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Full Length Research Paper

Identification of AFLP markers linked with cocoon weight genes in silkworm (*Bombyx mori* L.)

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DNA markers used in assisting selection method is a safe method in breeding process, due to deletion of environmental conditions, and it is an important tool in preparing linkage map and QTLs mapping. In mulberry silkworm that is, foundation of world sericulture, its major production- economic characteristics are polygenic. In this study, we want to determine QTL(s) affecting cocoon weight trait by AFLP markers. For this reason, we used 20 selected primer combinations from among 81 primers combinations of *PstI*/*TaqI* at the level of three F2 populations including 33, 36 and 34 offsprings sample, respectively. These populations were obtained by crossing two lines of Lemon Khorasan (as maternal) and 107 (as paternal). The parental lines, F1 and F2 individuals' DNA were extracted with phenol-chloroform method. Then they were digested by two restriction enzymes (*TaqI* and *PstI*) and amplified by using of appropriate adaptors. These amplified samples are transfered on annealed 6% polyacrylamide gels. After genotyping of individuals, the linkage maps of populations were drawn by Map manager/QTX and QTL Cartographer ver.2.5 softwares. Number of total and polymorphic bands that formed to 20 primer combinations in each populations were 930, 944, 810 and 142, 171, 178 bands, respectively. Therefore polymorphic frequencies were 15.27, 18.11 and 21.97%. The obtained linkage maps were included in 16, 18 and 24 linkage groups. The total length of this linkage maps and average distance between two markers were 2186.40, 2582.50 and 2392.60 cM, and 18.37, 16.45 and 14.95 cM, respectively. The detection of QTLs numbers of cocoon weight character in each F2 populations also showed 1, 6 and 1 loci in LRS>17 (LOD > 3.7) by compound interval mapping methods, respectively.

Key words: Silkworm, QTL analysis, linkage map, molecular markers and AFLP.

INTRODUCTION

Considering the increasing world population on one hand, and limited financial resources on the other hand, inventing new methods based on scientific principles and technology enhancements in different dimensions is necessary in order to increase food production which is needful for life. This industry has development capabilities because it has different potentials such as: job creation, profitability, increasing GDP and available fixed capitals (skillful men, moriculture, mulberry, and silkworm gene banks, rearing rooms, carpet broadloom, etc.) in the country. The reasons for these potentials are its thousand years of existence, history, new application fields and obtained advances in the biotech field. Also, it is used as bioreactor for production of recombinant proteins (Tomita et al., 2003) and in pest control.

The mulberry silkworm is an organism in insect class, Lipdoptera, Bombycidae and *Bombyx mori* (Tazima, 2001). This insect is the most important economic organism that sericulture industry is dependent on, to produce natural silk (Nguu et al., 2005). This is because more

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Abbreviations: QTLs, Quantitative traits loci; **RFLP,** restriction fragment length polymorphism; **AFLP,** amplified fragment length polymorphism; **PCR,** polymerase chain reaction; **CIM,** composite interval mapping; **LOD,** logarithmic of odds score; **LRS,** likelihood ratio statistic;**dNTPs,** deoxynucleoside 5'-triphosphate.

natural silk of the world is obtained by rearing of this insect that feeds only on mulberry plants (Zhan et al., 2009). In addition to this, we use it in basic and applied research widely. Genomic studies on this insect lead us to different linkage maps based on morphological (Doira, 1992) and different molecular markers (Tan et al., 2001). Today, the survival of this industry depends on increasing quantitative and qualitative production and diversifying of silk products. Also, silk will be increased if we use scientific rearing principles and genetic/breeding knowledge. Before this, for breeding and achievement of commercial lines, we used different selection strategies based on different traits amounts (Seidavi et al., 2008), selection index (Ghanipoor et al., 2006), control of selection severity and cross between breeded lines (Mirhoseini et al., 2004).

There are more than 3000 silkworm lines in the world. Many genotypes have same phenotype in spite of unique genetic characteristics or the same genotypes can be having different phenotype under different ecological conditions. Such similarities and differences have caused problems for silkworm breeders (Nagaraju and Singh, 1997). Using DNA markers in plants and animals breeding has created a new theme in agricultural section as molecular breeding.

