g)	Lower	Upper
pop 27 (VT-115 x OR-219)	44	80	57	102	22.5	10.6	39.5	16.4	4.0	44.2
pop 29 (VT-115 x PC-105)	38	111	93	130	59.7	7.2	106.5			
pop 30 (VT-115 x YS-143)	45	72	35	109	23.5	12.0	42.0	13.7	5.4	68.1
pop 32 (VT-115 x CC-146)	40	106	81	140	40.8	19.2	67.2	61.4	11.3	175.7
pop 34 (PC-175 x VT-115)	40	84	68	105	27.0	16.8	48.3	23.2	6.4	123.7
pop 36 (VT-115 x RC-144)	43	54	30	78	26.4	6.1	56.4			
pop 45 (CC-74 x VT-9)	42	113	78	140	25.9	15.7	37.6	17.8	6.7	41.2
pop 48 (CC-168 x VT-9)	42	105	84	126	31.3	14.9	66.6	40.2	6.1	218.0
pop 49 (VT-52 x VT-12)	38	113.5	94	136	53.8	21.5	101.8	131.2	15.6	474.5
pop 118 (VT-44 x WO-81)	44	83	37	113	22.5	2.9	45.2	18.5	8.0	65.6
pop 119 (WO-81 x VT-44)	47	87	46	113	21.7	10.2	36.1	19.2	4.4	61.2

As shown in table 4 the F_2 plants of population 29 (VT-115 x PC-105) have the largest average turnip width. Also the wide range of this population indicates a good segregation of the turnips trait. Also F_2 plants of populations 32, 48 and 49 from good turnips on average. In these populations the range is also wide and therefore these could be a good segregating population

for further analyzing the turnip trait. Another remarkable figure in table 4 is the average flowering time of population 36 (VT-115 x RC-144) it is the earliest flowering population. The short life cycle in this population makes it interesting for future research. Furthermore it forms on average remarkable turnips with a relative wide range, which makes it even more interesting.

In the distribution graphs of turnip width in figure 6 also the range in turnip size of different populations is shown. Here again the good population with large turnips and a wide range are given. In all three graphs the turnip width is approximately normally distributed, which indicates that multiple genes are involved in turnip width.

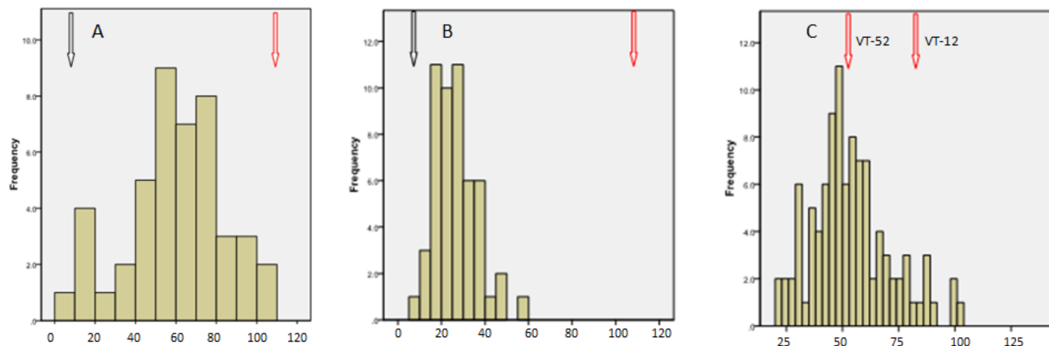

Figure 6 Distribution graphs of Turnip Width of population 29 (VT-115 x PC-105) (A), population 36 (VT-115 x RC_144) (B), population 49 (VT-52 x VT-12) (C). Black arrow indicates the non-turnip parent and the red arrow indicates VT-115 (A, B).

In figure 6B the distribution graph of population 36 (VT-115 x RC-144) is shown, here it is clear that the size of the turnip parent is much bigger than the average of the turnips in the F_2 population. In figure 6C it is shown that the turnips of the F_2 progeny of population 49 (VT-52 x VT-12) is both thinner and thicker than the parental lines; this is a clear example of transgressive segregation.

In figure 7 the distribution graphs of turnip weight of three populations are shown. In figures 7A

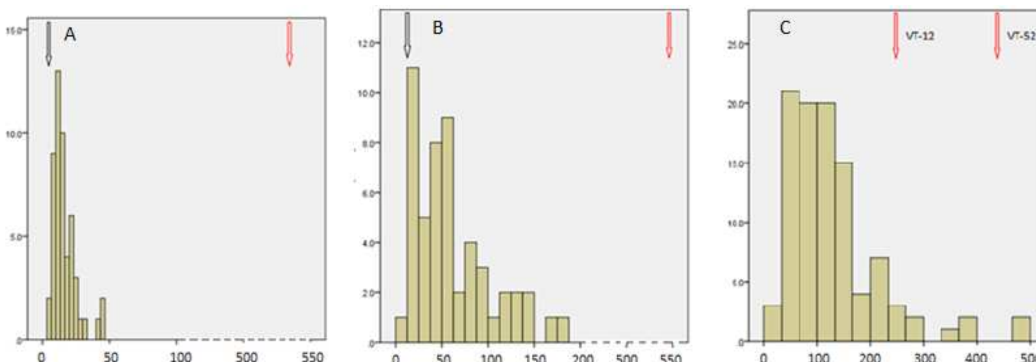

Figure 7 Distribution graphs of Turnip Weight of population 27 (A), population 32 (B), population 49 (C). Black arrow indicates the value of the non-turnip parent and the red arrow indicates the value of the turnip parent.

the distribution of population 27 (VT-115 x OR-219) is shown; it is clear that the average weight of the turnips in the F_2 is very low. In figure 7B the weight of the turnips are all lower than that of the turnip parent, but are bigger than in figure 9A.
In figure 7C the distribution of population 49 (VT-52 x VT12) is shown; a remarkable feature of this graph is that 90% of the population has a lower weight than turnips of both parents. A common remarkable thing of all distribution graphs of turnip weight have a preference for a fatter "tail" at the right side of the distribution where the graphs of turnip width seem to be more normally distributed. All distribution graphs of the rest of the population of FT, TuWidth and TuWeight are given in the appendix 2, 3 and 4 respectively.
In figure 8 the difference in size of turnips in different populations is shown. A turnip from population 29 (VT-115 x PC-105) (Figure 8A) is much bigger than the turnip from population 30 (VT-115 x YS-143) (Figure 8C). The turnip in figure 8C was not classified as real turnip in this thesis. Also shown is the intermediate size turnip of population 36 (VT-115 x RC-144) (figure 8B). In figure 9 the segregation in size, color and shape is shown. For example in figure 9B and 9C the large variation in color and shape in population 49 (VT-52 x VT-12) is visible. In figure 9B the parents are shown in the bottom left corner (VT-52) and bottom right corner (VT-12).

