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RESEARCH ARTICLE

Mapping of candidate genes involved in fatty acid synthesis in *Brassica rapa*

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SUMMARY

Oilseed crops are valuable sources of oil content and fatty acid constituents. *Brassica rapa* is a well-known oilseed and vegetable crop grown throughout the world. *B. rapa* has higher genomic similarities with model plant *A. thaliana*. The genetic regulation of fatty acid biosynthesis is well studied in *A. thaliana* as well as in *B. napus.* However, only little information on genetic regulation of fatty acid biosynthesis has been known in *B. rapa*. The objective of this work was to identify and map the genes involved in fatty acid biosynthesis process in *B. rapa* DH population (Yellow Sarson, YS -143 x Pak Choi, PC-175). 172 lines of DH68 population was used for genotyping of markers. Comparative mapping was performed to predict positions of genes in *B. rapa* using two approaches; homology with chiifu sequence and genomic block synteny *A. thaliana* and *B. rapa*. Primers were designed for 18 candidate genes and their 16 paralogs genes in *B. rapa* using Chiifu sequence. Among 31 markers genotyped, 13 markers were mapped in DH68 population are in agreement with predicted positions in genomic blocks. Thus, the research will be useful to co-localise QTLs with candidate gene, identification of putative genes as well as cis and trans positions of genes in future.

Key Words : *B. rapa*, DH population, Candidate gene, Fatty acid biosynthesis, Comparative mapping.

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Oilseed crops are valuable sources of oils and fatty acid constituents. Most vegetable oils are currently used for cooking, salad or margarine for human consumption. As much as 25 per cent of human calorific intake is derived from the vegetable oils in developed countries (Broun *et al.*, 1999). Brassica species are the third largest oil producing crops after soybean and oil palm (Huang *et al.*, 2009). *Brassica rapa* is one of the traditional oilseed crops in the Indian sub-continent. *Brassica rapa* has short life cycle and high oil content, which makes them an optimal choice for cultivation in some areas (Zhao, 2007). *Brassica rapa*, together with *B. napus*, *B. juncea* and *B. carinata* contribute to ~12 per cent of edible vegetable oil supply throughout the world, being B. napus the major contributor (Huang *et al.*, 2009). *Brassica* genus is well known for oilseed crops, vegetables and forage crops. Among *Brassica* crops, oilseed Brassicas is economically important due to their oil content. Nowadays, *B. rapa* and *B. juncea* are also receiving more attention as an oil crop due to rapidly developing global demand for canola oil. For oil crop improvement, it is necessary to understand the molecular basis of oil with their fatty acid composition.

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The biosynthesis of seed storage oil involves metabolic pathway which is located in several different sub cellular organelles. The overview of the fatty acid biosynthesis pathway with their candidate genes is shown in Fig. A. Fatty acid biosynthesis takes place in the plastids of developing seeds (Ohlrogge and Jaworski, 1997; Dyer and Mullen, 2005). The enzyme *ACCase* catalyzes the first step in the pathway resulting in formation of Malonyl-CoA. This Malonyl-CoA is transferred from CoA to a protein cofactor, Acyl carrier protein (ACP) before entering in the fatty acid biosynthesis pathway by *Malonyl-CoA ACP*

