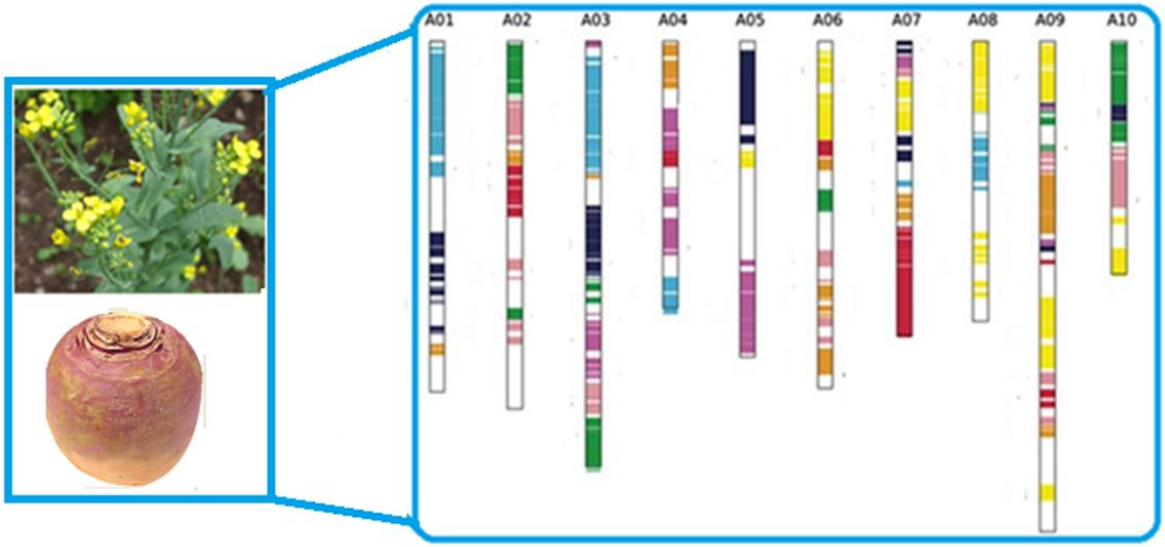
Construction of a Genetic Map and QTL Mapping for Turnip Tuber Traits Using an Recombinant Inbred Line Population from a Cross between Turnip and Rapid Cycling in *Brassica rapa*



Minor Thesis Report (PBR-80424)

Temesgen Menamo (Reg. No. 860717-557-050)

Supervisors: Dr. Ningwen Zhang
Dr. Guusje Bonnema

Laboratory of Plant Breeding, Wageningen University

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Abstract

This study described on construction of a genetic linkage map based on 110 SNPs and 14 InDels markers on *Brassica rapa*. It also described turnip tuber related traits QTLs for the *Brassica rapa*. An recombinant inbreed line population consists of 106 F6/7 individual lines derived from a cross between VT-115 and RC-144 were used for the present study. The Kosambi mapping function was used for linkage map to convert recombination frequencies into map distances (cM). A total of 61 markers were mapped over 13 newly constructed linkage groups. Linkage group 01, 02 and 09 had two linkage groups. The alignment and orientation of the newly constructed linkage groups were agreed with existing *B. rapa* linkage maps from chiifu genome. The map covered a total of 639.5 cM of the genome with an average linkage length (coverage) of 49.2 cM and an interval distance of 10.5 cM. The length of linkage groups ranged from 1.0 cM to 183.8 cM for L01_1 and L09_1 respectively. The turnip tuber related QTLs was made by Kruskal- wallis Mapping. A total of 94 turnip related QTLs were detected at significant level of ≤0.1 in all newly constructed linkage groups except L01_1 and L04. Out of the total 18 for Brix (total Soluble Sugar), 29 for turnip tuber fresh weight, 26 for turnip tuber dry weight and 21 for turnip tuber dry mass. Dry weight per turnip tuber, fresh weight turnip tuber and weight per turnip co-located together on linkage group A02 at flowering QTL loci. this paper would like to recommend making linkage and QTLs mapping with more number of individuals and markers. It gives accurate and precise linkage groups and QTLs maps.

Keywords: *Brassica rapa*, SNPs, InDels, linkage map, QTL

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1. Introduction

1.1. Background on Brassica

Brassica has a large plant family with 338 genera and 3,700 species, including a wide range of horticulture crops, which have great economic value and grown as vegetables, fodders, sources of oils and condiment (Al-Shehbaz *et al.*, 2006; Campo, 1999; Zhao *et al.*, 2010). The model plant *Arabidopsis* is also closely related to the genus *Brassica* (Ostergaard and King, 2008) which can be exploited to find orthologous genes from *Arabidopsis* with known function. This is more advantageous for genetic study for *Brassica* genus. This *Brassica* genus has six species including: *B. rapa, B. nigra, B. oleracea, B. juncea, B. napus* and *B. carinata*. The three diploid Brassica (*B. rapa, B. nigra* and *B. oleracea*) species forming the Triangle of U (figure 1), have hybridized in all possible combinations to produce the three allotetraploid species; *B. juncea, B. napus* and *B. carinata* (Nagaharu, 1935; Ostergaard and King, 2008). *Brassica Rapa* species has a diploid (2n=20) genotype and is a morphological diverse including vegetable and oilseed crops (turnip types, leafy types and oil types) such as: Chinese cabbage (*Brassica Rapa* subsp. *pekinensis)*, non-heading pak choi *(Brassica rapa* subsp. *Chinensis)*, turnip (*Brassica rapa* subsp*. rapa L.)*, turnip rape (*Brassica rapa*), and Sarson (*Brassica rapa* L. var. *trilocularis*) (Song *et al.*, 1995). Turnip (*Brassica rapa* subsp*. rapa L.)* is domesticated in very ancient time and was used for human consumption and animal feed (Vogl-Lukasser *et al.*, 2008). It believes that the turnip was the first domesticated *Brassica* species and naturally grown from the Western Mediterranean region to central Asia (Campo, 1999). Europe and Eastern Asia are one of the center of diversity for *B. rapa subsp. rapa*. It is grown popular in Europe starting from 2500-2000 BC, the Middle East is starting 1000 BC and Asia starts around 1800 B.C (Vogl-Lukasser *et al.*, 2008).

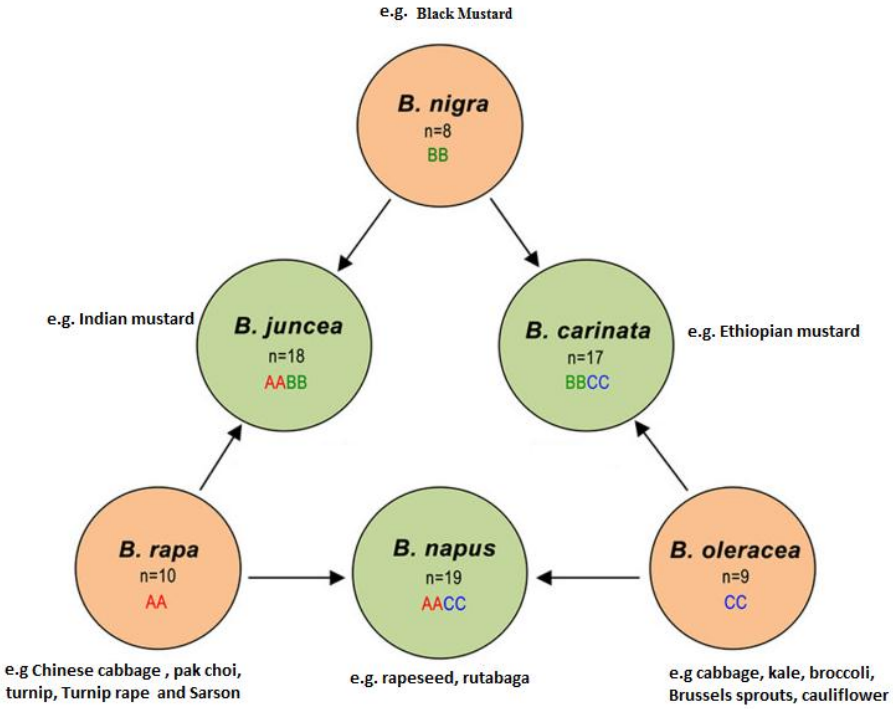


Figure 1. The "Triangle of U" diagram, showing the genetic relationships of six species in the genus Brassica *(Nagaharu, 1935).*

1.2. Economic importance

Brassica rapa is one of important crop among the six economically cultivated Brassica species of U's triangle. It is cultivated all over the world as a vegetable for food and fodder for animals. *Brassica rapa* var. Chinese cabbage and var. *chinensis* (leaf cabbage) are extensively grown as important market garden vegetables in China. The main economic important part of the turnip is hypocotyl with tap root but the leaves are also used for fodder as well as for food. The turnip greens (tops) are excellent chopped in salads or cooked briefly as a side dish. Turnip also used for flavor in ingredient soups and a winter salad. It also used in different dishes in some country like India and Pakistan. The nutrient composition of turnip (USDA, 2011), it has more calcium and sodium mineral, particularly rich in vitamin C, very low fat and proteins (USDA, 2011). Brassica vegetables are rich in glucosinolates, a chemical which can block the growth of human cancer cells (He *et al.*, 2012; Kushad *et al.*, 1999; Rosa *et al.*, 1997).

1.3. Morphological description

Flower agronomic traits are important for vegetative as well as for turnip which related to the quantity and quality of turnip tuber formation. The flowers are small, yellowish color and clustered at the top of the raceme. It produces pea-like pods which contain seeds. Turnip is a biennial crop but most commonly grown as an annual. The first year the plants grow to be used for human consumption in their hypocotyl with tap root and the second year produce flowers and set seed. Traditionally, turnip is called root crop but actually it is not a root at all. It consists mainly of the hypocotyl as well as main root. Hypocotyl is plant part that lies between the main root and the first seedling leaves (cotyledons) which grows below or above the surface of the soil. It also has little or no neck and a distinct tap root(Brassica group, Wageningen University).

1.4. *Brassica rapa* breeding objectives

Brassica rapa has a wide genetic variability, it helps to important traits and improvement in this crop. Recent advanced genetic marker techniques can supplement for improving of the agronomical important traits of this crop since conventional genetic analyses and breeding also could success in the improvement of many traits. Genetic analysis and identification of chromosomal regions which containing agronomic important morphological traits would be helpful in breeding for desired traits. Many root crops or root like crops can undergo considerable root radial growths which would be suitable for food. Turnip is one of fleshy and edible hypocotyl crop. So, it is important to improve the quality and quantity of the turnip yields.

1.5. Molecular Markers and Development of Genetic Linkage Maps

Most of agriculturally important traits (yield, flowering time and some of disease resistance) are controlled by many genes, called quantitative traits (polygenic) which contain genes associated with a particular quantitative trait are known as quantitative trait loci (QTLs) (Collard *et al.*, 2005). *Brassica Rapa* has a large number of morphological differences including flowering time and turnip tuber formation. Vegetative turnips have a very high morphological diverse among turnip genotypes (Yildirim *et al.*, 2010) including; flowering, turnip onset, turnip shape, size and so on.

There are several molecular linkage maps in *B. rapa* have been developed using RFLP, RAPD, AFLP, and SSRs molecular markers and mapping populations such as F2, DH, RIL. In Arabidopsis (El-Assal *et al.*, 2001) and some of B*rassica rapa (Lou et al., 2007)* studied at the co-location of QTL for

floral and other growth-related traits. Several studies are observed in QTL flowering time and turnip tuber formation in different populations in the *Brassica rapa at* Wageningen University by Brassica group members. The genetic of turnip tuber formation studied by SSR and InDel molecular markers to profile an F_2 population derived from a cross between a Vegetable Turnip (VT-115) and a Wutacai (PC-105) (Wang, 2011). He also studied morphological traits of the corresponding F_3 population in the field and the reliability of InDel markers and LightScanner. He observed phenotypic variation in F_3 population even unexpected variation also. For instance, red or purple color in leaves and/or turnips, since both of the parents (VT-115 and PC-105) didn't have this type of trait. He found ten markers to be associated with leaf traits eight of which are associated with leaf number, spreading over linkage group A01, A02, A03, A05, A09, and A10.

Vernalization caused delays of turnip onsets which unvernalized turnips are caused by the earlier turnip swelling onset (Visser, 2010). This study also showed that a strong correlation between turnip traits (diameter and width) and the swelling onset as significantly delayed by vernalization. In addition to vernalization, the study also had shown the turnip swelling onset different between genotypes. The study used three turnip growth candidate genes but only one marker (nw_60) was associated with turnip traits (diameter and width).