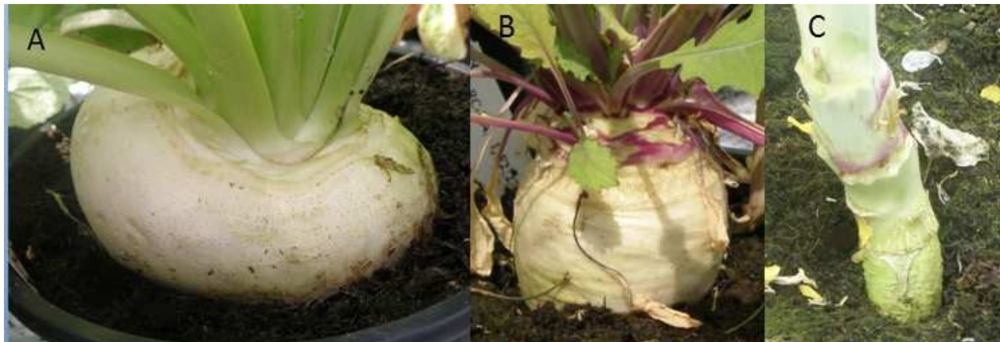

Figure 8 A: Turnip of population 29 (VT-115 x PC-105), B: Large turnip of population 36 (VT-115 x RC-144) C: Small "turnip" of population 30 (VT-115 x YS-143)

Figure 9 A: Segregation in size of Population 32 (VT-115 x CC-146), B: Segregation in size and color of population 49 (VT-52 x VT-12) C: Segregation in shape of population 49 (VT-52 x VT-12)

3.2. Lightscanner

A few markers were tested on the light scanner to compare the results with the Li-cor data. The markers 3, 95, e-72, 16, 94, e-17, e-15, e54 and e-78 were tested.

Of those markers only marker 3 could replicate the data obtained from the Li-Cor. All other marker didn't amplify well with the lightscanner PCR or the software program couldn't make a distinction between the three genotypes. Therefore the results of the lightscanner could not be directly used.

3.3. Correlations among traits

In previous studies Flowering Time (FT) and Turnip formation (TuF) were negatively correlated (Lou et al. 2007). In table 5 the correlations between FT and 3 different turnip traits are shown. TuWeight and TuWidth are different measurements for turnip formation and therefore have a high positive correlation (between 0.600 and 0.950, and all with a significance level below 0.000); therefore this correlation has no added value and is not shown in the table. In this table it is visible that in four populations the FT-TuWidth correlation and six populations the FT-TuWeigth correlation was present. Population 27 (VT-115 x OR-219) has a slight correlation but not significant. Only in population 32 (VT-115 x CC-146), 29 (VT-115 x PC-105) and 49 (VT-52 x VT-12) both the FT-TuWidth and FT-TuWeight correlation is absent.
In the column TuDays-TuWidth it is shown that in most populations a negative correlation between TuDays and TuWidth is present, which means that an early onset of turnip formation results in thicker turnips. In this column only one unexpected value is present and that is the value of population 30 (VT-115 x YS-143). This value is hard to explain.
When the data in table 5 are compared to previous studies where TuF and FT are negatively correlated it can be confusing. Most of the values of are positive numbers which indicate that the values measured are positively correlated. The actual correlation between these traits can still be described as negative as in a previous study (Lou et al. 2007). In 9 out of the 11 population the correlation between FT and TuWidth is positive. In other words, the later

Table 5 Correlations between flowering time (FT) and different turnip traits and TuDays-TuWidth correlation.

Population	Difference Parental FT	FT-TuWidth	FT-TuWeight	FT-TuDays	TuDays-TuWidth
pop 32 (VT-115 x CC-146)	2	-0.158	0.037	-0.068	-0.052
pop 29 (VT-115 x PC-105)	5	0.064	Nd	-0.101	-0.403[2]
pop 48 (CC-168 x VT-9)	26	0.246	0.369[2]	0.257	-0.647[2]
pop 27 (VT-115 x OR-219)	36	0.149	0.204	0.214	-0.328[1]
pop 45 (CC-74 x VT-9)	40	0.235	0.285[1]	-0.099	-0.499[2]
pop 49 (VT-52 x VT-12)	40	-0.09	0.166	0.147	-0.610[2]
pop 34 (PC-175 x VT-115)	46	0.409[2]	0.344[1]	0.044	-0.594[2]
pop 30 (VT-115 x YS-143)	48	0.267	0.619[2]	0.430[1]	0.355[2]
pop 118 (VT-44 x WO-81)	>45	0.389[2]	0.470[2]	0.016	-0.262
pop 119 (WO-81 x VT-44)	>45	0.657[2]	0.699[2]	0.008	-0.065
pop 36 (VT-115 x RC-144)	75	0.694[2]	Nd	0.290	-0.030

[1]Significance level of 0.05, [2] significance level of 0.001, nd = no data.

flowering plants in this F_2 population form on average bigger turnips than the early flowering plants. However this correlation is only significant 4 of the 11 population. Only populations where the difference in FT of the parents is larger than 45 days the FT-TuF correlation is present. In all populations where the difference in FT of the parents is smaller than 45 days the FT-TuF correlation is absent.

3.4. Marker Analysis

During the selection of suitable marker for the whole population screen, first 16 of the 44 possible markers were tested per population.

Table 6 Overview of markers analyzed in marker selection phase

Marker	Linkage group	pop 27 (VT-115 x OR-219)	pop 29 (VT-115 x PC-105)	pop 30 (VT-115 x YS-143)	pop 32 (VT-115 x CC-146)	pop 34 (PC-175 x VT-115)	pop 36 (VT-115 x RC-144)	pop 45 (CC-74 x VT-9)	pop 48 (CC-168 x VT-9)	pop 49 (VT-52 x VT-12)	pop 118 (VT-44 x WO-81)	pop 119 (WO-81 x VT-44)
3	3	P	-	P	P	P	P	P	N	P	P	P
4	4	-	P	-	-	-	P	-	P	P	-	-
5	5	N	P	-	-	-	P	N	P	N	P	P
7	7	-	N	N	-	N	-	N	N	N	N	N
16	9	P	P	-	P	P	P	P	-	-	P	P
21	3	P	P	P	P	-	P	P	P	P	N	N
23	5	P	P	-	-	-	-	-	N	N	N	N
27	3	P	P	P	-	-	-	P	P	-	P	P
40	2	-	N	-	P	-	-	-	-	N	N	N
73	6	P	P	P	P	P	P	P	P	P	-	-
85	3	P	P	P	P	-	-	P	-	P	P	P
94	2	-	N	N	P	-	P	P	P	P	N	N
95	10	-	-	P	N	N	P	P	P	P	P	P
176	6	-	-	P	P	P	P	-	-	P	-	-
272	10	-	N	-	-	-	-	-	N	-	-	-
362	10	-	N	-	-	-	-	P	P	-	-	-
363	2/10	P	P	P	-	P	P	P	-	-	P	P
e-13	8	-	-	N	-	-	-	-	N	-	N	N
e-15	9	N	-	P	-	-	P	-	-	P	-	-
e-17	2	-	P	P	-	-	N	-	N	-	P	P
e-50	3	-	-	-	-	-	N	-	-	-	-	-
e-54	3	N	-	P	N	N	P	-	N	N	P	P
e-71	?	-	-	-	-	-	P	-	-	-	-	-
e-72	5	P	-	P	P	P	P	P	P	P	-	-
e-78	4	P	N	P	P	N	P	P	P	P	P	P

Polymorphic markers are indicated with a "P". Non-polymorphic markers are indicated with a "N". Markers which were not tested are indicated with a "-".The "P" In green indicate the markers which were done on the whole population.