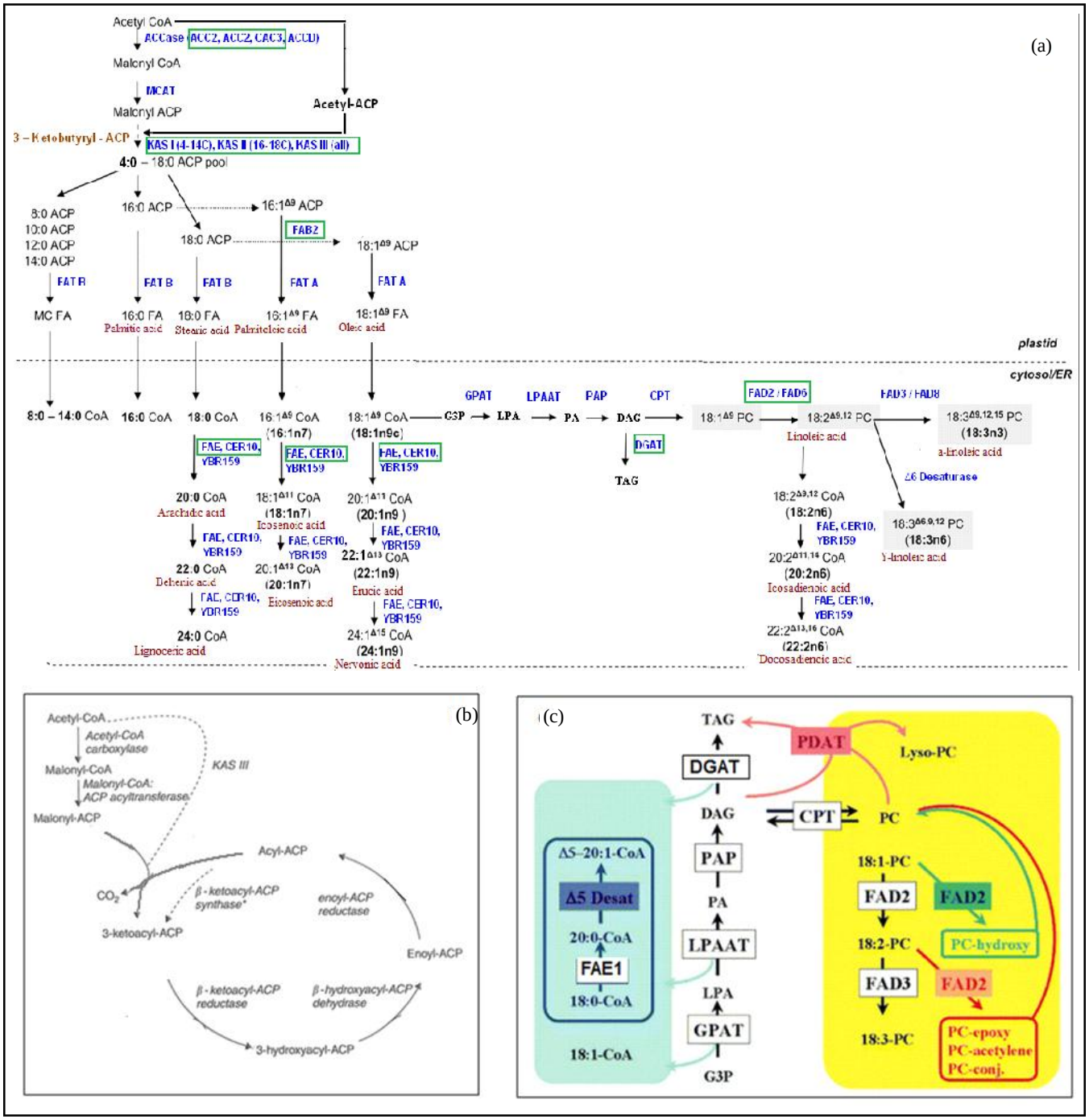

Fig. A : Overall fatty acid biosynthesis pathway with candidate genes (a) genes selected for primer design indicated with green color box; intermediate products and enzymes involved in synthesis of C4:0 C18:0 fatty acids (b) and TAG synthesis pathways with their candidate genes (c) (Source:Voelker and Kinney, 2001; Barker *et al.*, 2007 and Harwood, 2005 Edited in 2010)

transferase (Ohlrogge and Browse, 1995).This ACP act as a carrier for growing fatty acid chain. The activation of Malonyl-ACP with a second Acetyl-CoA is done by a condensing enzyme, *Ketoacyl- ACP synthase III* (KAS III). This reaction resulted in formation of a four carbon compound 3– Ketobutyryl – ACP which is an intermediate product. This 3-Ketobutyryl-ACP goes through set of three additional reactions to form saturated fatty acids (Fig. Ab). First, the reduction of 3-Ketobutyryl-ACP to hydroxyl group is performed by enzyme *3 -Ketoacyl-ACP reductase* using NADPH as the electron donor. Second reaction is dehydration of 3-hydroxyl-ACP to Enoyl-ACP by *3 - hydroxyacyl-ACP dehydratase enzyme*. While the third one is the reduction of dehydrated product again. The third reaction removes the double bound to form saturated fatty acid by the enzyme enoyl-ACP reductase (Ohlrogge and Browse, 1995).The result of the first cycle of fatty acid biosynthesis is a four carbon chain (C4:0). This four carbon chain is condensed with another Malonyl- ACP which results in six carbon side chain. The reaction is repeated as before. For each cycle, two carbon units are added at a time until a saturated fatty acid chain of 16 (C16:0) or 18 (C18:0) carbons in length is formed.

According to Ohlrogge and Browse (1995), three different isoforms of KAS enzyme are involved in fatty acid synthesis in plastids. The first condensation reaction between Malonyl-ACP and Acetyl-CoA is catalyzed by KAS III and results in formation of 3 Ketobutyryl-ACP, an intermediate product. The subsequent six condensations between growing Acyl side chains and additional Malonyl-CoA are catalyzed by KAS I to produce C6:0 – C16:0fatty acid. Finally, the elongation of 16 carbon palmitoyl-ACP to stearoyl-ACP is done by KAS II.