A total of 27 QTL detected which were affecting 20 morphological traits from population F2/3 crossing Rapid cycling vs. Chinese cabbage; double haploid from Yellow sarson, Pak choi, and a Japanese vegetable turnip variety. From those of it, eight linkage groups are detected for flowering time on R01, R02, R03, R06, R07, R08, and R10 in all populations (F2/3, DH-38, DH-30, and BC1) in different growing seasons (Lou *et al.*, 2007). It shows the major flowering time QTL (FLQTL-2) detected in all populations on linkage group R02 co-localized with BrFLC2. This study also detected a single QTL for the turnip traits at the top of R02 which co-localizes with the major flowering time QTL (FLQTL-2).

The major flowering time QTL detected in different *Brassica rapa* populations on linkage group R02 co-localized with BrFLC2. FLOWERING LOCUS T (FT) also identified in *Arabidopsis* as a main component of the florigen or mobile flowering promoting signal produced in the leaves (Abelenda *et al.*, 2011). This locus has a homologous gene in *Brassica rapa*. For instance, *BrFLC5* gene homologous to *FLC* regions to Arabidopsis (Schranz *et al.*, 2002).

1.6. Scope of the thesis

The two parental lines crosses VT-115 x RC-144 originated from Japan (VT-115) and USA (RC-144). Between these two parental lines there are different flowering time which RC-144 is an early flowering type than VT-115 (discussion with Ningwen) and they are different morphotype (VT-115 vegetative turnip; RC-144 oil type). The frequency distribution of this population had shown similar pattern in F2 and F3 progenies (Rémi, 2010). Phenotyping in F2 population could not repeated and the genotype comes from the combination of two parents while RILs are homozygous lines each harbours different combination of two parental genome fragments. QTLs mapping using of dominant F2 population is less accurate when compared to RILs (Ferreira *et al.*, 2006).

The scope of this thesis was:

- to construct a genetic map of 106 lines of an RIL population (F6/7)from a cross between VT-115 and RC-144
- to detect turnip tuber related QTLs to the constructed linkage groups

2. Method and Material

2.1. Parental material

The mapping population have been consisted of 106 RILs F6/7 populations developed by single-seed descent from the cross *Brassica rapa* accessions; Vegetable turnip VT-115 as female and Rapid cycling line RC-144 as male from a laboratory of Plant breeding, Brassica group, Wageningen University. VT-115 is vegetable turnip which originated from Japan and RC-144 is rapid cycling which originated from the USA. Between these accessions, the flowering time is different (discussion with Ningwen) which RC-144 line has earlier flowering time than VT-115 (Zhao *et al.*, 2005).

This population (36RIL) is flowering time between 30 and 78 days after sowing which it gives very early turnips formation. During F2 QTLs detection, it showed that all markers the average turnip size of plants with the "a" genotype (VT-115) was always bigger than the group with the "h" genotype, "h" heterozygous progenies have been always bigger than the "b" genotype (PC-144) (Vos, 2009). So, this indicates the co-dominance of turnip tuber related genes.

2.2. DNA extraction

The samples of 150 populations RIL36 F6/7 were collected from greenhouse grown condition. The protocol for DNA isolation (Gert van Arkel & Maarten Nijenhuis) was adapted from laboratory of plant breeding, Wageningen University. The leaf samples already were collected and put in the 96 micronic tubes with 2 steel balls in a blue holder in liquid nitrogen. Two hundred ml of isolation buffer was prepared with 4mg/ml of RNase. The isolation buffer was put in water bath with 65^{O}C until used. The samples were fixed in the RETCH machine with the blue holder without the transparent lid into a black and white adapter. The samples Milled for 30 seconds at 30 Hz for three times with the changing of the direction of the holders. After 1 ½ minute the entire blue holder with the tubes was back into liquid nitrogen. The boxes were Centrifuge briefly in order to get the powder in the bottom of the tube. The isolation buffer (400 µl) was added to each the tube. For safety purpose each chemical added in the fumehood. The blue holder with samples and without the transparent lid placed in the specially designed clamp and tight the 4 nuts (This will prevent that the lids not pop off after incubation at 65°C) for incubate at 65°C in a water bath for 60 minutes. The clamp containing the tubes cooled down for 15 minutes on ice-water. Four hundred µl of chloroform: isoamyalcohol (24: 1) was added to the tubes and mixed by inversion for 5 minutes by machine. The phases separated by centrifuging at 4600 rpm for 40 minutes. The water phases were Pipettes

2X175µl in to the new tubes and 280 µl isopropanol was added. It had mixed carefully by the inversion blue holder with tubes. The DNA pelleted by centrifuging for 25 minutes at 4600 rpm. The supernatant was discarded and 300µl of 70% ethanol was added. The samples again centrifuge for 20 minutes at 4600rpm for pellet the DNA. The supernatant was discarded and the pellet dried overnight in the fumehood. The DNA concentration was checked by NANOdrop. The quality of DNA was also checked on a 1% agarose gel by putting of the 2µl DNA sample, 8µl MilliQ and 2µl of loading buffer; total 12µl.

2.3. LightScanner

The LightScanner machine is high-resolution DNA melting curve (HRM) which provides post-PCR detection of mutations SNPs in genomic DNA. It detects high-density information of the fluorescence as a function of temperature which contain double-strand DNA binding dye LCGreen Plus. LCGreen has the ability to detect the presence of heteroduplexes formed during PCR if the sample is heterozygous for a particular mutation when compared to other DNA dye (e.g. SYBR Green). The main advantage is faster (1.5 hour for PCR and half an hour for measuring and analysis) when compare to Li-Cor procedure (3 hours for PCR and 2 hours Li-Cor) which eliminating the need for DGGE gels. SNP genotype scoring by high-resolution melting analysis is required only PCR, a DNA dye (LCGreen), and melting instrumentation which completed within 5 minutes after completion of PCR. It is simple, rapid, inexpensive and no-need of post PCR reagents. It also detects the SNPs (sequence variations) without the need for dye-labelled probes.

The Lightscanner has specific master mix and PCR program. The master mix was prepared: MillQ water 4.5 µl, reaction buffer for phire 2 µl, dNTP 0.4µl, primer (diluted) forward and reverse 0.5 µl, LC-green 1µl, and Phire enzyme 0.1µl per master mix. Finally, 9 µl of master mix, 1 µl DNA and 20 µl mineral oil were added into PCR plate in black-white with 96-wells for amplification of the DNA. The mineral oil helps to avoid evaporation of the samples during Hi-Res Melting.

Hi-Res Melting is shown a melting curve of the DNA which changes of the shape of the curve within one marker, it is indicating of the presence of sequence variances in the PCR product. The first step in the analysis defined subsets which define by DNA markers. Negative filter also was done to identify any samples that were included on the plate but failed PCR reactions. It helps to avoid the possibility of generating false positive data during the next step, normalization. Normalization is important before doing of analysis of the data. The two regions were selected one before and one after the major transition. The region shouldn't show any melting curves difference after the selected region. It should be shown a straight line out of selected regions. The final step is grouping of the DNA templates according to melting curves. The grouping of genotyping was made to automate

clustering when different curve shapes can be clearly distinguished. However, if confusing curve shapes happen, the automated clustering changed to manually select samples that share similar curve shapes based on difference temperature of melting peaks .

2.4. Pre-Screening markers with polymorphism

Pre-screening of the markers was made by LightScanner; testing the polymorphism of the markers. Before starting genotype scoring of the whole RIL36 $F_{6/7}$ population, pre-screening of the polymorphic marker test was made on 6 progenies with parents (VT-115 X PC-144). A total of 137 markers were pre-screened. Those markers were developed Dong Xiao to detect flower related QTL on DH-68 population of *Brassica rapa subspp rapa* . Simultaneously, genotype scoring of those of 6 progenies was done when they were shown a polymorphic for specific markers.

2.5. Markers and Genotyping

Flowering traits

A total of 137 markers were selected by Xiao Dong for his study on flowering traits of F1 double haplod from crossing yellow Sarson (YS-143) and Pak Choi (PC-175) population(Xiao *et al.*, Unpublished). He develop the markers based on *arabidopsis* genes which are involved in flowering time control. He also BLAST the Arabidopsis those genes sequences in Chinese cabbage Chiifu genome sequence; Brassica genome database; to get paralogues in Brassica rapa. I screened those 137 markers on 144 RILs C36 populations.

InDels and SNPs

Genome-wide *Brassica rapa* short insertion/deletion length polymorphisms (InDels) (<5 bp) were detected by comparing whole genome re-sequencing data from two *B. rapa* accessions, L144 and Z16, to the reference genome sequence of Chiifu-401-42. Previously tested 14 InDel polymorphisms (4~5 bp in length) between L144 and Z16 also showed polymorphisms between VT-115 and RC-144, and therefore included in the map construction of this study. SNPs markers were generated from re-sequencing data which compared five re-sequenced genomes (Chiifu, DH-VT-117, RC144, DH-YS143 and a Wutaicai DH line) and detected SNPs between each of the two genomes. The genotyping using these SNP markers was done through KARSPar SNP genotyping system from Kbioscience (Hoddesdon, Hertfordshire, UK). Genotyping data of the same RIL population with 51 SNPs that randomly distributed in *B. rapa* genome was included in the map construction in this study as well.

2.6. Linkage map construction

For construction of a genetic linkage map a total of 124 markers (SNPs & InDels) in 106 F6/7 population were used. Before linkage analysis, chi-square tests (X^2) were performed on both all markers for goodness of fit to the expected Mendelian 1:1 segregation ratio of each locus. Linkage analysis was performed with the Joinmap 4.1 and the Kosambi mapping function (Manly *et al.*, 2001) was used to convert recombination frequencies into map distances (cM). Initially, the maximum of 0.20 recombination frequency was used to identify linkage groups. Linkage maps were drawn using MapChart software (Voorrips, 2002). A framework physical map of FT markers was previously constructed by Xiao dong, which corresponds to the linkage groups of Brassica genome. The 51 SNPs and 14 InDels markers also incorporated into the frame based on their physical position in the reference genome of Chiifu-402 retrieved from BRAD database.

2.7. Phenotype and QTLs mapping

Seeds were sown on 30 July 2012 in greenhouse and plants were transplanted into field on 21 August 2012. For each line, three blocks with five plants in each block according to a complete randomized block design. Harvesting took place in three patches according to their flowering time. Because turnip growth turns slower after flowering and it might not be fair to harvest plants all in once. So harvest made in three patches to allow different lines reaching the maximal size while not losing much of fresh weight and not differ too much for other metabolic changes. First harvest was on 1-oct 2012, second time was on 12-oct and the last harvest was on 31-oct in the same year. The flowering turnip only harvested the first two harvests. The lastly, all leftover plants harvested, including the ones that were not flowering yet. QTL map was made, nonparametric mapping method, it uses the rank sum test of Kruskal- wallis MapQTL6 mapping statically software (Ooien *et al.*, 1996) with 0.1 a significant level of ≤0.1. Four turnip tuber traits were used for QTLs.

3. Results and discussion

3.1. Pre-Screening of marker with polymorphism and Genotyping

Construction of a genetic linkage map using a bi-parental segregating population needs to develop many molecular markers that show polymorphism between the parents as well as in the progeny. In laboratory of the plant breeding WUR, Dong Xiao has developed and mapped 137 flowering related markers using a doubled haploid population DH68 which was derived from a cross between YS-143 (oil type) and PC-175 (leafy type). Those 137 markers were developed from *B. rapa* sequences with high homolog (>75% nucleotide sequence identity) to flowering time related genes in *Arabidopsis thaliana (Xiao et al., Unpublished)*. Polymorphism test was made on those 137 flowering markers on 6lines from F6/7 RIL36 population. The DNA of the real parents was missing. The DNA of a plant from another accession of the parents was used as references which the parents consider as genetic similarity. These parents are representative but not really identical with the real parent genotypes. Therefore, selection of markers was made based on screening of two parents representatives and six RIL progenies. As a result, 59 markers out of 137 showed polymorphism in the RIL population of a cross between VT-115 and RC-144 (Appendix 1). The polymorphism was identified when two groups were formed on those 6 progenies and parents. For instance, BrSPA1P2a was one of marker showed polymorphic between F6/7 progenies (Figure 2).