In table 6 the results of those analyses are shown. In the table is shown which markers are polymorphic (P) and which are not polymorphic (N) in each population. Polymorphic markers in green are the markers which are done on the whole populations.

In table 7 the results of the statistical analysis of the correlation between markers and traits is shown. All markers indicated in green in table 6 are also present in this table. All significant correlations are indicated in a blue cell with a "+"sign. All markers which didn't correlate with FT or TuF are indicated in a pink cell with an "–"sign. The upper markers in the table are the markers that are mapped in genomic regions where flowering time (FT) and turnip formation (TuF) QTLs were mapped.

Table 7. Correlations between markers and traits

		Trait	***FT***								***TuF***							
		Population	***Pop 27 (VT-115 x OR-219)***	***Pop 29 (VT-115 x PC-105)***	***Pop 30 (VT-115 x YS-143)***	***Pop 32 (VT-115 x CC-146)***	***Pop 36 (VT-115 x RC-144)***	***Pop 45 (CC-74 x VT-9)***	***Pop 48 (CC-168 x VT-9)***	***Pop 49 (VT-52 x VT12)***	***Pop 27 (VT-115 x OR-219)***	***Pop 29 (VT-115 x PC-105)***	***Pop 30 (VT-115 x YS-143)***	***Pop 32 (VT-115 x CC-146)***	***Pop 36 (VT-115 x RC-144)***	***Pop 45 (CC-74 x VT-9)***	***Pop 48 (CC-168 x VT-9)***	***Pop 49 (VT-52 x VT12)***
Marker Labcode	*Marker Name*																	
94	FLC2	R02				-	+		-					-	+		-	+
e-17	e-17	R02		-								-						
363	ENA13	R02/ R10	-	+	-		+	-			+	-	-		+	-		
95	FLC1	R10			-		+	+	-				-		-	-	-	
362	EJU6R10	R10							-								-	
3	BRMS-043	R03			-		-	-		+			-		-	-		+
21	BRMS-050	R03		-			-		-	+		-			-		-	+
27	BRMS-008	R03			-				-				-				-	
85	Ol11B05	R03	-	-							-	-						+
e-54	e-54	R03					-								-			
e-78	e-78	R04				-	-		-					-	-		-	+
4	Na10D09t	R04		-			+					-			+			
5	BRMS-034	R05		-			-					-			-			
e-72	e-72	R05	-		-		-		-		-		-		-		-	
73	Na12H07	R06	-	-		-	-		-		+	-		+	+		-	
176	BC7t	R06			-	-	-						-	-	-			
16	BRMS-051	R09		-			-	-				-			-	-		
e-15	e-15	R09					-								-			

+ = marker and trait are correlated for that population, - = marker and trait are not correlated in that population. Blank cell = no information

Markers 94 (R02), 95(R10) and 363 (02 and R10) are mapped in those regions. All the correlations of those markers are in populations where the Asian turnip VT-115 was the turnips parent. Besides the expected markers also markers on R03 (SSRs 3, 21 & 85), R04 (SSR e-78) and R06 (SSR 73) showed correlation with turnip formation and FT. The markers on R03 only show correlations in the population with the turnips from both centers of variation (VT-52 x VT-12).

Table 8. Detailed information of all marker-trait correlation.

Population	*Trait*	*Marker name*	*Lab Code*	*Linkage Group*	*a:h:b*[1]	*Present:*[2]*absent*	*χ-Square*[3]	*Significance Level*[4]
27(VT-115 x OR-129)	TuWidth	Na12H07	73	R06	13:31:7		0.151	0.045
		ENA13	363-3	R02/R10		46:5	0.012	0.031
	TuWeight	ENA13	363-3	R02/R10		46:5	0.012	0.013
29 (VT-115 x PC-105)	FT	ENA13	363-2	R02/R10		41:11	0.522	0.034
32 (VT-115 x CC-146)	TuWidth	Na12H07	73-2	R06		30:15	0.197	0.006
	TuWeight	Na12H07	73-2	R06		30:15	0.197	0.015
36 (VT-115 x RC-144)	FT	FLC2	94	R02	12:22:13		0.890	0.001
		FLC1	95	R10	9:28:12		0.505	0.003
		ENA13	363-1	R02/R10		40:12	0.749	0.020
		ENA13	363-2	R02/R10		44:8	0.109	0.010
		Na10D09	4	R04	16:23:13		0.595	0.020
	TuWidth	FLC1	95	R10	9:28:10		0.505	0.000
		ENA13	363-1	R02/R10		40:12	0.740	0.002
		Na12H07	73	R06	10:27:14		0.669	0.014
		ENA13	363-2	R02/R10		44:8	0.109	0.004
		Na10D09	4	R04	16:23:13		0.595	0.034
45 (CC-74 x VT-9)	FT	FLC1	95	R10	24:17:9		0.012	0.000
49 (VT-52 x VT-12)	FT	BRMS-50	21-3	R03		24:79	0.690	0.021
		BRMS-43	3	R03	44:39:16		0.000	0.041
	TuWidth	FLC2	94-1	R02		54:46	0.000	0.002
			94-2	R02		21:76	0.446	0.003
			94-3	R02		72:25	0.860	0.007
		Ol11B05	85-5	R03		54:50	0.000	0.043
		e-78-1	e78-1	R04		39:75	0.003	0.000
		BRMS-50	21-2	R03		17:86	0.046	0.000
			21-3	R03		24:79	0.690	0.001
	TuWeight	FLC2	94-1	R02		54:46	0.000	0.012
			94-2	R02		21:76	0.446	0.027
			94-3	R02		72:25	0.860	0.041
		Ol11B05	85-5	R03		54:50	0.000	0.005
		BRMS-43	3	R03	44:39:16		0.000	0.022
		e-78-1	e78-1	R04		39:75	0.003	0.000
		BRMS-50	21-2	R03		17:86	0.046	0.001
			21-3	R03		24:79	0.690	0.007

[1]Number of plants per marker genotype (a, h and b) for co-dominant markers. [2]Number of plants per genotype (present:absent) for dominant scored markers. [3]χ^2-value connected to the ratios in a:b:h or present:absent columns. [4]Significance of marker-trait correlation in the ANOVA test.