In plants, the process of elongation of fatty acids in plastids can be halted in various ways. In most cases, Acyl-ACPs such as Palmitoyl-ACP or Stearoyl-ACP are hydrolyzed by an *Acyl-ACP thioesterase* and the free fatty acids are released outside the plastid. The *Fat B thioesterase* enzyme has highest activity with saturated Acyl-ACPs.The production of Oleoyl-ACP from Stearoyl-ACP is catalyzed by *Fatty Acid Biosynthesis* (FAB2) enzyme while *FatAthioesterase* enzyme preferentially hydrolyze Oleoyl-ACP and releases the Oleic acid (C18:1). Alternatively, the *acyltransferase* in plastids directly uses the Acyl-ACPs producing glycerol-3-phosphate (G3P).There are various pathways for formation of triglycerides (TAG). The transfer of acyl chain from CoA to sn-1 position of G3P is initiated by *glycerol-3-phosphate acyltransferase* (GPAT) which results in the production of lysophosphatidic acid (LPA). Further, the transfer of acyl chain to sn-2 position is catalyzed by *lysophosphatidic acid acyl transferase* (LPAAT) creating phosphatidic acid (PA). Diacyl-glycerol is formed after removal of phosphate group from PA by *phosphatidic acid phosphatase* (PAP). Finally, DAG is converted into triglycerides (TAG) by *Diacyl glycerol acyltransferase enzyme* (DGAT). During this conversion, an acyl group from acyl-CoA is transferred to sn-3 position of DAG (Dahlqvist *et al.*, 2000).The second pathway involves the *choline phosphotransferase* (CPT), an enzyme normally involved in membrane biosynthesis. The phosphatidylcholine (PC) is formed after esterification of sn-3 position of DAG by CPT. Acyl group of fatty acid is directly transferred from PC to DAG by P*hospholipid: diacylglycerolacyltransferase* (PDAT) which results in formation of lyso-PC and TAG (Ståhl *et al.*, 2004). These esters of fatty acids (TAG) are stored in the form of oil bodies in endoplasmic reticulum (ER) (Fig. Ac).

PC serves as a substrate for various lipid modifying enzymes like *Fatty Acid Desaturase 2* (FAD2), FAD3, FAD6, FAD8 etc. FAD2 enzyme introduces a double bond into the fatty acid acyl side chains of phosphatidylcholine (PC) which converts oleic acid (18:1) to Linoleic acid (18:2). A subsequent insertion of double

Table A : Composition of master mix used for PCR reactions

Sr. No.	Components	Volume of 1 x (ul)
1.	Phire enzyme	0.1
2.	Reaction buffer for Phire (5x)	2
3.	LC-green	1
4.	dNTP	0.4
5.	Primer Fw (5mM)	0.5
6.	Primer Rev (5mM)	0.5
7.	MQ	5.45

bond at ω3 position of 18:2 PC is performed by FAD3 enzyme which results in production of α-Linolenic acid (ALA, 18:3n3) (Arondel *et al.*, 1992; Yadav *et al.*, 1993) from linoleic acid whereas insertion of double bond at ω6 position of 18:2 PC by Δ6 Desaturase produces γ-Linolenic acid (GLA, 18:3n6) (Vrinten *et al.*, 2007). In addition to TAG and polysaturated fatty acids, very long chain fatty acids (VLCFA) chain length > 20 carbons are also found in the storage oils of the member of family *Brassicaceae* (Voelker and Kinney, 2001). The production of VLCFA takes place in ER. VLCFA are resulted from elongation of Oleoyl-COA by a membrane bond elongase complex (Harwood, 2005). A membrane bond elongase complex contains a group of genes which includes: FAE1, CER10, PAS2 and YBR159 (KEGG database). VLCFA are finally stored as esters of glycerol (TAG) by DGAT enzymes.

Brassica genus and *Arabidopsis thaliana* are developed from a hexaploid ancestor with (n=8) karyotype (Hong *et al.*, 2008). According to Yang *et al.* (2006), Brassica genus and A. thaliana are separated around 14.5-20.4 million years ago from a common ancestor. Hence, species from brassica genus have high genetic similarity or maybe better say synteny with *A. thaliana*. *A. thaliana* provides good opportunity for identification of candidate genes in *B. rapa* due to the known genetic sequence and homology with brassica genus. *B. rapa* genome is 4 to 5 times larger (529 mb) as compared to A. thaliana (157 mb)(Schranz *et al.*, 2006). The synteny of homologous genomic blocks of A. thaliana is triplicated (with few blocks replicated 2-5 times) on different chromosomes of B. rapa (Schranz *et al.*, 2006 and Wang *et al.*, 2011). Wang *et al.* (2011) also reported these blocks in *B. rapa* by using segmental co-linearity based on genome sequence of *A. thaliana* and *B. rapa*.