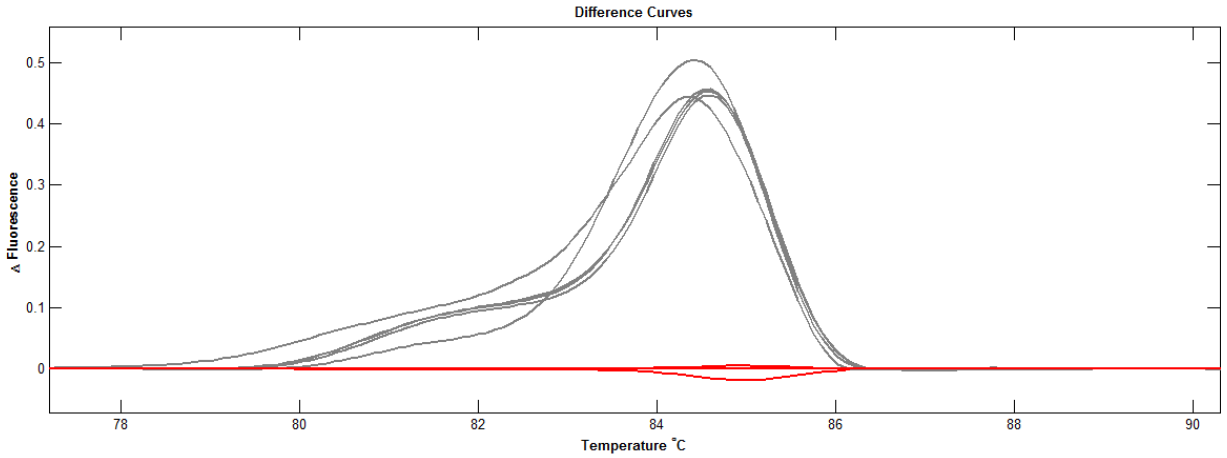


Figure 2. pre-screening of BrSPA1P2a marker which shows a polymorphic within 6 progenies and parents(brown from VT-115 parent and red from RC-144parent).

Genotyping of the polymorphic markers during pre-screen as well as on all RIL population was made. However, the genotyping was divided into three parts: easy, medium and too difficult to genotype scoring (Figure 3). The easy and too difficult one was rear but most of the markers were medium; there were manual scoring of the genotype. The lightScaner give the scoring directly for the easiest markers. The lightScaner sometimes gives different colour but they look like belong to one group (Figure 3B). Figure 3B shows that BrPHYAP1g markers had 3 colours. The green colour was belong to red colour. This type of marker/s made some correction manually such as making green colour belong to red colour. Some markers also showed polymorphic but not easy to score e.g. BrRGAP2c (F9gure 3C).

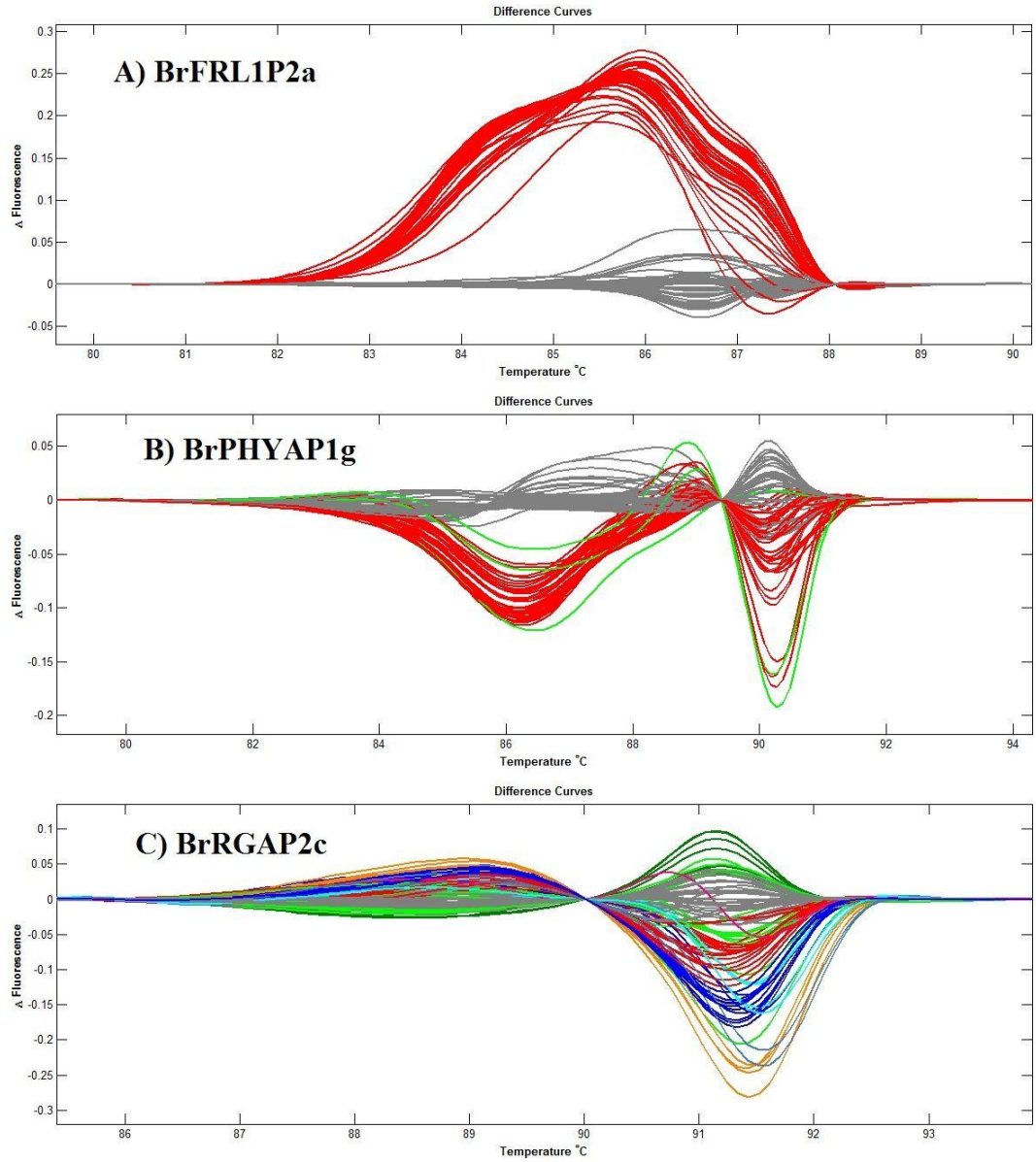


Figure 3. genotyping style A) easy, B) medium and C) difficult to make genotype scoring.

3.2. Linkage map construction

A genetic linkage map was constructed based on 124 markers in 36RILs populations of 106 F6/7 progeny lines. In total, 79 markers (60% of total markers) were scored as 1:1 ratio (5 to 2% heterozygous loci) with equal contribution from both parents. A total of 11 InDels and 34 SNPs showed significant distortion from the expected Mendelian gene frequencies. The chi-square tests confirmed that on those 11 InDels and 34 SNPs markers the frequencies of individual parental alleles in the F6/7 population indicated a significant deviation ($P \leq 0.05$) from the expected 1:1 ratio. Theoretically, F6 population is considered a RIL and presents an exception of 1:1 ratio for allele segregation among individuals with 3% of individuals should show as heterozygous. As a result, 9 InDels and 17 SNPs markers were distributed to the skewed segregation with highly significance at P value 0.0001. In most of *Brassica rapa* linkage maps skewed segregation of markers is a common feature (Choi *et al.*, 2007; Xinhua, 2011; Kapoor *et al.*, 2009). Some researchers were reported that might be due to loss of chromosomes, the presence gene conversion event and homologous recombination.

The markers which showed distorted segregation were distributed randomly along all the linkage groups. In total 61 markers were grouped into 13 linkage groups, with the number of markers per linkage group ranged from 2 (linkage 1,2 and 10) to 12 (linkage 9), and covered a total of 639.5 cM of the genome (Figure 3). This might be due to insufficient linkages and lack of polymorphism spanning those regions. Lower number of individuals showed higher stress values and higher distance variances as well as wide deviation from estimates of the genome size(Ferreira *et al.*, 2006).The thirteen linkage groups had the average interval distance of 10.5 cM. The number of markers which used for mapping per linkage ranged from 6 (linkage 4) to 24 (linkage 9), with an average of 11 markers per linkage group. On linkage L02_1, L02_2 and 10 have been shown only 2 markers were found per linkage group. The length of linkage groups ranged from 1.0 cM for group L01_1 to 184 cM for group L09_1. Markers BrPHYAP1g, BrVRN1P1b and BrRGAP2c showed polymorphism in the population, but were excluded during mapping analysis as the scoring of light scanner output was too difficult and not reliable.

All linkage groups contained region which the adjacent marker larger than 15 cM except in linkage groups L01_1, L06, L09_2and L10 (Table 1). The linkage groups placement and order for 124 of the SNPs and InDels markers were in good agreement with *Brassica rapa* flowering markers map (Xiao dong, DH68). However, markers PV_Br_SNP_0125 were placed on linkage group L07, while it was predicted to locate on A02 in the reference Chiifu genome. It might be many reasons, such as

small population size, lack of recombination between the limited number of markers, true inversion or translocation of between genotypes.

Two linkage group was observed in linkage 1 and 9. However, the first group was only contained two markers in linkage group L01. On linkage group 2 also showed two linkage groups with only two markers per linkage group. On linkage groups 3, 4, 6, 8 and 10 were from only SNPs marker.

The two linkage groups on L09 the placement and order were agreed with the physical map of *Brassica rapa* physical map which developed from chiifu genome except loci BrSVPP1c and PV_Br_SNP_0796 (Figure 3).

Table 1. Summary of a genetic linkage map construction on *Brassica rapa* using SNPs and InDels markers.

Linkage group	Number of markers assigned	Number of interval[a]	Number of gaps[b]	Length of linkage groups(cM)
L01_1	2	0	0	1.0
L01_2	6	6	2	65.3
L02_1	2	2	2	33.5
L02_2	2	2	2	24.8
L03	3	3	3	50.3
L04	3	3	3	63.8
L05	6	6	2	43.4
L06	4	4	0	23.0
L07	6	6	2	56.0
L08	7	7	1	41.8
L09_1	12	12	7	183.8
L09_2	6	6	0	39.2
L10	2	2	0	12.4

[a]Adjacent markers > 1cM [b]Distance between adjacent markers ≥ 15cM (Choi *et al.*, 2007; Kapoor *et al.*, 2009b; Wang *et al.*, 2011)