More detailed information on the different trait-marker correlations is shown in table 8. In this table the segregation ratios are given in the columns a:h:b and present:absent. Values are present in the a:h:b column when the marker segregates as expected with 3 clear distinguishable classes a, b and h. The present:absent contains values when markers with unexpected segregation were scored. In these markers every band was scored separately as a dominant marker.
In this table also the significance of the marker-trait correlation and the χ^2-value for the segregation are shown. This χ^2 value is connected to the expected segregation. The expected segregations are 13:26:13 (a:h:b) for a co-dominant marker and 13:39 or 39:13 for the dominant markers. If the χ^2 value is below the 0.05 threshold the segregation was skewed. The last column shows the significance level of the ANOVA test.
During this thesis four populations were considered as more promising than the other seven based on the phenotypic data regarding turnip size (large), turnip range (wide range) and the flowering time. Therefore the focus of the marker analysis was on these four populations. As shown in paragraph 3.1 populations 29 (VT-115 x PC-105), 36 (VT-115 x RC-144), 48 (CC-168 x VT-9) and 49 (VT-52 x VT-12) were the better population and were selected for the more dense marker analysis.
In figure 10 pictures of a gel of every of those four populations is shown. The expected segregation of a SSR marker is shown in figure 10B. This marker is an example of a marker in population 36 (VT-115 x RC-144). All F_2 plants of this population contain alleles which are present in one of the two parents. For this type of clear segregation the three different genotypes are distinguishable. The homozygous genotype equal to that of the VT-115 parent was scored as "a", the heterozygous genotype which consist of one allele from both parents was scored as "h" and the genotype which is the homozygous equal to the non-turnip parent was scored as "b". All markers in population 36 (VT-115 x RC-144) show approximately a 1:2:1 ratio of the "a", "h" and "b" genotypes respectively. Other populations however did show some unexpected results. Bands with unexpected sizes occur in some population. The bands with unexpected sizes are indicated with arrows in figures 10A, 10C and 10D. For example the arrows in figure 10A indicate bands which only occur in two of the 52 F_2 plants, which is rare. The origin of this unexpected band can be traced however, because this band occurs also in one of the VT-115 plants. In Figure 10C however one of the unexpected bands cannot be traced, the upper arrow indicates a band that occurs in the CC-168 parent, but the lower arrow indicates a band which cannot be linked to any of the parents. In Figure 10D even four unexpected band show up of which two occur in parental lines, but the origin of the other two cannot be explained. These kinds of discrepancies complicate the scoring and the reliability of the data, because it is unknown from where these bands originate. This type of results makes the scoring and analysis of the marker data complicated, because the origin of the bands cannot be traced.

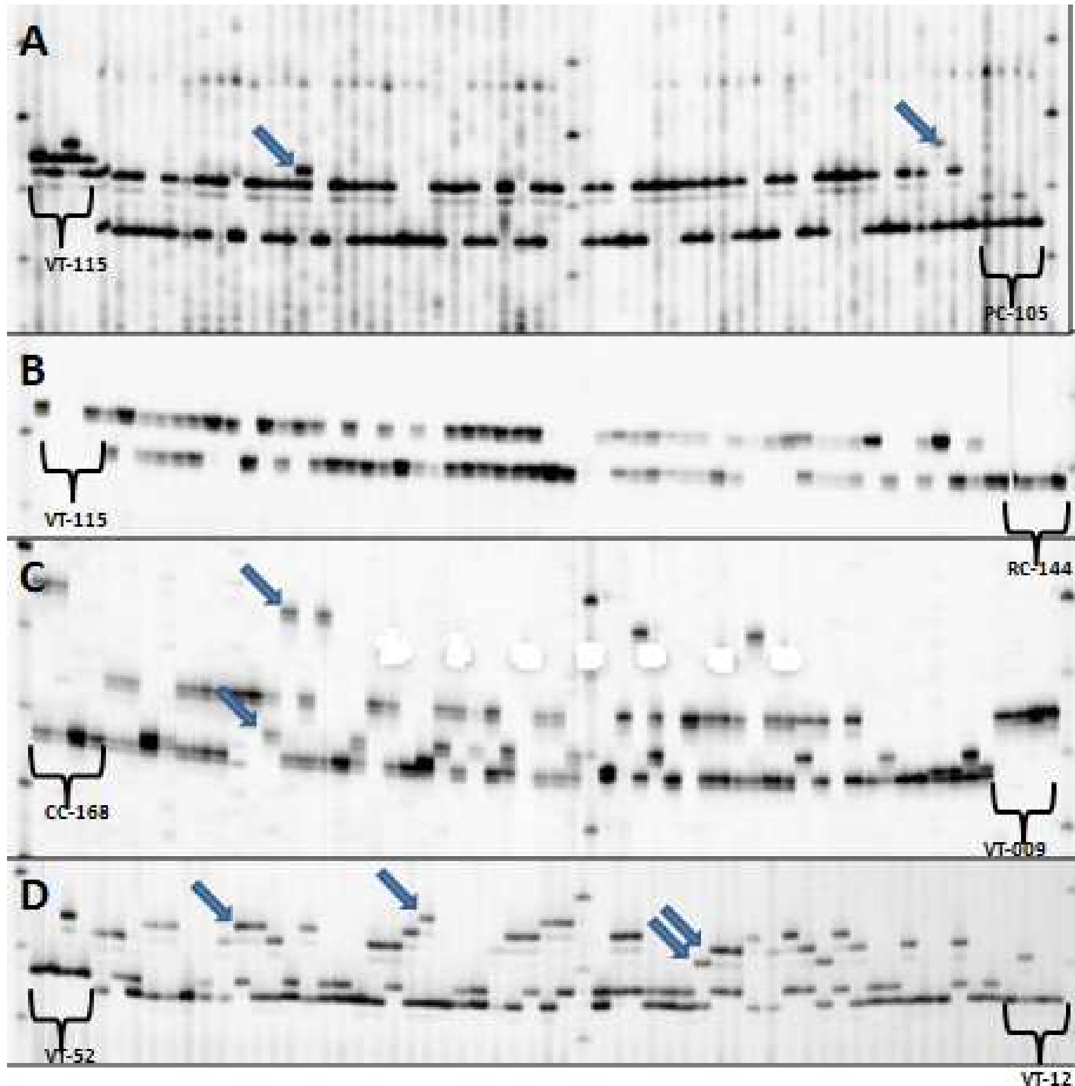

Figure 10 Li-Cor gel pictures of the emphasized populations A: Pop 29 (VT-115 x PC-105), marker Ol11B05 (85). B: Pop 36 (VT-115 x RC-144), marker e-71. C: Pop 48 (CC-168 x VT-9), marker Na12H07 (73). D: Pop 49 (VT-52 x VT-12), marker Ol11B05 (85). On each side 4

The unexpected bands were not scored when only one or two F_2 plants contain the band. Which was the case in population 29 (VT-115 x PC-105). When more individuals in a population contain a band which was not expected, the bands were scored separately in a dominant (present versus absent) manner. This was done in populations 45 (CC-74 x VT-9), population 48 (CC-168 x VT-9) and population 49 (VT-52 x VT-12). For a gel as shown in figure 10D this resulted in a scoring of six individual bands.