Since *B. rapa* is widely consumed as vegetable or oil throughout the world, and it is a diploid model for the amphidiploid B. napus, it is necessary to increase oil content by regulating genes involved in fatty acid biosynthesis pathway. Hence, a better understanding about genetic basis of fatty acid biosynthesis genes is needed. Storage compounds are accumulated during seed filling process and stored in seeds. These storage compounds consist of carbohydrates, fatty acids and proteins. The objective of this work was identification of candidate genes regulating oil content and quality and their mapping in *Brassica rapa* genetic linkage map of a DH68 population (cross between YS-143 and PC-175).

MATERIAL AND METHODS

Plant materials :

172 lines of DH-68 population derived from a cross between male parent Pak Choi, PC-175 (cv. NAI BAI CAI; accession number VO2B0226) and yellow Sarson, YS-143 as female parent (accession number FIL500) were used. Under different projects of *Brassica* research team of Wageningen University, 708 markers including Amplified Fragment Length Polymorphism (AFLP), Myb transcription factor, tocopherol and carotenoids, and flowering time gene targeted markers and simple sequence repeat (SSR) were genotyped in DH-68 genetic map. Those markers were also included in linkage map construction. In this study, gene targeted markers were developed for the candidate genes of fatty acid biosynthesis pathway and mapped in DH-68 population of *Brassica rapa*. The DNA of all DH-68 genotypes were isolated by RETEH method. Total DNA concentration of all samples was measured with Nano Drop ND-1000 UV-VIS spectrometer (Nano Drop Technologies) to check the quality and quantity of DNA. All samples were diluted to 20-50ng/µl DNA for analysis according to light scanner manual.

Selection of candidate genes from A. thaliana and Primer design :

Brassica rapa is a close relative of A. thaliana. Therefore, candidate genes involved in fatty acid biosynthesis were selected from already published literature (Beisson *et al.*, 2003; Dyer and Mullen, 2005 and Mu *et al.*, 2008) and the "*Arabidopsis* information resource" TAIR. For each selected gene, sequence was extracted from "the *Arabidopsis* information resource and used for searching sequence homology of *B. rapa* in brassica database (BRAD) and on homology with Chiifu sequence (*http://137.224.100.210:443/dev/f?p=118*). "*Bra*" is the annotation of *B. rapa* gene as like "AT" in Arabidopsis. Three sets of unlabelled primers were designed by using online software, primer3plus. All primers were designed by considering light scanner program criteria as this program not able to detect polymorphism in product size > 500bp. Therefore, the parameters were set for targeted product size of 100-300bp with minimum primer size 18bp and maximum 22bp lengths. The melting temperature (Tm) ranges from 58°C

(minimum) to 60°C as an optimum. The GC content was maintained at 40 per cent as an optimum. In case of paralogs of a gene, all sequences were aligned by using Megalign software to find differences in base pairs. By considering at least 3 base pairs differences, primers were developed for each paralogs of a gene. All designed primers were checked back to confirm they were able to amplify the targeted gene as well as to know the expected physical position, linkage group and scaffold number using "primer match information" feature on (*http://137.224.100.210: 443/dev/f?p=118*). The unlabelled primers were ordered and then diluted with different amounts of MilliQ water as given by primer developing company (Biolegio BV) to prepare "stock solution". A working solution of 5mM concentration was prepared from 100mM concentration of stock solution. PCR was done in 10µl volumes of PCR reaction for each pair of primers. For PCR reaction of primers, 9.95µl of master mix and 1µl of DNA amounts were used. In addition 20µl of mineral oil was used in each sample.

Polymorphism test and screening of progenies :

The polymorphism test was performed for each primer pair on parents of DH68 lines and 6 more randomly chosen DH lines. After PCR reaction, plates were transferred to a 96-well LightScanner (Idaho technology) for high resolution melting (HRM) analysis. Light scanner is a program used for high resolution melting (HRM) analysis which provides post-PCR detection of mutation and SNPs in genomic DNA. Samples were melted from 70 to 98°C, at default melting rate (0.1°C/ sec.). The gradual heating of PCR plate in light scanner resulted in dissociation of double stranded DNA with release of LC green dye. During the PCR amplification, the LC green plus dye were placed between each annealed base pair of the double stranded DNA molecule. These LC green dyes were excited at 470nm and after that it emitted fluorescence at 510nm. The fluorescence intensity was reflected by camera present in light scanner equipment. The melting curves were resulted from temperature dependent fluorescence intensity. The generated melting curves were normalized by selecting approximately 1°C temperature intervals above and below the melting region. A negative filter was applied to remove unamplified samples. Finally, grouping of samples were done by using "auto group" function and adjusting a sensitivity level that best differentiate the samples. The grouping results for amplicon were viewed in difference plots. In addition, after LightScanner analysis, the PCR products also loaded on Agarose gel to confirm identified polymorphic primer pair by size difference. 1.5 per cent Agarose gel (w/w) was prepared with TBE buffer to separate the PCR products of unlabeled primers. 1 µl of orange loading buffer were added to 10 µl of PCR products before loading on Agarose gel. The samples were run for 2 hours at 100 volt electric power. The primer pair identified as polymorphic was used for screening of whole set of DH lines. The same PCR reaction and program was used for screening of progenies with specific primer pair (Table A). The PCR products were again analyzed for melting temperature curves using light scanner (Idaho technology) and same procedure was followed for genotyping of individual lines.