Important economic traits in silkworm such as cocoon weight, cocoon shell weight, cocoon shell percentage, length and thickness of silk, reproduction and resistance are quantitative traits. This is because each of these traits is affected by many small genes with small effects and environmental conditions. Our information is insufficient on the number of genes of these traits and their QTLs and products. The identification of these loci with appropriate molecular markers that have such close conjunction with desired traits or characteristics is required to access the information, to reduce choice risk and to prevent waste of fund and time (Nagaraju and Goldsmith, 2002).

Today, molecular breeding is considered as a major success in plant and animal species (Stuber et al., 1987; Williams, 2003). At present, many plant and animal breeding centers accept such capacity for development and selection by markers.

Mulberry silkworm is an important economic resource for about 30 million farmers in the world (Miao et al., 2005). This insect has various applications in pharmaceutical sector (Tomita et al., 2003), agriculture and medicine industry (Yamamoto et al., 2006). Cocoon weight and shell weight are the most important or main traits evaluated for productivity in sericulture and these characters have been used for breeding more than half a century (Gaviria et al., 2006). The genomic study on silkworm has recently begun by molecular markers. Following them, some researches have been done by sericultural researchers. Now, different linkage maps and QTL(s) have been presented by using of various silkworm segregation populations and molecular markers methods such as: AFLP (Tan et al., 2001; Lu et al., 2005), RFLP (Goldsmith, 1991), SSR (Damodar et al., 1999), SNP (Yamamoto et al., 2006). Silkworm breeding in this way is still in its early stages. Because the formation of high density Linkage map will encounter some difficultly, such continuity plans will include more genes loci (Sima et al., 2006).

Amplified fragment length polymorphism (AFLP) method is a polymerase chain reaction (PCR) based technique that avoids the laborious steps involved in other methods such as restriction fragment length polymorphism (RFLP). It generally shows a much higher level polymorphisms production potency and informativeness. Therefore, we used this method for finding linked markers of cocoon weight loci by composite interval map-ping analysis in this study.

MATERIALS AND METHODS

The determination of quantitative traits loci (QTLs) involves two steps: a) Linkage map constraction and b) QTL analysis.

a) Linkage map construction

Linkage map construction presents the staring point for further molecular-based research on silkworm. The map builds the foundation for thoroughly exploring the entire genome represented by a large number of mapped AFLP markers. It creates a framework for anchoring morphological or other molecular markers and identifying of quantitative trait loci (QTL) for taxon-diagnostic, geographically varying and economically important traits. This map also can be utilized to locate genes of interest and to develop DNA probes, SNPs, STS/expressed sequence tags or other codominant DNA markers with a wider range of applications (Wang and Porter, 2004).

The three steps of linkage map constraction are: 1. Production of a mapping population; 2. identification of poly morphism; and 3. linkage analysis of markers (Collard et al., 2005).

Production of a mapping population

The parents selected for mapping population must differ for one or more traits of intrest. In a segregation population, there is a mixture of parental and recombinant genotypes. The frequency of recombinant genotypes can be used to calculate recombination fractions which may be used to infer the genetic distance between markers. By analyzing the segregation of markers, the relative order and distance between can be determined (Collard et al., 2005). This study focused on F2 populations. The advantage of this kind of population is its easy availability (time and cost).

i) Silkworm materials

Individuals from pure-breeding families of two species, Khorasan pink (Iranian local race that has a lower weight of cocoon, as maternal) and P107 (breeded Japonese line that has a higher weight of cocoon, as paternal) were randomly crossed to produce F1 families. Then, some of their pairs are randomly selected to produce three F2 populations (for determination more QTLs). The crossing experiments were performed in the Iran Silkworm Research Center, located in Rasht. It must be noticed that all rearing stages are done in standard conditions. Mapping was also performed with

Table 1. Adapters used in AFLP analysis.

Name		Sequence
Adapters/*Pst*I	*Pst*I top strand	5′-GACGTGACGGCCGTCATGCA-3′
	*Pst*I bottom strand	3′-GCACTGCCGGCAGT-5′
*Taq*I/Adapters	*Taq*I top strand	5′-GACGATGAGTCCTGAG -3′
	*Taq*I bottom strand	3′-TACTCAGGACTCGC-5′

Table 2. Preamplification primers used in AFLP analysis.

Name		Sequence
*Pst*I	P_{000}	5′-GACGGCCGTCATGCAG-3′
*Taq*I	T_{000}	5′-GATGAGTCCTGAGCGA-3′

Table 3. Selective amplification primers (based on bolded selective nucleotide) Used in AFLP analysis.