4. Discussion

During the growth of the eleven populations, it was clear that a few populations showed a good segregation for turnip formation and others did not form obvious turnips. Examples of good segregating population are populations 29 (VT-115 x PC-105), 32 (VT-115 x CC146), 36 (VT-115 x RC-144), 48 (CC-168 x VT-9), and 49 (VT-52 x VT-12). The plants in these populations show a wide range in turnip size and therefore are considered as good populations. Populations with less obvious turnip formation are populations 27 (VT-115 x OR-219), 30 (VT-115 x YS-143), 34 (PC-175 x VT-115), 45 (CC-74 x VT-9), 118 (VT-44 x WO-81) and 119 (WO-81 x VT-44). In this discussion most populations will be discussed separately.

4.1. Population 29 (VT-115 x PC-105)

This population is a good population for future research. It is the only population with VT-115 as turnip parent of which the F_2 makes turnips near the size of VT-115 itself (figure 8A). Also the range (table 4) and distribution (figure 6A) of the turnip size is sufficient to classify this population as good. Another interesting characteristic of this population is that the correlation between FT and Turnip formation is absent. Therefore it can be a good population to study the relation between FT and TuF. A possible explanation for the absence of the FT-TuF correlation in this population is that the parents do not differ in FT. Remarkable is that the FT is segregating in this population although the parents do not differ in FT. This could mean that both parental lines contain recessive alleles which influence flowering time. Besides the positive aspects of this population it has one negative aspect. The population seems to have a high susceptibility to abiotic and biotic stress, because it is the only population where plants died (17%) during the growth in the greenhouse. This can have three reasons the first is that at the early growing stage leafminers (*Liriomyza spp.)* were present in the greenhouse this population suffered most of those insects. The second reason was that a few plants seem to suffer from an unknown kind of rot; this can be caused by the high humidity of the soil. The third reason was that it could be due to genetic incompatibility between parental genomes which causes dwarf or lethal plants.
From the marker analysis of this population only marker 363-2 (ENA13) showed significant correlation with the FT trait. This marker has two segregating loci, one on the top of R02, the other on the bottom on R10 both near the FLC locus. Another marker mapped in the R02 region of FLC is e-17 (7cM away); in contrary to marker 363 it does not show any correlation with the FT trait. This suggests that this marker (363-2) is located on linkage group R10 and thus that FT is correlated with the genomic region near this marker. Taking all this into account this population is suitable for further research in genetic dissection of the turnip trait; on the other hand it is needed to pay special attention to the growing conditions of this population to obtain reliable results.

4.2. Population 36 (VT-115 x RC-144)

This population has two main advantages it segregates for the turnip trait and it is very early flowering. The range of FT is between 30 and 78 days after sowing which means that there can be sown 3 generations per year. Although the turnips of this cross are not as big as in population 29 (VT-115 x PC-105) the turnips are very well recognizable compared to other early flowering populations (VT-115 x YS-143 and PC-175 x VT-115). It is remarkable that in this very early flowering population you see turnips, since it was expected that the negative correlation between FT and TuF would mask turnip formation. In this population all polymorphic markers (14) were profiled. All markers segregated as expected (1:2:1), which is another positive characteristic of this population compared to populations 48 (CC-168 x VT-9) and population 49 (VT-52 x VT-12). Four markers are correlated with the FT (4 (R04), 94 (R02), 95 (R10) and 363 (R02 and R10), three of them (94, 95 and 363) are located near the FLC loci on R02 and R10 so the results are as expected. Marker 4 which is located on R04 indicates that there is another FT regulating QTL on R04. Also four markers show correlations with turnip width, again markers 95 (10) and 363 (R02 and R10) are involved which support that the QTLs for FT and TuF are located in the same genomic region. Also for TuWidth two additional markers show correlations with turnip width, markers 4 (R04) and 73 (R06). Since FT and TuF are correlated in this population marker 4 is expected because it is also correlated to FT. In all former mentioned markers the average turnip size of plants with the “a” genotype was always bigger than the group with the “h” genotype, which was always bigger than the “b” genotype. Only marker 73 shows a deviation of this pattern, the heterozygous genotypes (h) have bigger turnips than the RC-genotype (b) which had bigger turnips than the VT-genotype (a). The non-turnip parent RC-144 is contributing positively to turnip size. Two phenomena can cause this kind of results; the first is called overdominance, the second is heterosis. This is also shown in tomato where wild dwarf tomatoes contribute to bigger tomatoes (Lippman and Tanksley 2001).

4.3. Populations 32 (VT-115 x CC-146) and 48 (CC-168 x VT-9)

Chinese cabbage is in both populations one of the parents. Turnips of F_2 plants from population 32 (VT-115 x CC-146) and population 48 (CC-168 x VT-009) both have a wide range and intermediate size. Therefore these two are possible good populations for further research based on the phenotype. There is though one remark of population 32 a number of F_2 plants formed purple colored turnips which are not present in one of the parents. Beside the unexpected purple turnips in population 32 also the marker data of both populations is not as expected. In figure 10C is shown that not all alleles in the segregating F_2 are present in the parental lines. In many markers of these populations more than the expected two alleles are present. And most unexpected alleles are rare in the F_2 population. Therefore the origin of these unexpected alleles cannot be traced. The quality of the markers itself is good though

because in the VT-115 x RC-144 these same markers do not show the unexpected segregation. Every marker in these populations show this inheritance pattern with rare unexpected bands and therefore both populations are not useful for future research after all.

4.4. Population 49 (VT-52 x VT-12)

Population 49 (VT-52 x VT-12) shows transgressive segregation for turnip size. This population is a cross between two parents of different centers of variation. The transgressive inheritance of the turnip trait in this cross can indicate that different genes in different centers of variation influence turnip formation and growth.