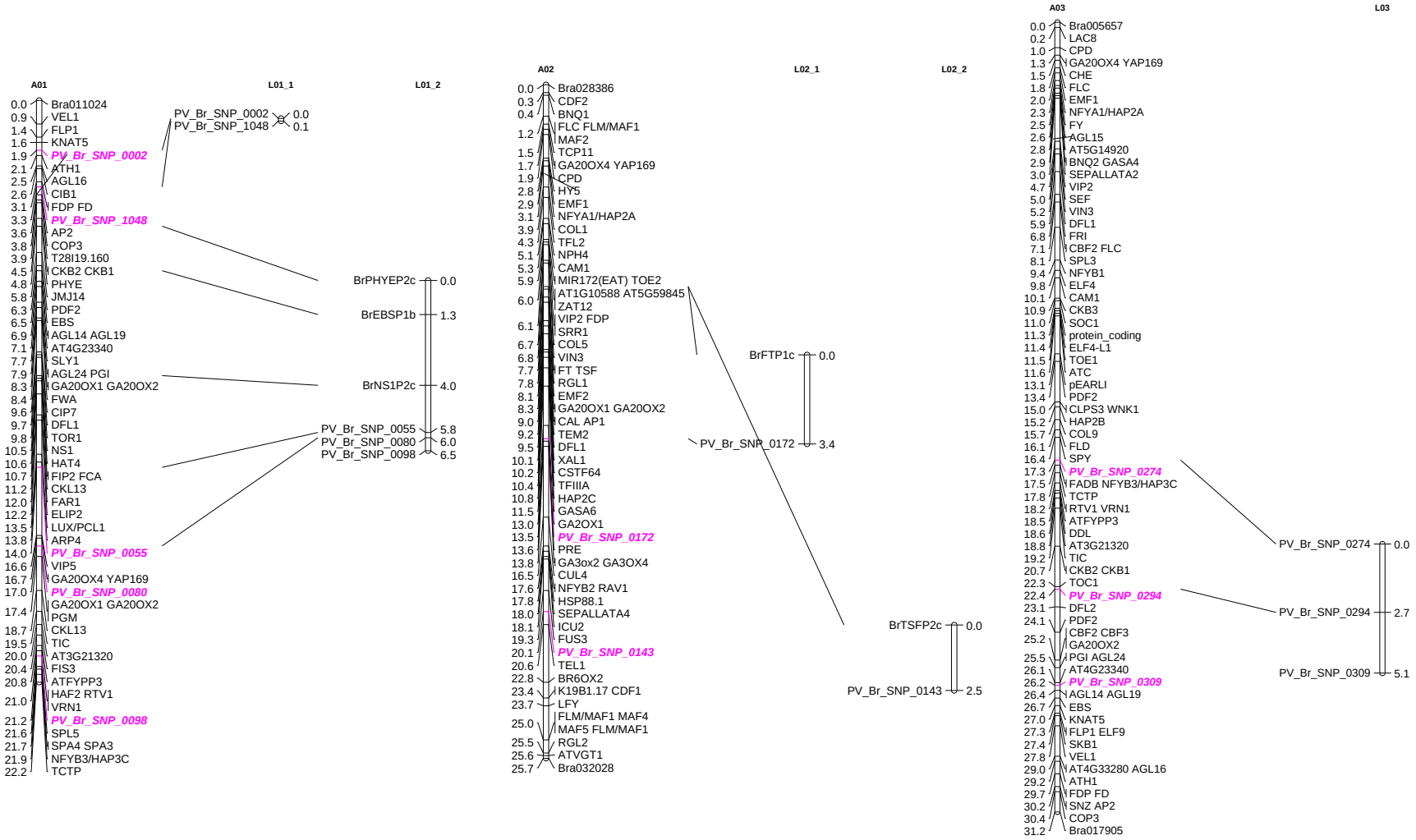
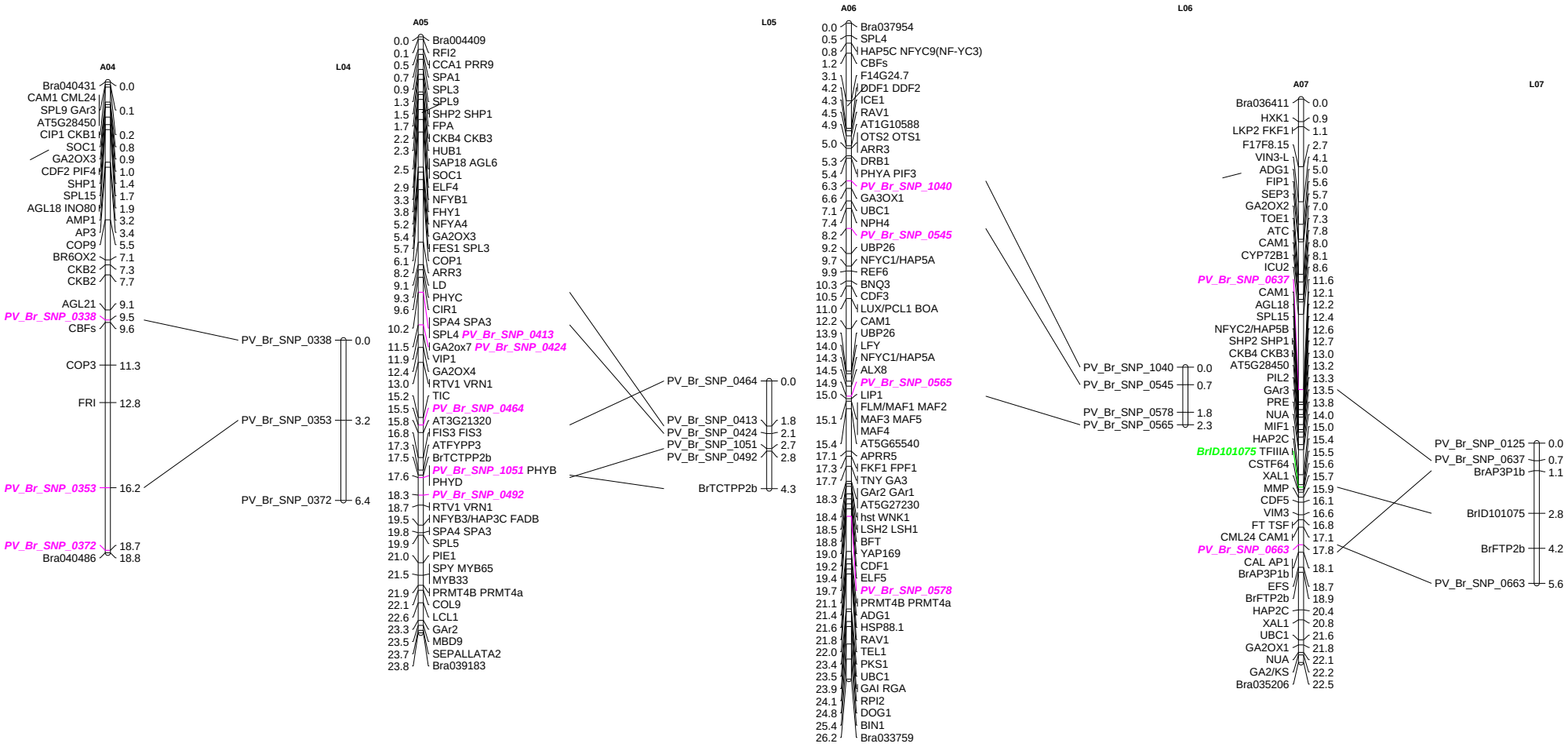
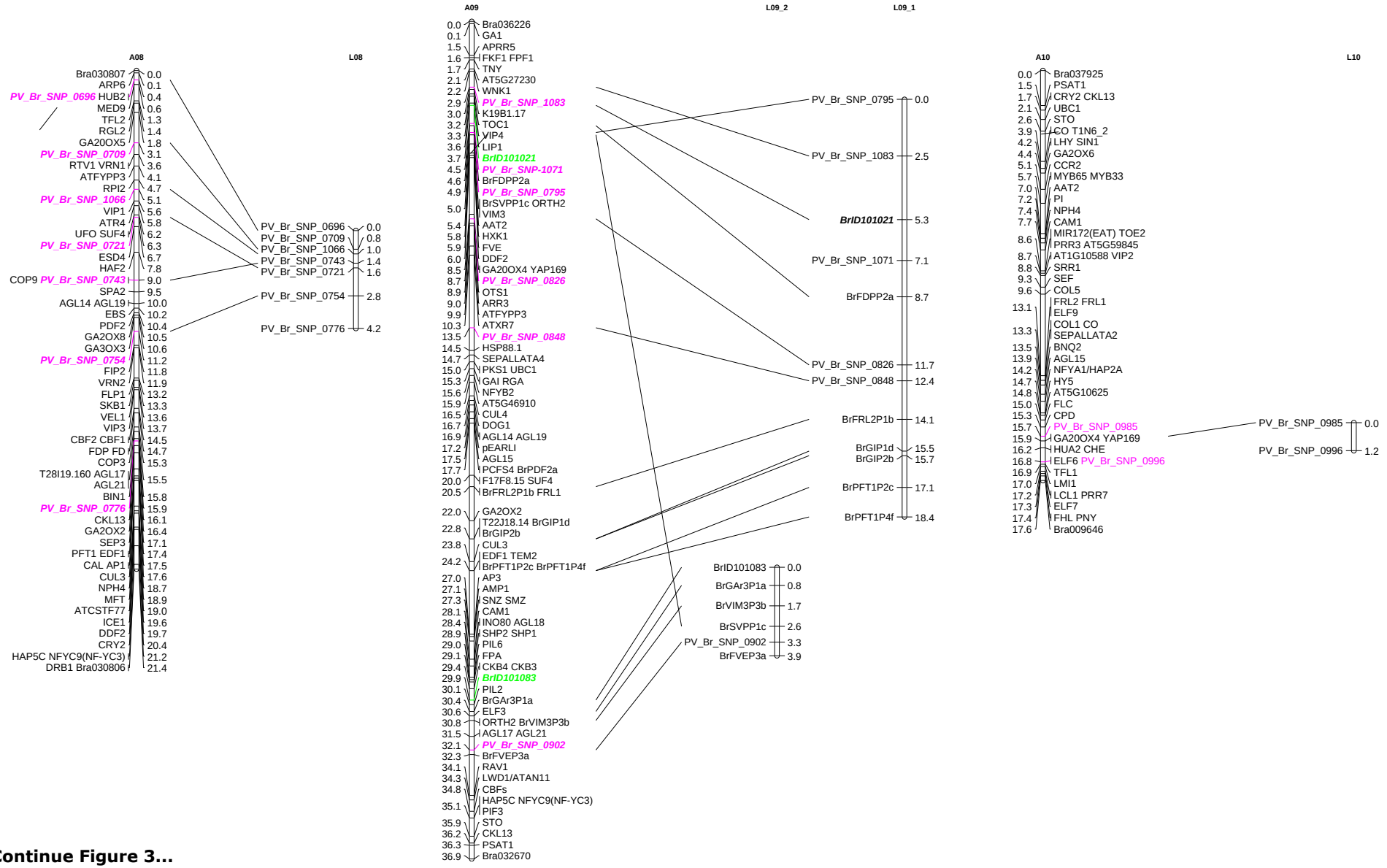


Figure 3. Comparing of newly constructed linkage groups (right side(Lx)) to *Brassica rapa* physical map(left side(Ax)); The length of each genetic linkage groups are indicated in centimorgans (cM) and the length for the physical map is in million base pairs (Mbp, A01-A10) .The cM of LX linkage groups was 0.1cM instead of 1CM to make it easy; because the physical map was constructed based on Mbp with maximum 50. The genetic map was greater than 50cM. The colour markers indicate the newly SNPs(rose) and InDels (green) markers.



Continuo Figure 3. Comparing newly constructed linkage groups (right side(Lx)) to Brassica rapa physical map(left side(Ax)); The length of each genetic linkage groups are indicated in centimorgans (cM) and the length for the physical map is in million base pairs (Mbp, A01-A10) .The cM of LX linkage groups was 0.1cM instead of 1CM to make it easy; because the physical map was constructed based on Mbp with maximum 50. The genetic map was greater than 50cM. The colour markers indicate the newly SNPs(rose) and InDels(green) markers.



Continue Figure 3...

3.3. Frequency distribution of turnip tuber traits

Average turnip tuber fresh weight, dry weight, dry mass and brix (total soluble sugar) were showed no normal distribution, the skewedness was shifted to positive (Figure 4). A distribution has values of skew which the results were above 0 . This indicates a deviation from normal distributions. The mean result of turnip tuber fresh weight, dry weight and dry mass were indicated most of progenies (population F6/7) shifted to VT-115 parent (Appendix 4).

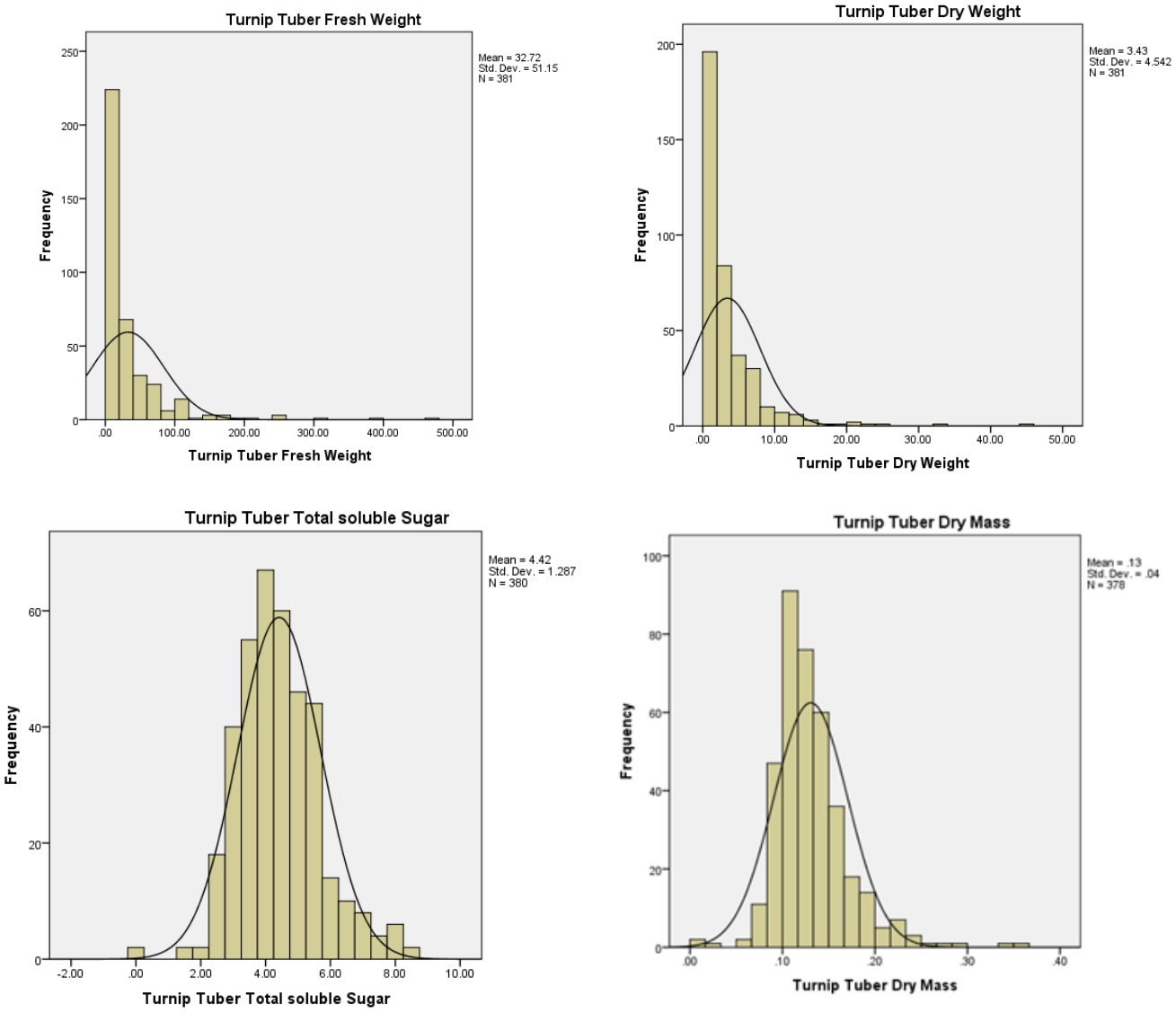


Figure 4. Frequency distribution of turnip tuber morphological traits in 36RIL F6/7 population.