Linkage analysis :

The linkage analysis and map construction was carried out by using the Joinmap 4.0 (Van Ooijen, 2006) in regression approach. All the marker data were first pre-processed based on missing values more than 60 per cent, segregation distortion from 1:1 ratio, and weak linkages below LOD score 5. The markers with acceptable quality were used for linkage map construction. In addition, individual DH lines were also screened for missing values. The DH lines with more than 60% missing values were discarded from analysis. The grouping of markers was done at a wide range of LOD scores (3.0 to 20.0). The Haldane mapping function was used to obtain genetic map distance from recombination frequencies. To overcome segregation distortion, 10 neighboring markers and 0.40 recombination threshold was considered. Based on linkage map constructed for all 10 linkage groups in IOP-nutrigenomics project and in Public database, the allocation of linkage group and their orientation was performed.

RESULTS AND DISCUSSION

Arabidopsis exhibits extensive conserved genome synteny with *B. rapa* as both species belongs to the family Brassicaceae. Availability of a complete genome sequence of *A. thaliana* provides opportunity to perform comparative mapping between many related or unrelated species. Nowadays, *B. rapa* genome sequence based on Chinese cabbage line (Chiifu -401) is also available on Brassica Database (BRAD) which increased the

scope and possibility of comparative mapping within *Brassica* species. Comparative mapping combined with genetical genomics approach has become a powerful tool to study genetic basis of many phenotypic traits in *Brassica* species.

Comparative mapping :

The criteria's used for comparative mapping were genomic block synteny of *B. rapa* and *A. thaliana* and *B. rapa* gene information based on *Chiffu-401* (Chinese cabbage type) sequence. However, homology with Chiifu sequence is more reliable criteria. The previous studies on comparative mapping between B. rapa and A. thaliana defined 24 genomic blocks in B. rapa based on BAC clone end sequence named as A-X (Schranz *et al.*, 2006). Genomic block positions as well as expected linkage group were predicted for 33 genes and paralogs of genes. The genomic block synteny based on BAC clone sequence (Schranz *et al.*, 2006) and block synteny based on segmental co-linearity between genome sequence of *A. thaliana* and *B. rapa* (Wang *et al.*, 2011) almost predicted the same block position for all genes except for DGAT, ABI3, KAS-III, PKL and SUS3 genes (Table 1). For example, DGAT gene was predicted in H block. According to BAC clone genomic synteny, H block was present on only A07 while based on genome sequence; it was present in A07 and A09 *B. rapa* chromosomes. Furthermore, based on Chiifu sequence information, i DGAT gene is predicted on A07 and A09. The block C, F, J and U had been predicted with two genes of the pathway while the block E, V, D, H, N, I, B, O and Q had predicted with only one gene (Fig. 1). Based on *Chiifu* sequence information, 7 genes predicted to map on A06, 6 genes on A09, 4 genes on A03, 4 genes on A05, 4 genes on A07, 3 genes on A02, 3 genes on A08 and 2 genes on A01 (Table 1).

Screening and polymorphism :

Out of 33 candidate genes and paralogs, only 21 gene tagged markers was screened on DH68 population (Table 1). The primer pairs of 12 genes were not amplified during PCR process. Genotypes were scored in two classes; a, b and for missing values in Light Scanner software. The allelic frequency classification with their significance level is given in Table 2.The marker KASP1_2, KAS1P2_2, KAS1P3_1, DGATP2_3, FAE1P1_3 and PKL_2 had highly skewed alleic frequency and they are significantly different at 0.0001. When these markers were scored second time by incresing sensitivity level in lightscanner, the allelic frequency completely reverse in case of KAS1P2_2, KAS1P3_1. This happened due to increased sensitivity level for grouping. Since, some DH lines showing third group but prsent in minority scored as missing values while someDH lines was shifted from "a" allele to "b" allele. Hence, there is difference between allelic frequency of marker scored first time and second time.