The marker data of this population 49 (VT-52 x VT-12) shows the same kind of abnormalities as population 32 (VT-115 x CC-146) and population 48 (CC-168 x VT-9). In figure 10D a marker is shown of which the F_2 population contains six different alleles. These six alleles cannot be product of a self-pollination of one F_1 plant. I suspect that either this population is a mixture of several selfed F_1 plants or admixture of pollen of other *Brassica rapa* accessions has occurred. A mixture of several selfed F_1 plants can produce a F_2 population that results in a gel like in Figure 10D, because most parental lines of this thesis are heterozygous Due to the heterozygosity of the parental lines the F_1 plants can differ in the genotype for a marker and thus also the segregation of F_2 population of different F_1 plants can differ. Taking this into account population 49 (VT-52 x VT-12) can be a very interesting population for research in differences of the genetic basis of turnip formation in the two main centers of variation, but due to the bad marker data the population available now is difficult to use. Although the marker data of this population is difficult to analyze, the TuF QTL on R02 is supported with the correlation of marker 94 with both turnip weight and width. FT is not correlated with this marker, which is as expected because the FT-TuF correlation is absent in this population. Besides the marker on R02 also markers on R03 (3, 85-5, 21-2 and 21-3) show correlation with turnip weight and width. Markers 3 (60cM) and 85(64cM) are mapped 4cM apart from each other and marker 21 (115cM) is mapped 60cM away, which indicate that two regions on R03 show correlations with the turnip trait. In the study of Schranz et al. (2002) two FLC genes are mapped on R03, FLC3 on the top of and FLC5 20cM lower. There are no common markers between the study of Schranz et al. (2002) and markers used in this study, furthermore is the length of R03 in the study of Schranz et al. (2002) 53cM in total which is small compared to the 115cM where the marker 21 is mapped (integrated map of DH-38 and DH-68). Therefore a clear correlation between the map positions of the markers used in this thesis and the map positions of the FLC genes the study of (Schranz et al. 2002) can't be made, but it suggests that the two different FLC genes mapped on R03 are linked to TuF QTLs and that those FLC genes are close to the map positions of the markers in this thesis.

4.5. Populations 118 & 119 (VT-44 x WO-81), (WO-81 x VT-44)

Examples of populations with little or no turnip formation are the reciprocal populations 118 (VT-44 x WO-81) and 119 (WO-81 x VT-44). Both populations were expected to be interesting for research in the FT-TuF relation, because a late flowering oil type was crossed with a vegetable turnip. The difference between FT of both parents was still more than 45 days though, which resulted in a correlation between FT and TuF. Therefore the purpose of this population is not as expected.

The goal of maintaining 80 plants in the greenhouse of both populations was to combine the populations to one big population for QTL mapping.

The populations differ in germination rate, population 118 (VT-44 x WO-81) had only a germination rate of 80% while WO-81 x VT-44 had a germination rate of almost 100%. In population 118 (VT-44 x WO-81) also had 4 of the 80 plants were dwarftypes and 5 of the 80 plant had a yellowish phenotype. Due to these differences in those reciprocal crosses it is not recommended to combine the populations. A common feature of these populations is that they did not form any turnips, only a small thickening of the root was observed and therefore these populations were recommended not to be used in future research at all.

4.6. Populations 27(VT-115 x OR-219), 30(VT-115 x YS-143), 34(PC-175 x VT-115) and 45(CC-74 x VT-9)

These populations did not form large turnips. All population had a few plants which did form turnips, but on average the turnips were too small to continue with those populations. Population 30 and 45 also showed some unexpected bands in het marker analysis, which makes it even more unfavorable for future research.

Also the absence of any marker-trait correlation in population 30 (VT-115 x YS-143) is remarkable, because one of the previous studies (Lou et al. 2007) in turnip formation was mainly based on DH and BC crosses of VT-115 and YS-143.

4.7. TuF-FT correlations

The TuF-FT correlation was present in 7 of the 11 populations. These correlations support the former studies. The populations which did not show the TuF-FT correlation are the populations where the flowering time of the parents differ less than 40 days. For example in population 29 (VT-115 x RC-144) the difference in flowering time between the parents is five days and no FT-TuF correlation is shown therefore this population could be an important one for further dissection of this TuF-FT correlation.

4.8. Different QTLs underlie TuF in two centers of variation

As discussed under population 49 (VT-52 x VT12) markers on R03 showed correlations with the turnip trait. The marker data is not sufficient but it is likely that the genomic regions around those markers are close to the FLC3 and FLC5 genes. Since on R02 a TuF QTL is mapped near the FLC2 gene it is likely that around FLC3 and FLC5 also TuF paralogues are located. Since only in the population with the European turnip markers on R03 showed correlation with turnip formation it is likely that different TuF paralogues control turnip formation in the different centers of variation. The paralogues have probably the same function but different paralogues are active in the two centers of variation. Therefore we can conclude that turnip formation in the two centers of variation is controlled by different genes (paralogues).

This conclusion is still fragile because only one population with a few markers supported this conclusion. To verify the statement new crosses should be made as discussed in the future recommendations.

5. Concluding remarks and future direction

Taking all data together two populations were recommended for further research in the turnip trait. Population 36 (VT-115 x RC-144) is good for its early flowering and wide segregation of turnip size. RILs (Recombinant Inbred Lines) will be made from this population by self pollination for five to six generations. A map will be constructed for this F_2 population and the future RIL population. To obtain a good quality map, more F_2 individuals need to be sown to increase the population size. Within 3 years a RIL population with a dense map should be available for this population.

Population 29 (VT-115 x PC-105) is a good population because of the wide segregation for turnip size. Doubled haploid will be generated for this population since PC-105 reacts good on DH production (personal communication Ningwen Zhang) so the problem of heterozygous plants as in this thesis will not occur anymore. New F_1 are already sown for this DH production.

Also the combination of the populations is good for further research. Because the FT-TuF correlation is absent in VT-115 x PC-105 and present in VT-115 x RC-144. More insight can be obtained in the FT-TuF correlation in comparing the two populations.

Although two populations are already lots of work it is recommended to make new crosses between a European turnip and PC-105 or RC-144 as the non-turnip parent. These “new” crosses can be compared with the already available population 29 (VT-115 x PC-105) and 36 (VT-115 x RC-144) and to reveal the genetic basis of the turnip formation. The comparison between the “new” VT-52 x RC-144 and the existing VT-114 x RC-144 can be used for verifying the final conclusion of this thesis.