3.4. QTL analysis

Four turnip tube related traits were evaluated; average turnip tuber fresh weight, dry weight, dry mass and brix (Total soluble Sugar). Non linkage group also included in QTLs detection. Some markers were unlinked but they have contribution for QTLs (Figure 5). A total of 94 turnip tuber related traits QTLs were detected at significant level ≤0.1 on 13 newly constructed *brassica rapa* linkage groups except linkage group L01_1 and L04 (Figure 5). As a result, 18 Brix (total soluble sugar) related QTL was detected on all newly constructed linkage groups except L02_1, L02_2 and L10. Average turnip tuber fresh weight related QTLs(29) were also detected on all linkage group except L03. Twenty six turnip tuber dry weight and 21 turnip tuber dry mass percentage QTLs were also detected on all newly constructed linkage groups except L08. The interesting thing was in linkage group 2, turnip tuber dry weight and fresh weight co-located together which all were affected by BrFT loci. Turnip related traits QTLs were detected on the top of A02 in DH-30 and the BC1 population which were co-located with flowering QTL ((Lou *et al.*, 2007)). In F2 segregation population study, it showed turnip width and flowering markers correlated each other on linkage groups of A02 and A10 (Vos, 2009). Average turnip tuber fresh weight, dry weight and dry mass were co-located on linkage group L02, L05, L06, L07, L09 and L10. F2 population study on linkage group L02 didn't show any turnip related traits QTLS but in this study some QTLs were detected. Only linkage groups A01, A05 A06 A08 and A10 were detected turnip related QTLs F2/3 of this population (RIL 36C) (Rémi, 2010). The explained variation per QTL was less than 16.5%.

I was working major thesis on turnip tuber traits genes expression *in vitro* condition. I have found some genes which differentially expressed related to turnip tuber formation. For instance, DDF1(delay and dwarf flowering 1), VTuni_02 and VTuni_14 (unique for DHVT-117 turnip) genes were differentially expressed in turnip tuber induced medium. The physical position of DDF1 and VTuni_02 is A09 at 34.51Mbp and 36.99Mbp, respectively on Chiifu genome. The newly constructed L09_2 linkage map had 14 turnip tuber related QTLs. The mega base pair size was not far from DDF1 and VTuni_02 genes. If those genes included in the linkage map, they might be contribute in turnip tuber QTLs.

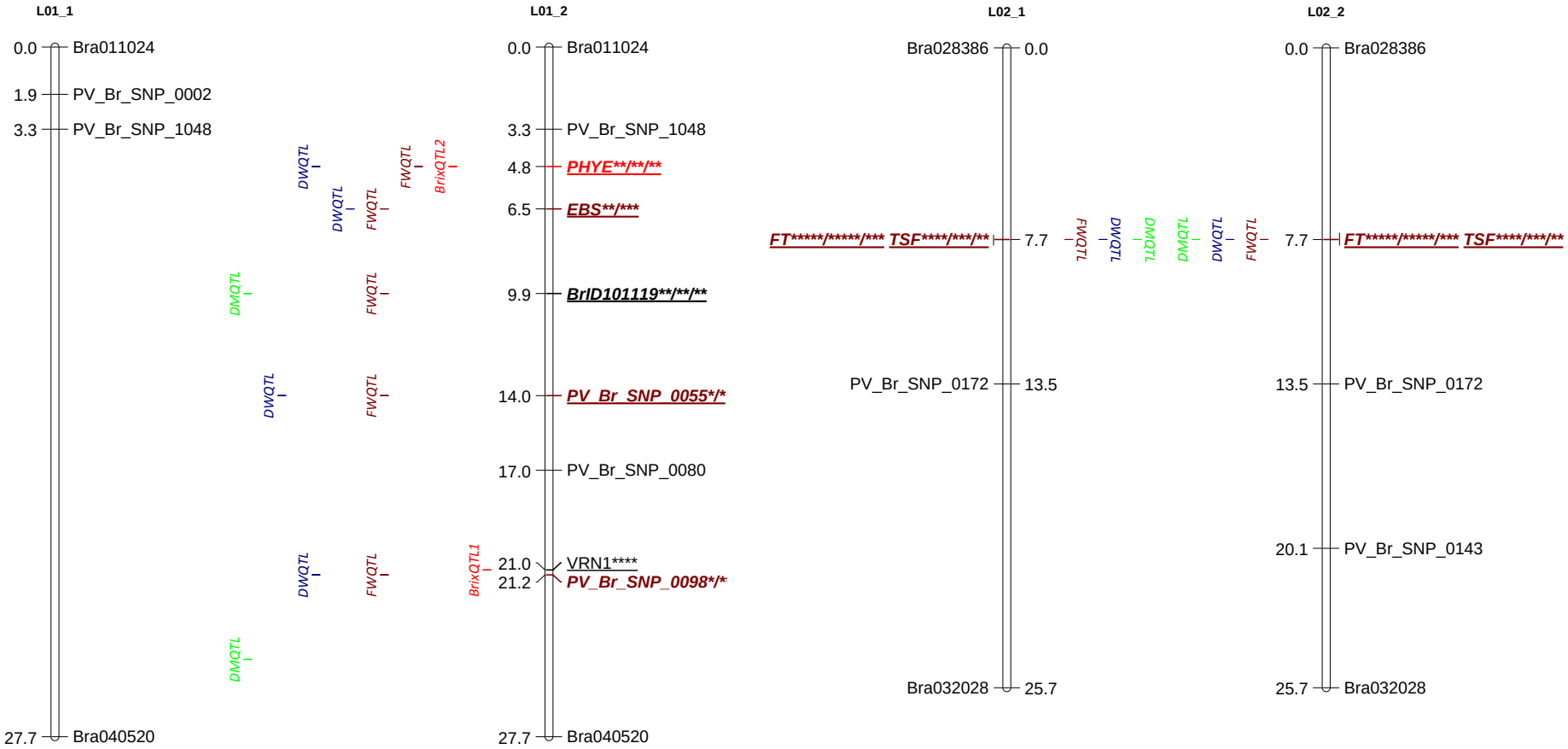
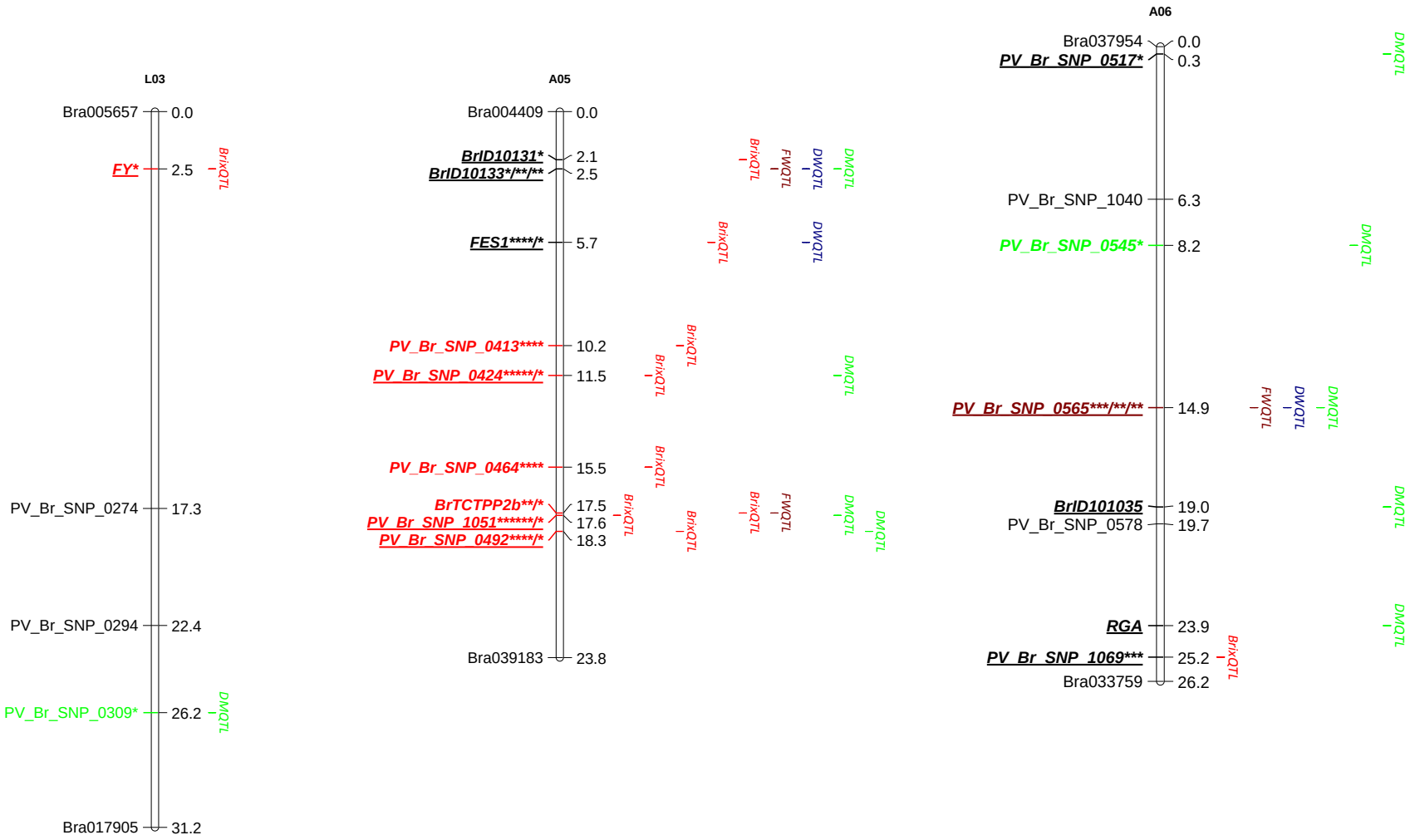
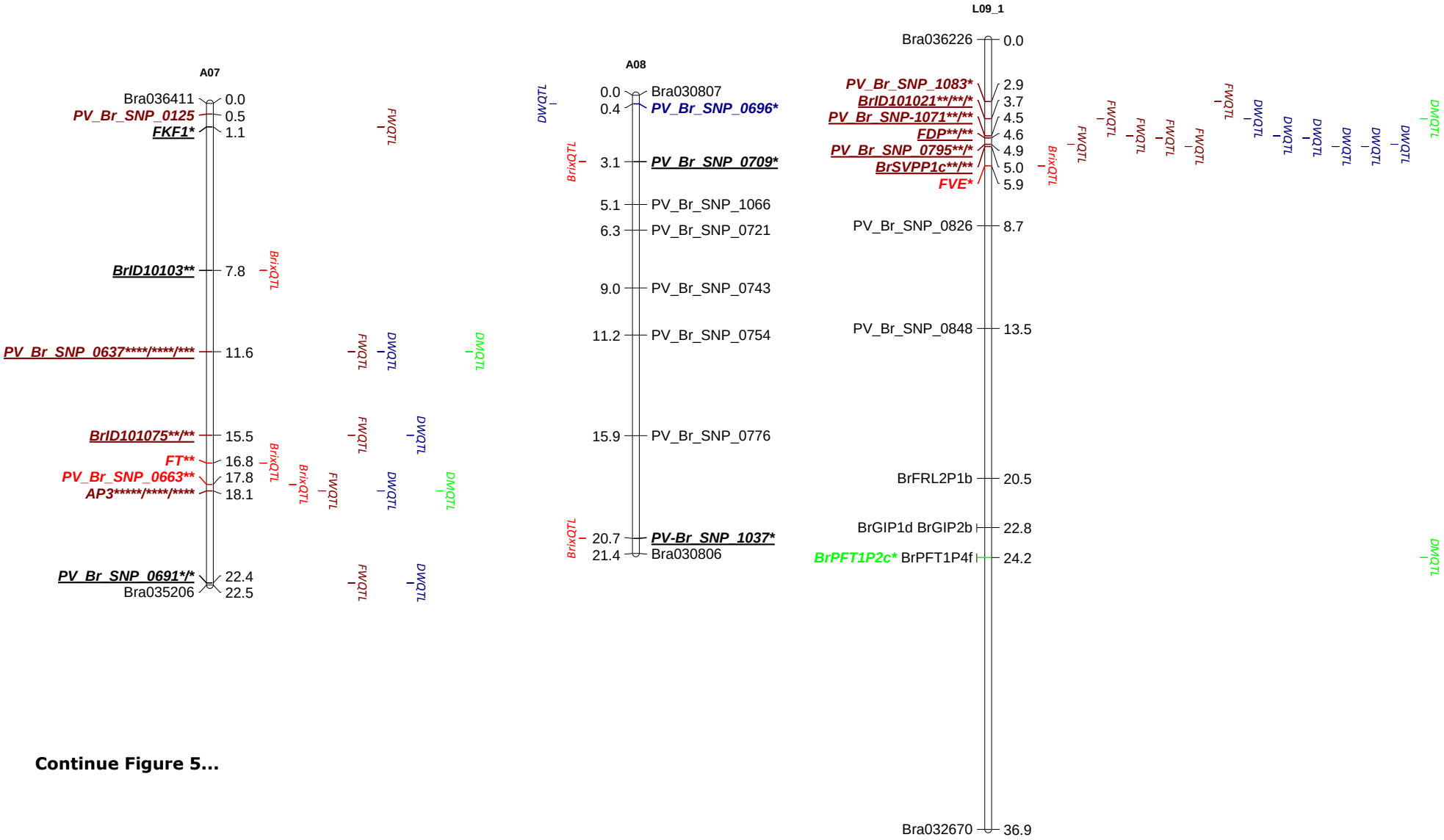