The PCR product of remaining 12 genes namely KAS-II, FAD6, FAB2, LEC1and SUS3 and their paralogs were not amplified during PCR process. During testing of primer of KAS-II, FAD6, FAB2, LEC1, SUS3 and their paralogs for polymorphism in LightScanner, stable fluorescence intensity lines were observed instead of melting peaks. Also on Agarose gel, DNA bands were not observed within 100-300bp region. For some primers of FAD6, DNA bands were observed above 500bp that is not our targeted base pair size. Therefore, melting peak curves were not observed in LightScanner. As primers were designed to amplify the targeted product size of 100-300bp. On Agarose gel, the primer dimers for primer pairs which were not amplified were observed, hence we are sure about that there is no mistake in preparation of PCR sample. Few reasons were speculated for no amplification of primers. First, the DNA quality of those randomly chosen DH lines was not good because for some primers DNA diluted by other person was used. In that case, we not checked DNA quality. However some primer pairs were screened with DNA solution made by us. Second, the primer pairs were designed based on Chiifu sequence, not on parents of DH68 (YS-143 or PC-175). Therefore, these markers may be able to amplify on Chiifu DNA but not in DH68 lines. Thus, no band was observed on Agarose gel. It was suggested that, to screen those markers that were not amplified on Chiifu along with few DH68 lines to observe DNA band pattern. In this study, due to the unavailability of Chiifu DNA, we were unable to screen markers that were not amplified.

Linkage map construction :

For linkage map construction, 739 markers were used. These markers contained a group of 708 markers developed under differeent projects of Brassica research team of WUR and a group of 31 designed markers genotyped during this research work. In total, 21 genes of fatty acid biosynthesis were genotyped. Out of which,

10 genes were scored two times with different allelic frequency. Two time scoring was done because the first scoring showed high segregation distortion. This might be due to the forcing the genotype in grouping in lightscanner procedure. A duplicate marker was indicated with two digits at the end of gene name for example, KAS1P1_21 (Table 2). 10 linkage groups were selected based on position of anchor markers to represent 10 chromosomes of B. rapa. Out of 31 new markers, 13 markers were mapped in DH68 with LOD score higher than 5.0 except FAE1P2 (LOD score = 4.4). Marker FUS3P1 was mapped on A02 at the middle region (100.7cM) which corresponds to the prediction made based on Chiifu sequence as well as on genomic block synteny comparison (Schranz et al., 2006) and segmental co-linearity (Wang *et al.*, 2011) between A. thaliana and B. rapa (Table 1). Similarly, CAC3P1 (at the top of A03), ABI3 (at the bottom of A03), FAD2 (at the middle of

Table 1 : List of selected genes of fatty acid biosynthesis pathway with their number of paralogs in B. rapa, expected linkage group based on chiifu sequence, current map position in DH68 population, genomic blocks in A. thaliana with their position in B. rapa based on two studies on comparative mapping and idenitified QTL for different fatty acids compounds containing markers in significant region

Gene	Paralogs	Chiffu LG	Map postion in DH68	Genomic block in Arabidopsis	Block position in B. rapa based on BAC clone sequence (Schranz *et al.*, 2006)	Block position based on genome sequence (Wang *et al.*, 2011)
ACC1	1	A08	A08	C	A05, A06, A08	A05, A06, A08
	2	A06	A06	C		
ACC2	1	A06	A06	C	A05, A06, 108	A05, A06, 108
	2	A08	Not mapped	C		
CAC3	1	A03	A03	J	A03, A04, A05	A03, A04, A05
	2	A05	Not mapped	J		
KAS I	1	A02	Not mapped	V	A02, A06, A09	A02, A06, A09
	2	A06	Not mapped	V		
	3	A09	A09	V		
KAS-II	1	A07	n.a.	E	A02, A07	A02, A07
	2	A07	n.a.	E		
	3	A06	n.a.	E		
	4	A02	n.a.	E		
KAS-III		A09	Not mapped	D	A09	A01, A09
FAB2	1	A03	n.a.	J	A03, A04, A05	A03, A04, A05
	2	A05	n.a.	j		
FAD2		A05	A05	F	A01, A03, A05	A01, A03, A05, A07
FAD6	1	A01	n.a.	U	A01, A03, A08	A01, A03, A08
	2	A08	n.a.	U		
DGAT1	1	A09	A09	H	A07	A07, A09, A10
	2	A07	Not mapped	H		
FAE1	1	A01	Not mapped	U	A01, A03, A08	A01, A03, A08
	2	A03	A03*	U		
CER10		A09	A09	N	A04, A07, A09	A04, A07, A09
ABI3		A03	A03	F	A01, A03, A05	A01, A03, A05, A07
FUS3	1	A02	A02	L	A02, A06	A02, A06
	2	A06	A06	L		
PkI		A09	Not mapped	I	A03, A04, A07, A09	A03, A04, A05, A07, A09
LEC1	1	A05	n.a.	B	A05, A06, A07, A08, A09	A05, A06, A07, A08, A09
	2	A07	n.a.	B		
SUS3	1	A09	n.a.	O	A03, A09	A03, A05, A09
	2	A06	n.a.	O		
SSI1		A06	A06	O	A02, A06, A09	A02, A06, A09

Note: *: FAE1P2 gene was mapped on A03 linkage group but removed during QTL analysis; n.a.: not aplicable.