Name		Sequence
*Pst*I	P_1	5′-GACGGCCGTCATGCAG**TA**
	P_2	5′-GACGGCCGTCATGCAG**AT**
	P_3	5′-GACGGCCGTCATGCAG**TC**
	P_4	5′-GACGGCCGTCATGCAG**AC**
	P_5	5′-GACGGCCGTCATGCAG**AAC**
	P_6	5′-GACGGCCGTCATGCAG**AAG**
	P_7	5′-GACGGCCGTCATGCAG**AGA**
	P_8	5′-GACGGCCGTCATGCAG**ATG**
	P_9	5′-GACGGCCGTCATGCAG**TAT**
*Taq*I	T_1	5′-GATGAGTCCTGAGCGA**TA**
	T_2	5′-GATGAGTCCTGAGCGA**AT**
	T_3	5′-GATGAGTCCTGAGCGA**TC**
	T_4	5′-GATGAGTCCTGAGCGA**TG**
	T_5	5′-GATGAGTCCTGAGCGA**AAT**
	T_6	5′-GATGAGTCCTGAGCGA**ACA**
	T_7	5′-GATGAGTCCTGAGCGA**AAG**
	T_8	5′-GATGAGTCCTGAGCGA**AGC**
	T_9	5′-GATGAGTCCTGAGCGA**TAC**

three F2 populations including 33, 36 and 34 male individuals, respectively. Because genetic recombination occurs only in males in *B. mori* and other lepidopteran insects, therefore this Lepidoptera-related phenomenon leads to the fact that marker loci derived from the female and male parents cannot be integrated into a single map (Tan et al., 2001). As a result, we used male individuals only.

ii) Quantitative data

Cocoon weight was determined in each case. An electronic balance was used and the data were obtained to two significant figures.

Identification of polymorphism

It is critical that sufficient polymorphism exists between parents in order to construct a linkage map (Young, 1994). In many cases, parents that provide adequate polymorphism are selected on the basis of the level of genetic diversity between parents (Collard et al., 2005). When we identified polymorphic markers, they must be screened across the entire mapping population, including the parents (and F1). This is known as marker "genotyping" of the population.

i) AFLP analysis

Genomic DNA of individual silkworms was extracted from moth as described by Suzuki et al. (1972) and as modified by Nagaraja and Nagaraju (1995). In this way it was purified by extraction with phenol/chloroform, precipitated by ethanol and resuspended in TE buffer (10 mm Tris-HCl, pH 8.0, 1 mm EDTA).

AFLP analysis was conducted according to the procedures and reaction conditions described by Vos et al. (1995) with some modifications. The used restriction enzymes were *Pst*I (six base cutters) and *Taq*I (four base cutters). According to report of Tan et al. (2001), these enzymes can produce polymorphic DNA fragments in silkworm.

The adaptors, preamplification primers and selective amplification used in AFLP analysis are seen in Tables 1 - 3.

Among 81 selective amplification primer, combinations are tested on parents, only 20 primers combinations had the best polymerphisms. Therefore, we used these combinations in our experiment.

The 50 µl digestion reaction system contained 10 µl (50 ng/µl) extracted DNA, 0.5 µl (10 u/µl) of restriction enzymes, 5 µl (10X) PCR buffer and 34 µl double distilled H_2O. Then this mixture was stored at 37°C in 17 - 18 h.

The 50 µl ligation reaction system contained 40 µl digested DNA, 1 µl (50PM/µl) *Taq*I adapter, 1 µl (5PM/µl) *Pst*I adapter, 1 µl (1 u/µl) T4 DNA ligase, 1 µl (10X) PCR buffer and 5 µl double distilled H_2O.

The 25 µl PCR preamplification reaction system contained 2 µl of digested and ligated DNA diluted 1:5 V/V double distilled (ddH_2O), 1 µl (60 ng/µl) of both primers (*Pst*I-P_{000} and *Taq*I-T_{000}), 0.2 µl (5 u/µl) *Taq* DNA polymerase, 2.5 µl (2 mM) of dNTPs, 1 µl (50 mM) $MgCl_2$, 2.5 µl (10X) PCR buffer and 14.8 µl ddH_2O. The preamplification reaction conditions and steps employed were as described by Vos et al. (1995). After this step, we diluted preamplificated mixture 1:5 V/V with double distilled H_2O (ddH_2O).