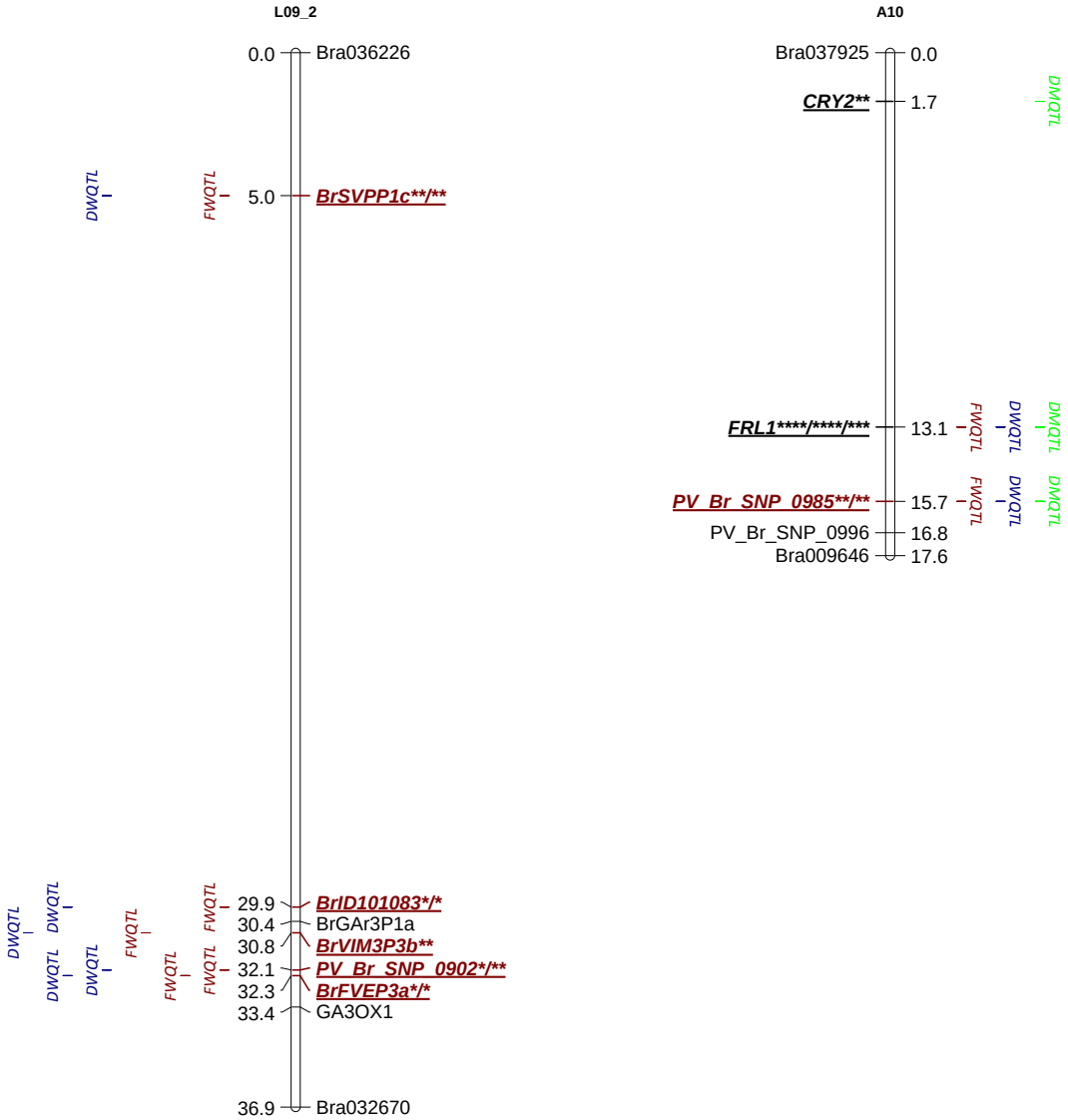
Figure 5. Turnip tuber related QTLs on *Brassica rapa* linkage groups on flower time related markers of physical map including SNPs and InDels markers . The coloured loci shows significantly(<0.001) for correspondence QTL; red colour markers for brix QTLs, brown colour for fresh weight QTLS, blue for dry weight and green for dry mass percentage; stars with markers indicates the significant different(*=0.1, **=0.05, ***=0.01, ****=0.005, *****=0.001 and ******= 0.0005, *******=0.0001) from hypothesis(no QTLs for linkage group); colour and underline indicates the markers contribute for more than one QTLs; black colour and underline indicated the markers which didn't assigned any linkage group but it contribute for correspondence QTL.



Continue Figure 5. ...



Continue Figure 5...



Continue Figure 5...

4. Conclusion and recommendation

A genetic linkage map provides the basic and essential information to study the *B. rapa* genome and to conduct marker-assisted selection in genetics and breeding. Genotype scoring was not easy for some of the markers which excluded during mapping. I was able to locate more than ten linkage groups to ten corresponding *Brassica rapa* linkage groups. The size of population were reduced from 144 to 106 to make correlation with different markers. Size and number of marker can affect linkage group and QTLs analysis. If there are more markers included, those loci might be linked. After running the JoinMap program for each group, a linkage map on each linkage group was determined based on recombination frequency.

A total 61 markers were grouped into 13 newly constructed linkage groups. The orders and orientations of the markers on each 13 newly constructed linkage groups were the same as physical the map map of *Brassica rapa* from chiifu genome except BrTSFp2c (A02), Pv_Br_SNP_0464 (A05), PV_Br_0578 (A06), BrID101075 (A07), PV_Br_SNP_0663 (A07), PV_Br_SNP_0743 (A08), PV_Br_SNP_0795(A09) and BrSVPP1c (A09). Of the 124 markers assigned to the linkage groups, 49 showed a deviation from the expected segregation ratios 1:1. This might be due to common feature markers skewed segregation *Brassica* linkage maps. Generally, 90% of loci had homozygous genotypes, 4% heterozygous and 6% missing values during scoring or unamplified genotypes. I also observed the differential genes expressed in tuber formation and physical position of those gene to related to the newly constructed QTLs. It showed some of genes (e.g. VTuni_02) were not far from in L09 linkage groups. This supported turnip tuber traits are polygenic. A total of 94 turnip related QTLs were detected in all linkage groups; 18 for brix(Total soluble Sugar), 29 for turnip tuber fresh weight, 26 for turnip tuber dry weight and 21 for tuber dry mass percentage. Dry weight and fresh weight co-located together on linkage group A02 at flowering QTL loci. The linkage map reported here is therefore help as a resource in undertaking future linkage mapping on C36 RIL population. Finally, I would recommend to make linkage and QTLs mapping with higher number of individuals and markers. It gives accurate and precise linkage groups and QTLs maps.

References

Abelenda, J. A., C. Navarro and S. Prat (2011). "From the model to the crop: genes controlling tuber formation in potato." Current Opinion in Biotechnology **22**(2): 287-292.

Al-Shehbaz, I., M. Beilstein and E. Kellogg (2006). "Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview." Plant Systematics and Evolution **259**(2): 89-120.

Campo, C. G.-. (1999). Biology of Brassica Coenospecies. Madrid, ELSEVIER SCIENCE B.V.

Choi, S. R., G. R. Teakle, P. Plaha, J. H. Kim, C. J. Allender, E. Beynon, Z. Y. Piao, P. Soengas, T. H. Han and G. J. King (2007). "The reference genetic linkage map for the multinational Brassica rapa genome sequencing project." TAG Theoretical and Applied Genetics **115**(6): 777-792.

Collard, B. C. Y., M. Z. Z. Jahufer, J. Brouwer and E. C. K. Pang (2005). "An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts." Euphytica **142**(1): 169-196.

El-Assal, S. E. D., C. Alonso-Blanco, A. J. M. Peeters, V. Raz and M. Koornneef (2001). "A QTL for flowering time in Arabidopsis reveals a novel allele of CRY2." Nature genetics **29**(4): 435-440.

Ferreira, A., M. F. da Silva, L. C. Silva and C. D. Cruz (2006). "Estimating the effects of population size and type on the accuracy of genetic maps." Genet Mol Biol **29**(1): 187-192.

He, H., L. Ping, G. Bonnema, M. Dekker and R. Verkerk (2012). "Genetic Variation in Glucosinolate Content within Brassica rapa Vegetables." Acta Hort.: 944.

Kapoor, R., S. S. Banga and S. K. Banga (2009a). "A microsatellite (SSR) based linkage map of Brassica rapa." New Biotechnology **26**(5): 239-243.

Kapoor, R., S. S. Banga and S. K. Banga (2009b). "A microsatellite (SSR) based linkage map of< i> Brassica rapa</i>." New biotechnology **26**(5): 239-243.

Kushad, M. M., A. F. Brown, A. C. Kurilich, J. A. Juvik, B. P. Klein, M. A. Wallig and E. H. Jeffery (1999). "Variation of Glucosinolates in Vegetable Crops of Brassica o leracea." Journal of Agricultural and Food Chemistry **47**(4): 1541-1548.

Lou, P., J. Zhao, J. S. Kim, S. Shen, D. P. Del Carpio, X. Song, M. Jin, D. Vreugdenhil, X. Wang and M. Koornneef (2007). "Quantitative trait loci for flowering time and morphological traits in multiple populations of Brassica rapa." Journal of Experimental Botany **58**(14): 4005-4016.

Manly, K. F., J. Cudmore, R.H. and J. M. Meer (2001). "Map Manager QTX, cross-platform software for genetic mapping." Mammalian Genome **12**(12): 930-932.

Nagaharu, U. (1935). "Genome analysis in Brassica with special reference to the experimental formation of B. napus and peculiar mode of fertilization." Jap J Bot **7**: 389-452.

Ooien, J. W., M. Boer, R. Jansen and C. Maliepaard (1996). "MapQTL 4.0. Software for the calculation Of QTL positions on genetic maps." Order **501**: 6204.

Ostergaard, L. and G. J. King (2008). "Standardized gene nomenclature for the Brassica genus." Plant Methods **4**(10): 1-4.