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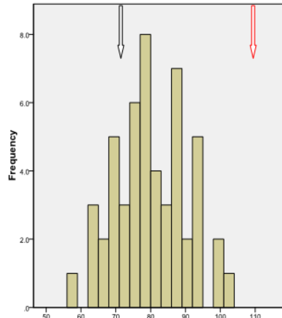
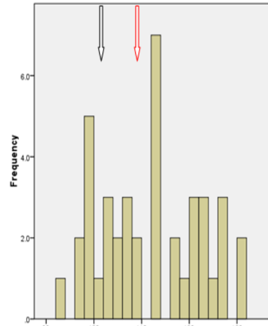
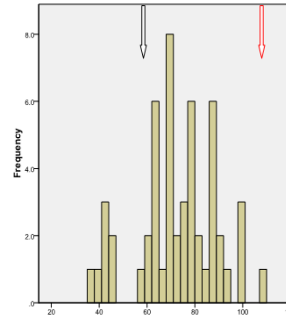
Appendix 1 List of all available markers

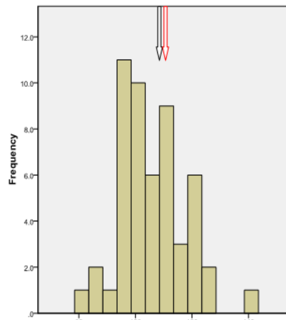
Labcode	Marker	Group	Linkage group	Position (cM)
94	BRH04D11flc2t	A	2	10.293
est-17	Br323	A	2	13.74
est-20	Br326t	A	2	56.439
40	Na12H09t	A	2	65.149
272	chsssr13	A	10	2.5
95	BRH80A08flc1t	A	10	58.0
362	EJU6R10	A	10	63.1
27	BRMS-008	B	3	0
3	BRMS-043t	B	3	59.089
21	BRMS-050	B	3	115.319
256	F3H-SSR2t	B	3	124.467
5	BRMS-034t	B	5	16.007
23	BRMS-007t	B	5	41.863
7	BRMS-040	B	7	23.4
15	BRMS-018t	B	7	25.7
16	BRMS-051t	B	9	69.2
363	ENA13i	C	2	22.428
367	ENA23h	C	2	42.248
363	ENA13h	C	10	83.9
367	ENA23l	C	6	62.5
est-2	Br308t	D	1	4.4
198	BC105t	D	1	5.8
56	BRMS-031t	D	1	64.409
est-54	Br360	D	3	31.456
85	Ol11B05	D	3	64.019
est-18	Br324t	D	3	67.193
est-50	BR356t	D	3	68.34
210	BRMS-042-2t	D	3	120.123
4	Na10D092	D	4	14.991
4	Na10D09t	D	4	15.621
est-78	BR384t	D	4	38.961
est-19	BR325	D	4	47.384
est-72	BR378t	D	5	28.742
176	BC7t	D	6	0.0
est-38	Br344t	D	6	60.5
73	Na12H07t	D	6	62.8
est-66	Br372	D	7	12.4
est-13	BR319t	D	8	33.351
8	Ra2E12t	D	8	77.732
est-37	BR343t	D	9	61.0
est-15	BR321	D	9	67.4
est-34	Br340t	D	9	80.7
198	BC105t	D	9	155.0
14	Na12A01l	E	3	160.082
14	Na12A01h	E	3	160.378
14	Na12A01m	E	6	70.2

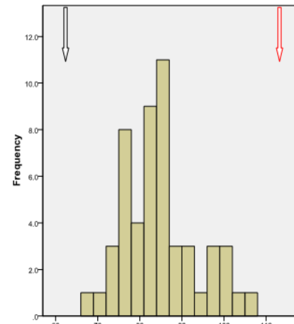
Appendix 2: Distribution graphs Flowering Time (FT) in all populations.

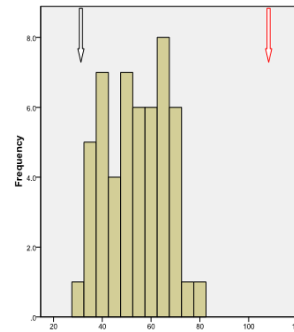


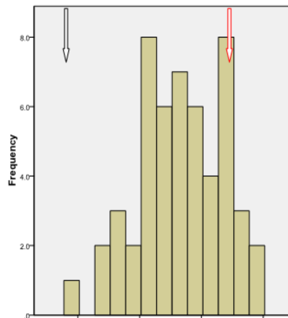


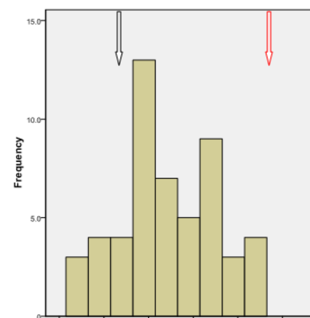


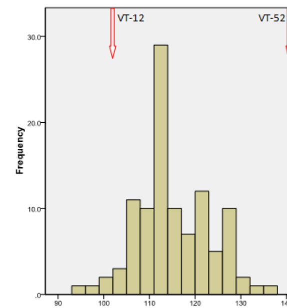


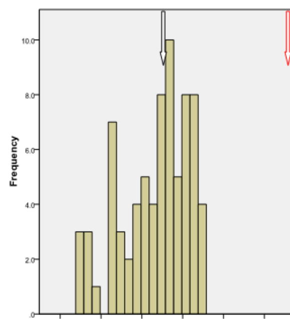


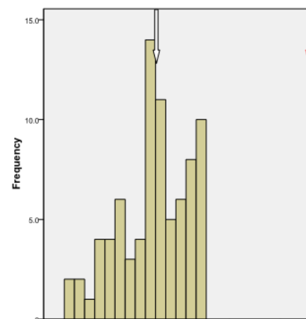




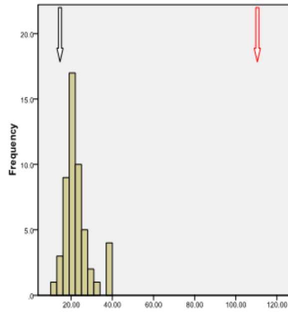


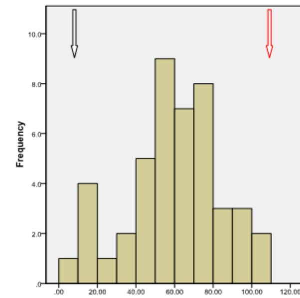


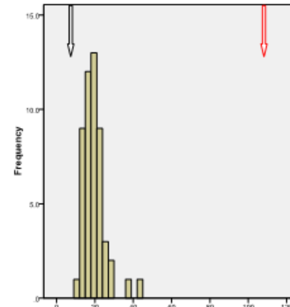


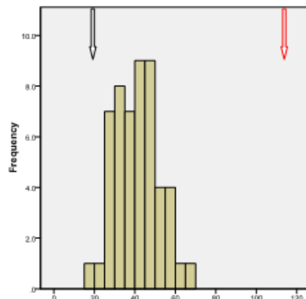


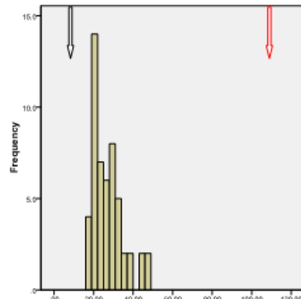
Appendix 3 Distribution graphs Turnip Width of all populations.