Now, the 15 µl PCR selective amplification system contained 2 µl of product from the diluted preamplification reaction, 0.6 µl (60 ng) of both selective primers, 0.2 µl (5 u/µl) of *Taq* DNA polymerase, 1.5 µl (2 mM) of dNTPs, 0.6 µl (50 mM) $MgCl_2$, 1.5 µl (10X) PCR buffer and 8 µl ddH_2O. All amplification reactions were performed in

Touch gene model thermocycler.

ii) Gel analysis

The products of the end step of AFLP method were added with loading buffer (formamide 10 ml, Xylene cyanol FF 10 mg, parameters were set to 75 watt, 50℃ and a run time of 1.5 - 2.0 h.

Linkage analysis of markers

This step involves coding data for each DNA marker on each individual segregated population (F2 offspring) that follows by using computer programs (Collard et al., 2005).

i) Coding

Gel images were scored by using a binary scoring system as 1 and 0 (presence and absence of bands respectively). Then these extracted information were inverted to codes D and B or C and A, according to SIS or TRANS dominant polymorphic markers in MAPMANAGER/QTX software manual (Manly et al., 2001).

ii) Mapping

The MAPMANAGER/QTX software program for mapping needs markers order and distance between them along a linkage group. It is done in terms of the frequeny of recombination between genetic markers (Paterson et al., 1996a). Because recombination frequeny and crossing over frequeny are not linearly related, we used kosambi mapping function (which assumes that recombination events influence the occurrence of adjacent recombination events).

b) QTL analysis

This step is based on the principle of detecting an association between phenotype and the genotype of markers. Markers are used to partition the mapping population into different genotype groups based on the presence or absence of a particular marker locus and to determine whether significant differences exist between groups with respect to the trait being measured (Tanksley, 1993). A significant difference between phenotypic means of the groups, depending on the marker system and type of population, indicates that the marker locus being used to partition the mapping population is linked to a QTL controlling the trait (Collard et al., 2005).

Detecting QTLs

The different methods can be used for detecting QTLs. An important and widely-used method is Composite Interval Mapping (CIM). This method combines interval mapping with linear regression and includes additional genetic markers in the statistical model in addition to an adjacent pair of linked markers for interval mapping (Zeng, 1994). The advantage of CIM is that it is more precise and effective at mapping QTLs compared to other method, especially when linked QTLs are involved. In this study, we used QTL Cartographer ver 2.5 (Wang et al., 2007) to perform CIM. The results of test statistic for CIM (as profile of likely sites for a QTL between adjacent linked markers) are presented using a logarithmic or LRS profiles are used to identify most likely position for a QTL in relation to the linkage map, which is the position where the highest of odds (LOD) score or likelihood ratio statistic (LRS). These LOD LOD value is obtained (Collard et al., 2005).

RESULTS

AFLP markers polymorphic distribution

The total AFLP bands appeared on electrophoresis gels per 20 primer combinations on all F2 individuals which were 930, 944 and 810. That gave us an average of 46.5, 47.2 and 40.5 bands per primer combinations for each population, respectively. Among total bands there were 142, 171, 178 polymorphic bands or there were 15.27, 18.11 and 21.97% polymorphism and on average, each primer combination generated 7.1, 8.55, 8.9 polymorphic fragments that conformed to a 3:1 segregation ratio, respectively. These amounts of polymorphic AFLP markers were distributed between 95 - 595 bp.

Linkage map construction and its characteristics

We obtained an AFLP linkage map for each F2 populations of *B. mori* but 119, 157, 160 polymorphic bands were allocated to each linkage maps, respectively. The others 23, 14 and 18 loci conforming to the 3:1 segregation ratio were not able to link and could not be used to construct linkage maps. Each linkage maps included 16, 18 and 24 linkage groups, with total length of 2186.40, 2582.50 and 2392.60 cM. And the average length of a linkage group in each linkage maps was 136.65, 143.47 and 99.69 cM. Meanwhile, number of markers in each three linkage maps varied from 2 to 48; 2 to 30 and 2 to 39. The average distance between markers also was 18.37, 16.45 and 14.95 cM, respectively.