Rémi, R. (2010). Mapping QTL for turnip formation and other morphological traits in Brassica rapa. Labaratory of Plant Breeding, Wageningen University. **MSc**.

Rosa, E., R. Heaney, G. Fenwick and C. Portas (1997). "Glucosinolates in crop plants." Horticultural reviews **19**: 99-215.

Schranz, M. E., P. Quijada, S. B. Sung, L. Lukens, R. Amasino and T. C. Osborn (2002). "Characterization and effects of the replicated flowering time gene FLC in Brassica rapa." Genetics **162**(3): 1457-1468.

Song, K., M. Slocum and T. Osborn (1995). "Molecular marker analysis of genes controlling morphological variation in Brassica rapa (syn. campestris)." TAG Theoretical and Applied Genetics **90**(1): 1-10.

USDA (2011). Potato, Flesh and Skin, Raw, National Nutrient Database.

Visser, J. d. (2010). Effect of vernalization on turnip formation and other morphological traits in Brassica rapa. Laboratory of Plant Breeding

Wageningen, Wageningen University. **MSc**.

Vogl-Lukasser, B., C. R. Vogl and H. Reiner (2008). "The turnip (Brassica rapa L. subsp. rapa) in Eastern Tyrol (Lienz district; Austria)." Ethnobotany Research & Applications **5**(0): 305-317.

Voorrips, R. (2002). "MapChart: software for the graphical presentation of linkage maps and QTLs." Journal of Heredity **93**(1): 77-78.

Vos, P. (2009a). Characterization of Brassica rapa turnip formation in multiple segregating populations. Laboratory of Plant Breeding,, Wageningen University. **Msc**.

Vos, P. (2009b). Characterization of Brassica rapa turnip formation

in multiple segregating populations. Laboratory of Plant Breeding. Wageningen, Wageningen University. **MSc**.

Wang, S. (2011). Assessment of phenotypic variation in an F3 population from a cross between a Japanese turnip and a pak-choi, and association to the F2 marker profiles. Laboratory of Plant Breeding, Wageningen University and Research Centre. Wageningen, Wageningen University. **MSc**.

Wang, Y., S. Sun, B. Liu, H. Wang, J. Deng, Y. Liao, Q. Wang, F. Cheng, X. Wang and J. Wu (2011). "A sequence-based genetic linkage map as a reference for Brassica rapa pseudochromosome assembly." BMC genomics **12**(1): 239.

Xiao, D., J. Zhao, R. K. Basnet, N. Zhang, D. P. d. Carpio, J. B. K. Lin, F. Cheng, X. Hou, X. Wang and G. Bonnema (Unpublished). "Genetic architecture of flowering time regulation in a *Brassica rapa* doubled haploid population."

Yildirim, E., N. Yildirim, S. Ercisli, G. Agar and H. Karlidag (2010). "Genetic relationships among turnip (Brassica rapa var. rapa) genotypes." Genetics and Molecular Research **9**(2): 987-993.

Zhao, J., A. Artemyeva, D. P. Del Carpio, R. K. Basnet, N. Zhang, J. Gao, F. Li, J. Bucher, X. Wang and R. G. F. Visser (2010). "Design of a Brassica rapa core collection for association mapping studies." Genome **53**(11): 884-898.

Zhao, J., X. Wang, B. Deng, P. Lou, J. Wu, R. Sun, Z. Xu, J. Vromans, M. Koornneef and G. Bonnema (2005). "Genetic relationships within Brassica rapa as inferred from AFLP fingerprints." TAG Theoretical and Applied Genetics **110**(7): 1301-1314.

Appendixes

Appendix 1. SNPs FT markers;

Abbreviation	Markers	Polymorphism in RIL36 (VT-115 X PC-144)	estimated position	DH-68 mapped position	C36 mapped position*	Annotation
AGL15	BrAGL15P2a	No	unassigned	A02	unassigned	AGL15 (AGAMOUS-Like 15) is a member of the MADS domain family of regulatory factors.
AGL24	BrAGL24P1b	No	A03	A03	unassigned	MADS-box protein
AGL24	BrAGL24P2c	No	unassigned	A03	unassigned	MADS-box protein
AP2	BrAP2P1d	No	A01	A01	unassigned	Encodes a floral homeotic gene
AP2	BrAP2P2a	No	unassigned	A03	unassigned	Encodes a floral homeotic gene
AP3	BrAP3P1b	Yes	A07	A07	A07	Floral homeotic gene encoding a MADS domain protein
APRR5	BrAPRR5P1a	No	A09	A09	unassigned	Encodes a pseudo-response regulator whose mutation affects various circadian-associated biological events
APRR5	BrAPRR5P1b	No	A09		unassigned	Encodes a pseudo-response regulator whose mutation affects various circadian-associated biological events
AS1	BrAS1P1c	Yes	A05	A03	ungrouped	Encodes a MYB-domain protein involved in specification of the leaf proximodistal axis
AS1	BrAS1P2a	Yes	unassigned	A03	ungrouped	Encodes a MYB-domain protein involved in specification of the leaf proximodistal axis
AT1G18450	Br450P1a	No	A06	A06	unassigned	Encodes a gene similar to actin-related proteins in other organisms
AtBRM	BrAtBRMP1d	No	A03	A03	unassigned	Encodes a SWI/SNF chromatin remodeling ATPase
ATVGT1	BrATVGT1P1a	No	A03	A01	unassigned	Encodes a vacuolar membrane-localized glucose transporter
BFT	BrBFTP1a	No	unassigned	A02	unassigned	brother of FT and TFL1 protein (BFT
BIN1	BrBIN1P2c	Yes	A08	A08	ungrouped	Encodes a plasma membrane localized leucine-rich repeat

						receptor kinase
CAL	BrCALP1g	No	A07	A02	unassigned	encoding a MADS domain protein homologous to AP1
CAL	BrCALP2a	Yes	unassigned	A02	ungrouped	encoding a MADS domain protein homologous to AP1
CBFs	BrCBFsP2b	No	unassigned	A06	unassigned	transcription factor activity
CCA1	BrCCA1P1c	Yes	A03	A09	ungrouped	Encodes a transcriptional repressor
CCA1	BrCCA1P3b	Yes	unassigned	A05	ungrouped	Encodes a transcriptional repressor
CDF1	BrCDF1P2e	Yes	unassigned	A06	ungrouped	Dof-type zinc finger
CO	BrCOP1f	No	A10	A02	unassigned	Similar to zinc finger
COL1	BrCOL1P1a	No	unassigned		unassigned	Homologous to the flowering-time gene CONSTANS.
COL1	BrCOL1P1c	No	unassigned	A10	unassigned	Homologous to the flowering-time gene CONSTANS.
COL2	BrCOL2P3aF	Yes	unassigned	A01	ungrouped	homologous to the flowering-time gene CONSTANS (CO) encoding zinc-finger proteins
CPD	BrCPDP1a	No	A10	A10	unassigned	Encodes a member of the CP90A family,
CRY1	BrCRY1P2d	No	unassigned	A09	unassigned	Blue-light photoreceptor
CRY1	BrCRY1P1c	No	unassigned	A09	unassigned	Blue-light photoreceptor
CRY2	BrCRY2P1a	Yes	A10	A10	ungrouped	Blue-light photoreceptor
CRY2	BrCRY2P4b	No	unassigned	A04	unassigned	Blue-light photoreceptor
CRY2	BrCRY2P2c	Yes	unassigned	A08	ungrouped	Blue-light photoreceptor
CRY2	BrCRY2P3a	Yes	unassigned	A08	ungrouped	Blue-light photoreceptor
CUL3	BrCUL3P1a	No	A08	A08	unassigned	Cullin, putative, similar to Cullin homolog 3
DDF1	BrDDF1P3a	No	unassigned	A06	unassigned	Encodes a member of the DREB subfamily A-1 of ERF/AP2 transcription factor family (DDF1).
DDF1	BrDDF1P2b	No	unassigned	A09	unassigned	Encodes a member of the DREB subfamily A-1 of ERF/AP2 transcription factor family (DDF1).
DRB1	BrDRB1P1d	Yes	unassigned	A06	ungrouped	Encodes a nuclear dsRNA binding protein delayed flowering
DRL1	BrDRLP3b	Yes	unassigned	A08	ungrouped	Encodes a homolog of the yeast TOT4/KTI12 protein. Yeast TOT4/KTI12
DRL1	BrDRLP1c	No	A09	A09	unassigned	Encodes a homolog of the yeast TOT4/KTI12 protein. Yeast TOT4/KTI12

DRL1	BrDRLP2b	No	A08	A08	unassigned	Encodes a homolog of the yeast TOT4/KTI12 protein. Yeast TOT4/KTI12
EBS	BrEBSP2d	Yes	A08	A02	ungrouped	Putative plant chromatin remodeling factor
EBS	BrEBSP1b	Yes	A01	A01	A01	Putative plant chromatin remodeling factor
EDF1	BrEDF1P2c	No	unassigned	A08	unassigned	Encodes a member of the RAV transcription factor family that contains AP2 and B3 binding domains.
ELF3	BrELF3P1c	No	A09	A09	unassigned	Encodes a novel nuclear protein
ELF5	BrELF5P1a	No	unassigned	A06	unassigned	Nuclear targeted protein
ELF8	BrELF8P1b	No	A03	A03	unassigned	Encodes a yeast CTR9 homolog
ESD4	BrESD4P1a	Yes	A03	A06	ungrouped	encodes a SUMO protease
ESD4	BrESD4P2b	Yes	unassigned	A08	ungrouped	encodes a SUMO protease
FD	BrFDP1g	No	A01	A01	unassigned	bZIP transcription factor
FDP	BrFDPP2a	Yes	unassigned	A09	ungrouped	bZIP transcription factor
FDP	BrFDPP1a	No	unassigned	A07	unassigned	bZIP transcription factor
FES1	BrFES1P2c	Yes	unassigned	A05	ungrouped	Zinc finger
FES1	BrFES1P3b	No	unassigned	A05	unassigned	Zinc finger
FKF1	BrFKF1P1c	No	unassigned	A07	unassigned	F-box protein
FKF1	BrFKF1P2d	Yes	unassigned	A07	ungrouped	F-box protein
FKF1	BrFKF1P3c	Yes	unassigned	A07	ungrouped	F-box protein
FLD	BrFLDP1c	No	A10	A03	unassigned	Encodes a plant homolog of a SWIRM domain containing protein
FLK	BrFLKP2b	No	unassigned	A03	unassigned	Nucleic acid binding
FLM/MAF1	BrFLMP1b	Yes	A02	A02	ungrouped	MADS domain protein
FLM/MAF1	BrFLMP2d	No	unassigned	A02	unassigned	MADS domain protein
FPA	BrFPAP1c	No	A09	A09	unassigned	regulates flowering time in Arabidopsis via a pathway that is independent of daylength
FPF1	BrFPF1P1d	No	A02	A06	unassigned	Small,12.6kDa protein
FPF1	BrFPF1P2a	No	unassigned	A06	unassigned	Small,12.6kDa protein
FRI	BrFRIP2c	No	unassigned	A10	unassigned	Vernalization response factor
FRL1	BrFRL1P2a	Yes	unassigned	A10	ungrouped	FRI-related gene