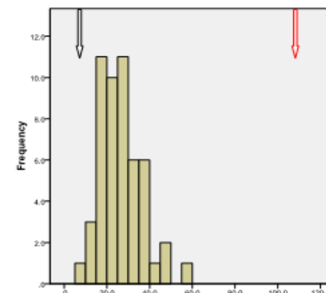


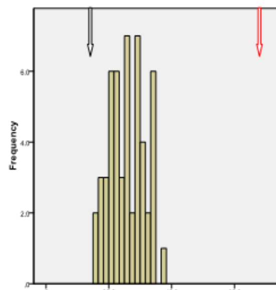


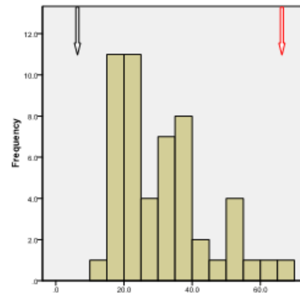


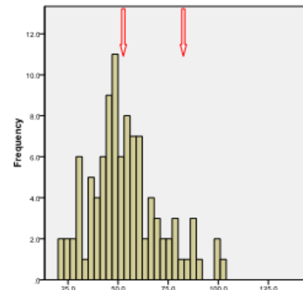


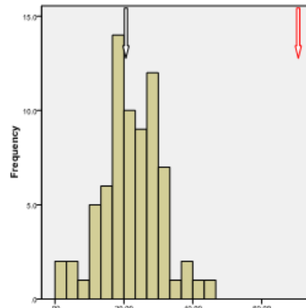


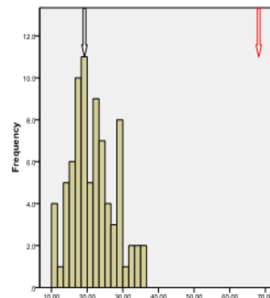




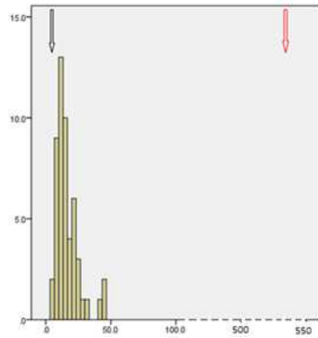


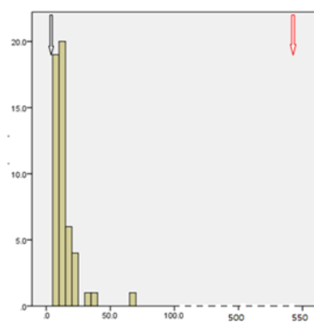


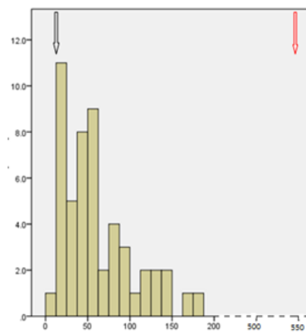


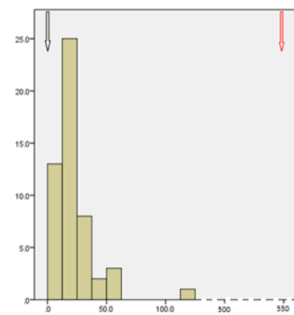


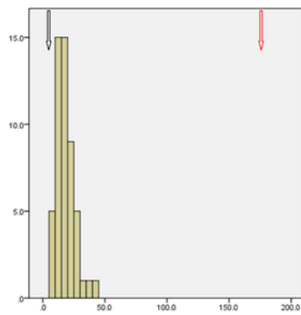
Appendix 4 Distribution graphs Turnip weight

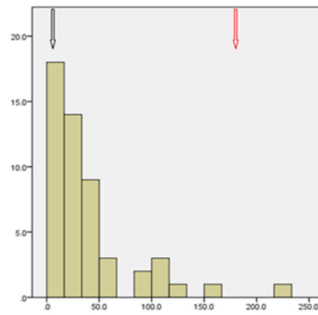


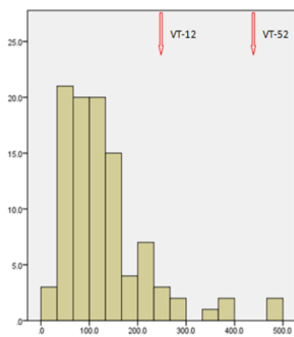


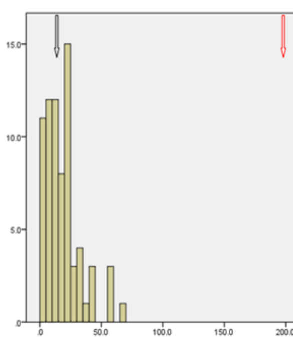


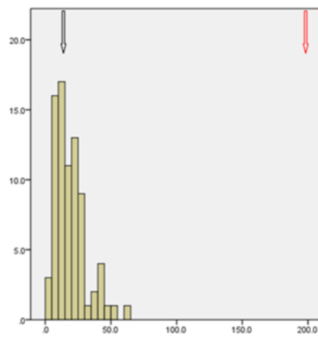












Appendix 5 DNA Isolation protocol

For this protocol around two leafdiscs of young leaf tissue were harvested and put in Liquid nitrogen immediately. The boxes with all leaf tissue were milled for 30 seconds at 30 Hz in the RETCH machine. After grinding 400µl of isolation buffer was added. Isolation buffer 200 ml**:** Lyses buffer 84.0 ml, Extraction buffer 84.0 ml, Sarkosyl 5% 33.5 ml, Sodium bisulfide 500 mg. Lyses buffer 500 ml: Tris-HCl 1M (pH 7.5) 100 ml, EDTA 0.5 M (pH 8.0) 5 ml, NaCl 5 M 200 ml, MQ 195 ml, CTAB 10g, Extraction buffer: Tris-HCl 1M (pH 7.5), EDTA 0.5 M (pH 8.0). MilliQ 445 ml, Sorbitol 31.9 g. The plant material together with the isolation buffer was incubated at 65°C for 1 hour. Then the samples were cooled down in icewater. Then 400 µl chloroform:isoamylalcohol (24:1) was added to the samples. Then the tubes were centrifugated at 6000rpm for 20 minutes. The supernatant was pipetted out and 280 µl isopropanol was added. The mixture was centrifugated again at 6000rpm for 8 minutes, the supernatant was discarded and 300 µl of 70% ethanol was added. Again the mixture was centrifugated for 5 minutes at 6000rpm, the supernatant was discarded and the pellet was airdried for 2 hours. The pellet was the dissolved in 100 µl MilliQ. After freezing and thawing the samples once the DNA concentration was measured on the NANOdrop.