Determined QTLs

Now, we used these frameworks (linkage maps) for anchoring of markers and distinguishing of important QTLs for different propose. As valued that cocoon weight is a very important economic trait in sericulture industry (Gaviria et al., 2006), therefore we tried to define QTL(s) present in three groups of F2 individuals linking it. In this relationship, the CIM statistic test detected to us 1, 6 and 1 QTLs that were placed on 11; 3, 9, 10, 13, 16 and 11 linkage groups (or chromosomes). The percentage of phenotypic variation explained by a QTL (R^2) for cocoon weight trait were 77.4, 0.019 - 77.4 and 33.7% in each studied segregation F2 populations, respectively (Table 4). These QTLs are designated as *cw*[x/y], where *cw* is cocoon weight, x is the linkage group and y is the AFLP marker closest to QTL (e.g: *cw*1/15).

It must be noticed that we used LOD > 3.7 or LRS = LRT = 17 as appropriate minimum threshold level for QTLs detection, based on permutation test (n = 1000) (Churchili and Doerge, 1994). According to Table 4 and h values (Stuber et al., 1987) in it, more detected QTLs in three F2 populations (7 no.) have underdominant or recessive effects ($h < 0$) but only one QTLs has over dominant

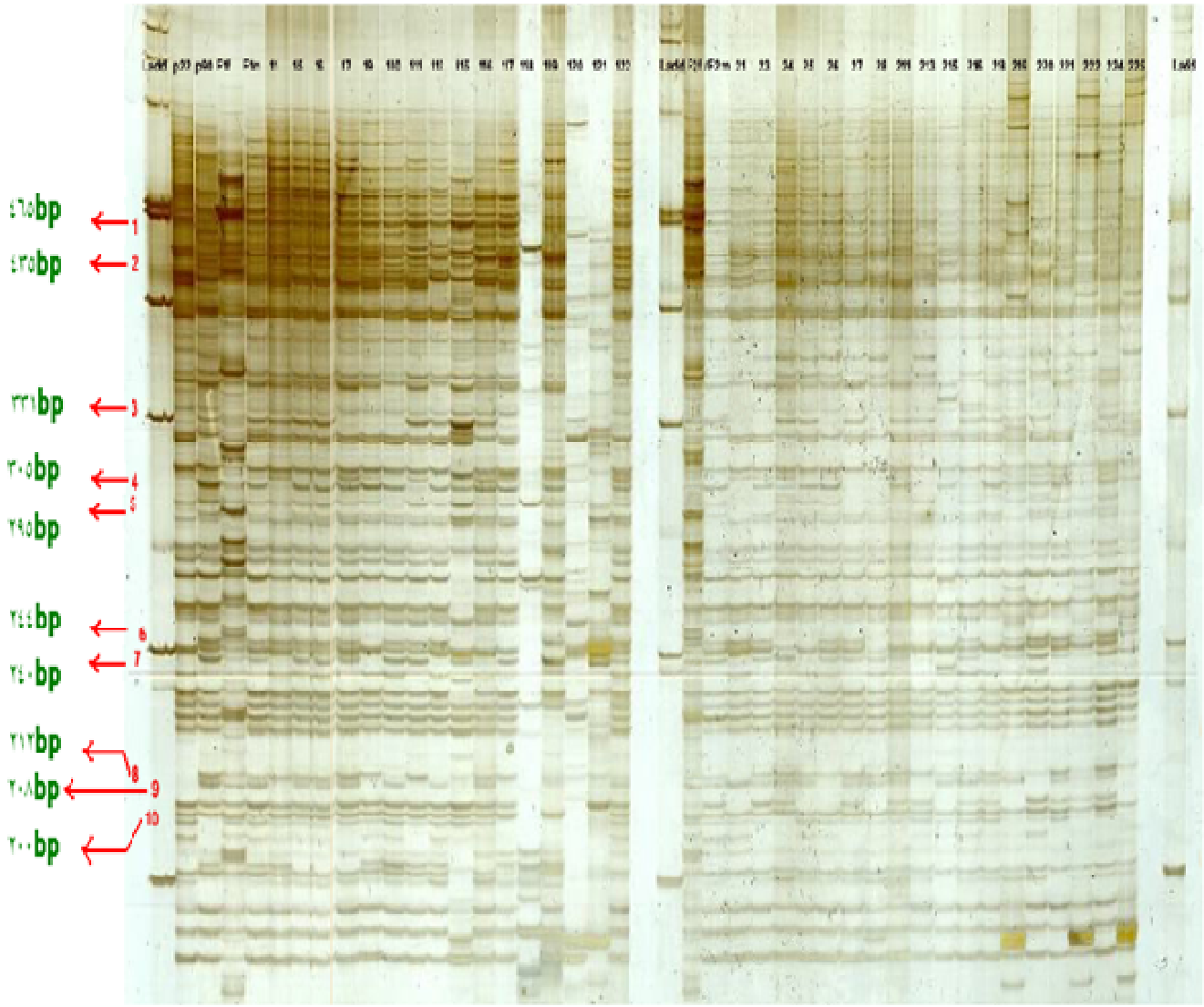

Figure 1. A portion of an AFLP polyacrylamide gel that generated by using T_{AAG} - P_{TA} Primer pair. Ladd equal Ladders or size marker, P equal to parent, F1 equal first generate and numbers on up side show us F2 individuals. Polymorphic bands are indicated by arrows on the left. The numbers on left side are size of each polymorphic band.

effects on expression of cocoon weight trait (h > 1.20).

DISCUSSION

AFLP markers are excellent to applied variety of agronomically important organisms breeding because they have been widely employed to construct linkage maps and to map genes in them (Tan et al., 2001). More studies in sericulture show that cocoon weight trait is most important component to economic evaluating for breeding (Gaviria et al., 2006).

Statistical threshold is the most important in QTL analysis because the numbers of detected QTLs would be different when using different thresholds in QTL analysis. Most thresholds employed in published QTL analysis have been between LOD 2 and 3. In this paper, our goal was to identifiy all possible putative QTLs, given that the F2 linkage map constructed is not saturated (Okogbenin et al., 2008). We used a threshold of LOD > 3.7 for QTL mapping based on permutation test (n = 1000). Also we applied three F2 populations in here. Because these kinds of populations have non-stable status in comparison together, however they have been originated from same parental lines. But its main advantage will be to detect more QTLs.