FRL2	BrFRL2P1b	Yes	unassigned	A03	ungrouped	FRI-related gene
FRY1	BrFRY1P1b	Yes	unassigned	A09	ungrouped	Encodes a bifunctional protein that has 3'(2'),5'-bisphosphate nucleotidase and inositol polyphosphate 1-phosphatase
FT	FT-P2a	No	A07	A02	unassigned	*TFL1* homolog;antagonist of TFL1
FT	BrFTP1c	Yes	A02	A02	A02	*TFL1* homolog;antagonist of TFL1
FT	BrFTP2b	Yes	A07	A07	A07	*TFL1* homolog;antagonist of TFL1
FUL	BrFULP1b	Yes	A09	A02	ungrouped	MADS box gene negatively regulated by APETALA1
FVE	BrPVEP2a	No	unassigned	A07	unassigned	Controls flowering
FVE	BrPVEP3a	Yes	unassigned	A09	A09	Controls flowering
FY	BrFYP1a	Yes	A03	A02	ungrouped	Encodes a protein with similarity to yeast Pfs2p
GA1	BrGA1P2c	Yes	A03	A03	ungrouped	Gibberellin biosynthesis
GAr1	BrGAr1P1d	No	unassigned	A06	unassigned	GA receptor homolog
GAr3	BrGAr3P1a	Yes	A03	A09	A09	GA receptor homolog
GAr3	BrGAr3P2b	No	unassigned	A09	unassigned	GA receptor homolog
GI	BrGIP2b	Yes	unassigned	A09	ungrouped	Circadian clock gene
GI	BrGIP1d	Yes	A09	A09	ungrouped	Circadian clock gene
GI	BrGIP3b	No	unassigned	A09	unassigned	Circadian clock gene
HOS1	BrHOS1P2a	No	unassigned	A04	unassigned	RING finger E3 ligase
HUA2	BrHUA2P1b	Yes	A02	A06	ungrouped	Transcription factor
LKP2	BrLKP2P1a	No	unassigned	A07	unassigned	encodes a member of F-box proteins that includes two other proteins in Arabidopsis (ZTL and FKF1).
MAF2	BrMAF2P1b	No	A10	A02	unassigned	Originally published as Agamous like MADS-box protein AGL31
MAF4	BrMAF4P1d	No	A02	A02	unassigned	Encodes MADS-box containing FLC paralog
MAF4	BrMAF4P2a	No	A02	A02	unassigned	Encodes MADS-box containing FLC paralog
MAF5	BrMAF5P1a	Yes	unassigned	A06	ungrouped	Is upregulated during vernalization and regulates flowering time.
MFT	BrMFTP1b	No	A08	A08	unassigned	Nuclease
MFT	BrMFTP2b	No	unassigned	A08	unassigned	Nuclease
NS1	BrNS1P2c	Yes	A01	A03	A01	Asparaginyl-tRNA synthetase protein
NS1	BrNS1P1d	No	A08	A08	unassigned	Asparaginyl-tRNA synthetase protein

PFT1	BrPFT1P2c	Yes	unassigned	A09	ungrouped	Transcription coactivator
PFT1	BrPFT1P4f	Yes	unassigned	A09	ungrouped	Transcription coactivator
PFT1	BrPFT1P3b	Yes	unassigned	A08	ungrouped	Transcription coactivator
PHYA	BrPHYAP1g	Yes	A08	A09	ungrouped	G-protein coupled red/far red photoreceptor
PHYA	BrPHYAP3b	No	unassigned	A04	unassigned	G-protein coupled red/far red photoreceptor
PHYA	BrPHYAP3c	No	unassigned	A04	unassigned	G-protein coupled red/far red photoreceptor
PHYE	BrPHYEP1b	Yes	A01	A06	ungrouped	G-protein coupled photoreceptor
PHYE	BrPHYEP1a	No	A01	A10	unassigned	G-protein coupled photoreceptor
PHYE	BrPHYEP2c	Yes	unassigned	A01	A01	G-protein coupled photoreceptor
PI	BrPIP2a	No	unassigned	A02	unassigned	Floral homeotic gene encoding a MADS domain transcription factor
PI	BrPIP1b	Yes	A10	A02	ungrouped	Floral homeotic gene encoding a MADS domain transcription factor
RGA	BrRGAP2c	Yes	A06	A09	ungrouped	VHIID/DELLA transcription factor
RGA	BrRGAP1a	Yes	A06	A06	ungrouped	VHIID/DELLA transcription factor
RGA	BrRGAP1c	Yes	A06	A06	ungrouped	VHIID/DELLA transcription factor
SEP3	BrSEP3P2a	Yes	unassigned	A07	ungrouped	Member of the MADs box transcription factor family
SEP3	BrSEP3P1a	No	A09	A07	unassigned	Member of the MADs box transcription factor family
SLY1	BrSLY1-P1b	No	A01	A01	unassigned	F-box protein involved in GA signaling
SNZ	BrSNZP2b	No	unassigned	A09	unassigned	Encodes a AP2 domain transcription
SOC1	BrSOC1-P1a	No	A01	A01	unassigned	Transcription factor
SPA1	BrSPA1P1a	Yes	A03	A05	unassigned	Encodes a member of the SPA (suppressor of phyA-105) protein family (SPA1-SPA4
SPA1	BrSPA1P2a	Yes	unassigned	A05	ungrouped	Encodes a member of the SPA (suppressor of phyA-105) protein family (SPA1-SPA4
SPL5	BrSPL5P1a	No	A03	A01	unassigned	Encodes a member of the SPL
SPL5	BrSPL5P2bF	No	unassigned	A05	unassigned	Encodes a member of the SPL
SVP	BrSVPP1c	Yes	A09	A09	A09	Transcription factor
SVP	BrSVPP2d	Yes	unassigned	A04	ungrouped	Transcription factor

T1N6_2	T1N6_2P1c	No	A09	A09	unassigned	Putative role in flower development
T1N6_2	T1N6_2P2a	Yes	A04		ungrouped	Putative role in flower development
TCTP	BrTCTPP1b	No	A03	A03	unassigned	Encodes a protein homologous to translationally controlled tumor protein (
TCTP	BrTCTPP2b	Yes	unassigned	A05	A05	Encodes a protein homologous to translationally controlled tumor protein (
TFL1	BrTFL1P2c	No	A02	A02	unassigned	Phosphatidylethanolamine binding
TFL2	BrTFL2P4b	No	unassigned	A10	unassigned	Chromatin maintenance protein
TFL2	BrTFL2P1c	No	A02	A10	unassigned	Chromatin maintenance protein
TFL2	BrTFL2P2a	No	unassigned	A10	unassigned	Chromatin maintenance protein
TOC1	BrTOC1P2b	No	mapped A09	A09	unassigned	Pseudo response regulator involved in the generation of circadian rhythms.
TOC1	BrTOC1P1b	No	mapped A03	A03	unassigned	Pseudo response regulator involved in the generation of circadian rhythms.
TSF	BrTSFP2c	Yes	unassigned	A02	A02	FT homolog
VEL1	BrVEL1P1e	Yes	A01	A08	Ungrouped	Encodes a protein with similarity to VRN5 and VIN3.
VIM3	BrVIM3P3b	Yes	A09	A09	A09	Encodes the VIM3/ORTH1 protein that is similar to VIM1. This protein has an N-terminal PHD domain and two RING
VIN3	BrVIN3P1a		A10	A10	unassigned	Homeodomain protein
VIN3	BrVIN3P2b	No	unassigned	A02	unassigned	Homeodomain protein
VIN3	BrVIN3P3a	No	unassigned	A03	unassigned	Homeodomain protein
VIP4	BrVIP4P1b	Yes	A09	A09	ungrouped	Encodes highly hydrophilic protein
VIP4	BrVIP4P2b	Yes	unassigned	A09	ungrouped	Encodes highly hydrophilic protein
VRN1	BrVRN1P1b	Yes	A01	A05	ungrouped	Required for vernalization.Essential for the complete vernalized plants.
VRN1	BrVRN1P3b	Yes	unassigned	A03	ungrouped	Required for vernalization.Essential for the complete vernalized plants.
VRN2	BrVRN2P4b	Yes	A08	A08	ungrouped	encodes a nuclear-localized zinc finger protein with similarity to Polycomb group (PcG) proteins

*unassigned =the markers didn't show polymorphism; ungrouped =markers showed polymorphism but didn't have linkage groups

Appendix 2. SNPs markers

SNP_ID	Estimated linkage group	VT-115X RC-144	Turnip vs.	Flanking-200bp SNPs
PV_Br_SNP_0002	A01	A01	L144,L143,WTC	0,0,0
PV_Br_SNP_0055	A01	A01	WTC,L143	0,0,0
PV_Br_SNP_0080	A01	A01	L144,L143	0,0,0
PV_Br_SNP_0098	A01	A01	L144,WTC	0,0,0
PV_Br_SNP_0125	A02	A07	L144,WTC	0,0,0
PV_Br_SNP_0143	A02	A02	L144,L143	0,0,0
PV_Br_SNP_0172	A02	A02	WTC,L143	0,0,0
PV_Br_SNP_0242	A03	Ungrouped	L144,L143	0,0,0
PV_Br_SNP_0274	A03	A03	L144,L143	0,0,0
PV_Br_SNP_0294	A03	A03	L144,L143	0,0,0
PV_Br_SNP_0309	A03	A03	L144,L143	0,0,0
PV_Br_SNP_0319	A04	Ungrouped	WTC,L143	0,0,0
PV_Br_SNP_0338	A04	A04	L144,L143	0,0,0
PV_Br_SNP_0353	A04	A04	L144,WTC	0,0,0
PV_Br_SNP_0372	A04	A04	L144,L143	0,0,0
PV_Br_SNP_0413	A05	A05	L144,L143	0,0,0
PV_Br_SNP_0424	A05	A05	L144,L143	0,0,0
PV_Br_SNP_0464	A05	A05	L144,L143,WTC	0,0,0
PV_Br_SNP_0492	A05	A05	L144,L143	0,0,0
PV_Br_SNP_0517	A06	Ungrouped	L144,L143	0,0,0
PV_Br_SNP_0545	A06	A06	L144,L143	0,0,0
PV_Br_SNP_0565	A06	A06	WTC,L143	0,0,0
PV_Br_SNP_0578	A06	A06	L144,L143	0,0,0
PV_Br_SNP_0637	A07	A07	L144,L143	0,0,0
PV_Br_SNP_0663	A07	A07	L144,WTC	0,0,0
PV_Br_SNP_0691	A07	Ungrouped	L144,L143	0,0,0
PV_Br_SNP_0696	A08	A08	L144,L143	0,0,0
PV_Br_SNP_0709	A08	A08	L144,L143,WTC	0,0,0
PV_Br_SNP_0721	A08	A08	L144,L143	0,0,0
PV_Br_SNP_0743	A08	A08	L144,L143	0,0,0
PV_Br_SNP_0754	A08	A08	L144,L143	0,0,0
PV_Br_SNP_0776	A08	A08	L144,L143	0,0,0
PV_Br_SNP_0790	A08	Ungrouped	L144,WTC	0,0,0
PV_Br_SNP_0795	A09	A09	L144,L143	0,0,0
PV_Br_SNP_0826	A09	A09	L144,L143	0,0,0
PV_Br_SNP_0848	A09	A09	L144,L143	0,0,0
PV_Br_SNP_0902	A09	A09	L144,WTC	0,0,0
PV_Br_SNP_0918	A09	Ungrouped	L144,L143	0,0,0
PV_Br_SNP_0921	A10	Ungrouped	L144,L143	0,0,0
PV_Br_SNP_0985	A10	A10	L144,L143	0,0,0
PV_Br_SNP_0996	A10	A10	L144,L143	0,0,0

PV_Br_SNP_1037	A08		L143	0,0,0
PV_Br_SNP_1040	A06	A06	L144	0,0,0
PV_Br_SNP_1041	A08	Ungrouped	L144	0,0,0
PV_Br_SNP_1048	A01	A01	L143	0,0,0
PV_Br_SNP_1051	A05	A05	L144	0,0,0
PV_Br_SNP_1066	A08	A08	L144,L143	0,0,0
PV_Br_SNP_1067	A10	Ungrouped	L144	0,0,0
PV_Br_SNP_1069	A06	Ungrouped	L143	0,0,0
PV_Br_SNP_1071	A09	A09	L144	0,0,0
PV_Br_SNP_1082	A03	Ungrouped	L144,WTC	0,0,0
PV_Br_SNP_1083	A09	A09	L143,WTC	0,0,0

Appendix 3. InDels markers

InDel_ID	Estimated linkage group	VT-115 X RC-144	Start	Stop
BrID10075	A10	Ungrouped	10184370	10184527
BrID10081	A10	Ungrouped	8588747	8588898
BrID10091	A10	Ungrouped	5042697	5042828
BrID101021	A09	Ungrouped	3696434	3696561
BrID101025	A05	Ungrouped	9323883	9324018
BrID10103	A07	Ungrouped	13590527	13590696
BrID101035	A06	Ungrouped	18959379	18959513
BrID101075	A07	A07	15492626	15492755
BrID101083	A09	A09	29890041	29890177
BrID101119	A01	Ungrouped	9895949	9896088
BrID10113	A07	Ungrouped	16124793	16124947
BrID10131	A05	Ungrouped	2101631	2101797
BrID10133	A05	Ungrouped	2506072	2506218
BrID10253	A06	Ungrouped	17658487	17658580

Appendix 4. Average mean of turnip tuber related traits in RIL F6/7 population

	brix	FW	DW	DM
No. of lines	94	94	94	94
Mean	4.49	9.36	1.00	0.127
Maximum	6.67	130.97	11.94	0.22
Minimum	2.33	0.24	0.06	0.06
VT-115	4.5	0.26	0.06	0.15
RC-144	5.33	130.97	11.94	0.09
Skewness	0.22	5.73	5.49	0.74
Std. Error of Skewness	0.25	0.25	0.25	0.25