A05), ACC1P2, ACC2P1 (at the top of A06), SSI1 and FUS3P2 (at the bottom of A06), ACC1P1 (at the top of A08), KAS1P3, DGATP1 (at the top of A09) and CER10 (at the bottom of A09) were mapped. These all genes were mapped at expected linkage group based on Chiifu sequence information and also in predicted blocks based on genomic block synteny (Schranz *et al*., 2006)and segmental co-linearity (Wang *et al*., 2011). In A03, FAE1P2 marker was expected to map at the bottom region next to the ABI3 based on *Chiifu* sequence homology. Also based on genomic block comparison, it is predicted in U-block and expected to map at bottom of A03. In this study, FAE1P2 gene was also mapped at the bottom of A03. To avoid underestimation of QTLs due to large gap of 20cM between ABI3 and FAE1P2, FAE1P2 marker was removed from analysis and also from linkage map. Furthermore, there was one more large gap identified on A07 (31cM at the middle region). There are several reasons for presence of a large gap on linkage map. First, there might be lack of sufficient linkage between markers due to low density of markers. Therefore, no neighbouring markers were available. Hence, there should be two linkage sub-groups of A07. Second, there may be no polymorphism between parents

Table 2 : Allelic frequency of markers scored in DH68 population with their significance level showing deviation from 1:1 ratio (segregation distortion). Map position in DH68 represents mapping status of markers while number of lines are total the DH lines used for genotyping of markers

Genes	Alleles of			Significance	Map position in DH68	Number of DH lines
	YS-143	PC-175	Missing values			
ACC1P1_3	97	42	33	*******	A08	172
ACC1P2_1	65	64	43	-	A06	172
ACC2P1_2	73	68	31	-	A06	172
ACC2P2_1	28	79	65	*******	Not mapped	172
CAC3P1_1	69	56	47	***	A03	172
CAC3P2_1	27	98	47	*******	Not mapped	172
KAS-IP1_2	9	126	37	*******	Not mapped	172
KAS-IP2_2	7	124	41	*******	Not mapped	172
KAS-1P3_1	12	103	57	*******	A09	172
KAS-III_1	63	88	21	*******	Not mapped	172
FAD2_3	113	24	35	*******	A05	172
DGAT1P1_3	36	99	37	*******	Not mapped	172
DGAT1P2_3	20	112	40	*******	Not mapped	172
FAE1P1_3	10	132	30	*******	Not mapped	172
FAE1P2_3	89	26	57	*******	A03	172
CER10_3	87	15	70	*******	Not mapped	172
ABI3_3	60	77	35	*******	A03	172
FUS3P1_2	53	87	32	***	A06	172
FUS3P2_1	42	64	66	**	A02	172
PkI_2	9	127	36	*******	Not mapped	172
SSI1_1	64	69	39	-	A06	172
KAS-1P1_21	23	91	58	*******	Not mapped	172
KAS-1 P2_21	93	22	57	*******	Not mapped	172
KAS-1 P3_11	65	31	76	*****	A09	172
DGAT1P1_31	66	49	57	-	A09	172
DGAT1P2_31	27	78	67	*******	Not mapped	172
FAE1P1_31	25	84	63	*******	Not mapped	172
FAE1P2_31	81	27	64	*******	Not mapped	172
CER10_31	71	18	83	*******	A09	172
AB13_31	69	54	49	***	A09	172
PkI_21	59	48	65	*******	Not mapped	172

Notes: **: significant at 0.05; ***: significant at 0.01; ****: significant at 0.005; *****: significant at 0.001; ******: significant at 0.0005; *******: significant at 0.0001; - : not significant