Negative and positive h values of the QTLs show that the recessive and over-dominant alleles controlled cocoon weight trait of mulberry silkworm and the alleles of identified QTLs which affected cocoon weight were transmitted to progenies from both parents. In total, the QTLs in each F2 populations which could be mapped,

Table 4. The determined QTLs of cocoon weight trait in three F2 populations of mulberry silkworm by Composite Interval Mapping (CIM) statistic test method at minimum threshold LRS (LRT) > 17.

Trait name	Linkage group	Nearest marker to QTL*	Adjacent markers to QTL	QTL	Likelihood Ratio Test (LRT)	Addetive effect (H1:a)	Dominant effect (H2:d)	h**	R^2
First F2 Population									
Cocoon Weight(CW)	11	1	TP05/260-TP20/630	*cw*11/1	19.934	0.0762	0.1489	1.954	0.156
Second F2 Population									
Cocoon Weight(CW)	3	1	TP01/295-TP08/395	*Cw*3/1	20.58	0.1902	- 0.2126	- 1.117	0.774
	9	2	TP15/111-TP19/172	*cw*9/2	18.552	0.1629	-0.2281	- 1.400	0.469
	10	17	TP05/190-TP20/570	*cw*10/17	17.342	- 0.0333	0.2528	- 7.591	0.019
	13	2	TP01/220-TP06/205	*cw* 13/2	17.830	- 0.0402	0.2652	- 6.594	0.029
	13	4	TP11/162-TP01/205	*cw* 13/4	17.919	- 0.0468	0.2651	- 5.676	0.039
	16	22	TP18/140-TP03/200	*cw* 16/22	17.276	0.0894	- 0.2133	- 2.385	0.145
Third F2 Population									
Cocoon Weight(CW)	11	2	TP08/135-TP08/285	*cw* 11/2	20.832	- 0.1491	0.1850	- 1.241	0.337

*The nearest place marker to QTL desired controller from the beginning side of linkage group.
**: h is equal to the ratio dominance/additive effects. So if h < 0, it show that QTL has underdominance or recessive effect on trait, if h = 0 - 0.20, it means that it has additive effect, if h = 0.21 - 0.80, it has partial dominance effect, if h = 0.81 - 1.20, it also has dominance effect and finally, if h > 1.20, it means that QTL has over dominant effect on trait (Stuber et al., 1987).

accounted for 15.6, 125 and 33.7% of phenotypic variance explained, respectively. The remaining variation in 1st and 3rd populations which could not be explained by the QTL model may be due to undetected QTLs with too small effects that are not resolvable by this experiment, interaction between these QTLs and interaction of individual F2 genotypes with environment in the experiment (Okogbenin et al., 2008).