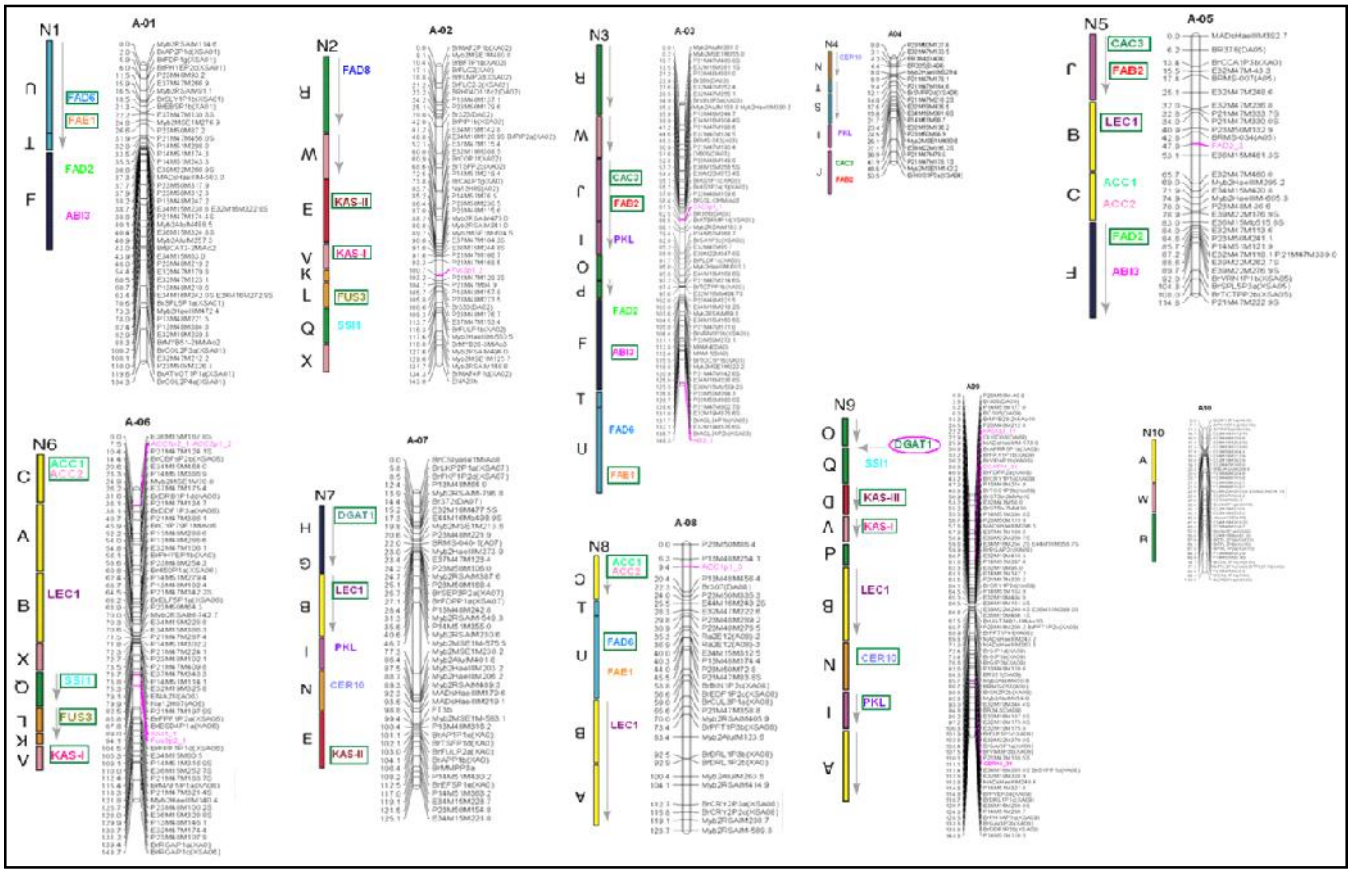

Fig. 1 : The linkage map of 10 B. rapa linkage group with their map position in centiMorgan (cM) and text on right side of linkage map indicates marker name. The markers developed in this project and were mapped on different linkage group indicated with pink colour. The markers boxed with green colour are expected to map on particular linkage group based on Chiifu sequence. The marker DGAT1 indicated with pink circle was expected to map on A09 based on Chiifu sequence but block corresponding to this marker is missing in A09

of DH68 population in that region due to the less meiotic events in DH population as compared to recombinant inbred lines (RILs) population. However, RILs population is not easy to create and maintain due to self-incompatibility of most B. rapa accessions. Third, it might be due to shortage of time, markers belongs large gap region were not scored. Fourth, polymorphic lines may not exist due to loss of DNA/seed material in the past or due to self-incompatibility of DH lines, there are no seeds and we lost these lines. Fifth, some DH lines did not respond to microspore culture and therefore, could not survive.

The 18 markers were not mapped in DH68 population. One of the possible reasons might be high segregation distortion with missing values. In all these markers, PC-175 had the highest allele frequency. In DH population, expected segregation ratio is 1:1 (Lou *et al.*, 2008). The deviation of markers (distorted markers) from expected segregation ratio arisen may be due to the natural selection during microspore culture because some lines are sensitive and can't survive. Another reason could be loss of DNA of some lines in past. Self-incompatibility might also be responsible for segregation distortion by not producing the seeds.

Conclusion :

The location of genes related to the different enzymes of fatty acid biosynthesis pathway of *Arabidopsis* were predicted in different linkage groups as well as in genomic blocks based on homology with Chiifu sequence and genomic block synteny. However, the comparative mapping based on Chiifu sequence and block position defined using segmental co-linearity is more reliable. In addition, the chromosomal region of A02 extending from top to middle will be interesting region to find the candidate genes and transcription factors for regulation of saturated fatty acids. The genes KAS-I and KAS-II might be candidate genes for synthesis of

saturated fatty acids. The markers FUS3P1 mapped at the middle of A02 might be involved in regulation of genes on A02 and responsible for synthesis of saturated fatty acids.

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