Some identified QTLs have small effects because they are dependent upon interaction with other loci. It must be noticed for more detecting of QTLs with small effects, we will need more number of progeny.

But additional variation in 2nd population that is explained by detected QTLs is due to additive effects between QTLs or error. Therefore, it is necessary that we repeat experiment with more primers and individuals.

On the basis of QTLs' tables (Table 4), we can conclude that content genomes (amount and combination) in each F2 generation families are different. It may be due to several factors: First, Lemon Khorasan and 107 parents that are two distinct silkworm lines. The former is Iranian bivoltine and latter is from Japanese bivoltine races. Second, the crossing overacts as a variation apparatus in here as others animals. Third, a large fraction of the silkworm genome consists of families of transposable elements and the distribution of these elements has varied between silkworm strains (Tan et al., 2001).

In recent years some studies were done in order to identify and characterize QTLs and genes controlling economical traits of silkworm (Tan et al., 2001; Lu et al., 2005; Sima et al., 2005; Li et al., 2006).

Conclusion

Ultimatly, these results can be used in marker assisted

selection or gene transmission in breeding programs after confirming these QTLs by other mapping populations and other molecular marker methods.

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REFERENCES

Chalhoub BA, Thibaclt S, Laucou V, Rameau C, Hofte H, Cousin R (1997). Silver staining and recovery of AFLP(TM) amplification products on large denaturing polyacrylamide gels. Biotechniques, 22: 216-218.

Churchili GA, Doerge RW (1994). Empirical threshold values for quantitative trait mapping. Genetics, 138: 963-971.

Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica, 142: 169-196.

Damodar K, Abraham EG, Nagaraju J (1999). Microsatellite in the silkworm, *Bombyx mori*: Abundance, polymorphism and strain characterization. Genome, 42: 1057-1065.

Doira H (1992). Linkage Maps and List of Genetical Stocks Maintained in Kyushu University (Institute of Genetic Resources, Kyushu University, Fukuoka, Japan).

Gaviria DA, Aguilar E, Serrano HJ, Alegria AH (2006). DNA fingerprinting using AFLP markers to search for markers associated with yield attributes in the silkworm, *Bombyx mori*. J. Insect Sci. 6(15): 1-10.

Ghanipoor M, Mirhosseini SZ, Shadparvar A, Seidavi AR, Bizhannia AR (2006). Comparison of different selection indices for genetic improvement of economic traits in silkworm, (*Bombyx mori* L.) Lines. Sericologia, 46(2): 137-143.

Goldsmith MR (1991). The *Bombyx mori* genome-mapping project, Sericologia, 31: 145-155.

Li B, Lu C, Zhao AC, Xiang ZH (2006). Multiple interval mapping for whole cocoon weight and related economically important traits QTL in silkworm (*Bombyx mori*). Agric. Sci. China, 5(10): 798-804.

Lu C, Li B, Xiang Z (2005). QTL mapping of economically important traits in silkworm (*Bombyx mori*). Yi Chuan. 27(3): 372-376.

Manly KF, Cudmore JR, Meer JM (2001). Map Manager QTX: cross platform software for genetic mapping. Mamm. Genome, 12: 930-932.

Miao XX, Xu SJ, Lia MH, Lia MW, Huang JH, Dai FY, Marino SW, Mills DR, Zeng P, Mita K, Jia SH, Zhng Y, Liu WB, Xiang H, Guo QH, Xu AY, Kong XY, Lin HX, Shi YZ, Lu G, Zhng X, Huang W, Yasukochi Y, Sugasaki T, Shimada T, Nagaraju J, Xiang ZH, Wang SY, Goldsmith MR, Lu C, Zho GP, Huang YP (2005). Simple sequence repeat-based consensus linkage map of *Bombyx mori*. PNAS 102: 16303-16308.

Mirhoseini SZ, Bizhnnia AR, Vishkaei S, Seidavi AR (2004). Estimation of combining ability and heterosis of two biological chracters of Iranian native and improved silkworm breeds. Proceeding of biology in Asia international conference Singapore.

Nagaraja GM, Nagaraju J (1995). Genome fingerprinting in silkworm *Bombyx mori*, using random arbitrary primers. Electrophoresis, 16: 1633-1638.

Nagaraju IG, Singh L (1997). Assessment of genetic diversity by DNA profiling and its sighnificance in silkworm, *Bombyx mori,* Electrophoresis, 18: 1676-1681.

Nagaraju J, Goldsmith MR (2002). Silkworm *Bombyx mori,* Electrophoresis, 18: 1676-1681.

Nguu EK, Kadono-Okuda K, Mase K, Kosegawa E, Hra W (2005). Molecular linkage map for the silkworm, *Bombyx mori,* based on restriction fragment lenghth polymorphism of cDNA colones. J. Insect Biotechnol. Sericol. 74: 5-13.

Okogbenin E, Marin J, Fregene M (2008). QTL analysis for early yield in a pseudo F2 population of cassava. Afr. J. Biotechnol. 7(2): 131-138.

Paterson A, Tanksley S, Sorrels ME (1996a). DNA markers in plant improvement. Adv. Agron. 44: 39-90.

Seidavi AR, Bizhnnia AR, Ghanipoor M, Qotbi AAA (2008). Investigation on Improvement possibility of resistance, production and reproduction traits in 3P, 2P and P generations in three Japanese pure lines of silkworm *Bombyx mori* L., using individual selection in 3P generation. Asian J. Anim. Vet. Adv. 3(6): 443-447.

Sima YH, Li B, Chen DX, Sun DB, Zho AC, Zhng L, Lu C, He SM, Xiang ZH (2006). Construction and analysis of an AFLP molecular linkage map of the silkworm (*Bombyx mori*). Chin. J. Agric. Biotechnol. 3(1): 25-31.

Sima YH, Li B, Xu HM, Chen DX, Sun DB, Zhao AC, Lu C, Xiang ZH (2005). Study on location of QTLs controlling cocoon traits in silkworm. Acta genetica Sinica 32(6): 625-32.

Stuber CW, Edwards MD, Wendel JF (1987). Molecular markers facilitated investigations of quantitative trait loci in maize. II. Factors influencing yield and its component traits. Crop Sci. 27: 639-648.

Suzuki Y, Gage LP, Brown DD (1972). The genes for fibrion in *Bombyx mori*. J. Mol. Biol. 70: 637-649.

Tan YD, Wan C, Zhu Y, Lu C, Xiang Z, Deng HW (2001). An amplified fragment length polymorphism map of silkworm. Genetics, 157: 1277-1284.

Tanksley SD (1993). Mapping polygenes. Annu. Rev. Genet. 27: 205-233.

Tazima Y (2001). Silkworm, an important laboratory tool. Kodash LTD. Tokyo.

Tomita M, Munetsuna H, Sato T, Adachi T, Hino R, Hyashi M, Shimizu K, Nakamura N, Tamura T, Yoshizato K (2003). Transgenic silkworms produce recombinant human type III procollagen in cocoons. Nat. Botechnol. 21: 52-56.

Vos P, Hogers R, Bleeker M, Reijans M, Lee TVD, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995). AFLP a new technique for DNA fingerprinting. Nucleic acids Res. 23: 4407-4414.

Wang B, Porter AH (2004). An AFLP- based interspecific linkage map of sympatic, hybridizing Colias Butterflies. Genetics, 168: 215-225.

Wang S, Basten CJ, Zeng Z (2007). Windows QTL caetographer version 2.5. Department of Statistics. NorthCarolina state university. Raleigh, NC.

Williams KJ (2003). The molecular genetics of disease resistance in barley. Aust. J. Agric. Res. 54: 1065-1079.

Yamamoto K, Narukawa J, Kadono-Okuda K, Nohta J, Sasanuma M, Suetsugu Y, Banno Y, Fujii H, Goldsmith MR, Mita K (2006). Construction of a Single Nucleotide Polymorphism Linkage Map for the Silkworm, *Bombyx mori,* Based on Bacterial Artificial Chromosome End Sequences. Genet. Soc. Am. 173: 151-161.

Young ND (1994). Constructing a plant genetic linkage map with DNA markers, P. 39-57. In: Ronald IKV and Philips L (Eds), DNA-based markers in plants. Kluwer, Dordrecht/Boston/London.

Zeng ZB (1994). Precision mapping of quantitative trait loci. Genetics, 136(1): 457-468.

Zhan S, Huang J, Guo Q, Zhao Y, Li W, Miao X, Goldsmith MR, Li M, Huang Y (2009). An integrated genetic linkage map for silkworm with three parental combinations and its application to the mapping of sigle genes and QTL. BMC Genomics, 10: 389.