Pak. J. Bot., 48(4):1579-1584, 2016

LINKAGE MAP CONSTRUCTION AND QTL ANALYSIS OF FRUIT TRAITS IN MELON (*CUCUMIS MELO* L.) BASED ON CAPS MARKERS

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Abstract

In the current experiment, the quantitative trait loci (QTL) analysis was done by composite interval mapping method to detect QTLs in edge, central parts and fruit shape of melon. In this context, 235 F_2 populations along with their parents were evaluated for fruit size, shape and color under replicated trail at Horticulture Experimental Station of Northeast Agricultural University, Harbin, China, during the growing year 2014. Moreover, 96 pairs of CAPS markers were used to construct a linkage map using F_2 population that was derived from the cross between two contrasting parents (MR-1 and Topmark). The total length of linkage map was found to be 4984.1cM with an average of 51.9177 cM between the markers. In a total, we detected ten QTLs, in which one was major, while others were minor. Five QTLs were detected in the edge part of melon fruit and three QTLs were detected in central parts of melon and all were considered as Brix content. Two QTLs were related with fruit shape. Our present genetic and QTLs mapping would be proved useful in plant breeding programs for the improvement of economically important horticultural traits.

Key words: Linkage map, CAPS marker, QTLs, Melon.

Introduction

Melon vegetable crop (*Cucumis melo* L.) is one of the most important vegetable crop in the world, especially in Asia, and is grown in temperate as well as in warm climates. This crop belongs to cucurbitaceae family that also includes pointed gourd, ash gourd, pumpkin, watermelon and squash,(Sebastian *et al.,* 2010). The carotenoids are natural class of compounds that are produced by most important photosynthetic living organisms, and are essential for both animals as well as for plants (Wong *et al.,* 2004). Melon is a highly nutritious vegetable crop and is an excellent source of natural β-carotene (Vitamin A). These carotenoid pigments such as β-carotene and α-carotene are important for human health (Mares-Perlman *et al.,* 2002) and carotenoids (e.g., β-carotene and lycopene) are known to prevent chronic diseases (Sugiura *et al.,* 2008). Due to its biological characteristics such as fruit ripening, the melon has become an attractive model crop for genetic studies when compared to other cucurbit crops (Pech *et al.,* 2008). The melon possesses high genetic variation within species, the morphologic diversity as well as the small genomic size; all these characteristics make it more useful for the molecular and genetic studies (Stepansky *et al*., 1999). Relating to scientific and the economic interest various genetic and molecular tools are developed that also include linkage maps (Díaz *et al.,* 2011). Several integrated genetic maps have been constructed in economically important horticultural and agronomical crop species such as grapevine (Vezzulli *et al.,* 2008), lettuce (Truco *et al.,* 2007) and maize (Falque *et al.,* 2005). The linkage maps are powerful tools that link genes and horticultural traits, and the high-density genetic linkage maps are useful for positioning and tagging genes of interest to facilitate marker-assisted breeding, and are also useful in gene cloning (Lee, 1995). The restriction fragment length polymorphism markers were used to construct first molecular marker-based genetic map in melon in (Baudracco-Arnas & Pitrat, 1996). The first genetic map positioned markers on 12 linkage groups (LG) were constructed few years later in Asia by using F_2 population of melon (Oliver *et al.,* 2001). Linkage maps are required for deep studies of plant genome (e.g. for dissecting quantitative traits, map-based cloning of agronomic traits and comparative genome studies among related species). The deep and thorough analysis of quantitative trait loci (QTLs) requires a detailed molecular marker genetic maps (Tanksley, 1993), and this analysis has been proved to be useful in identifying different genetic components of variation in economically important horticultural and agricultural crop traits. Molecular markers such as DNA and isozymes are useful tools to remove the undesirable traits in plant breeding program. Cleaved amplified polymorphic sequence (CAPS) is the latest marker for crops species and this marker can also be developed from a polymorphic restriction site that exists in PCR products (Konieczny & Ausubel, 1993). Our present study was aimed to construct the linkage map and QTLs mapping in melon crop. We believe that this study will be proved useful for plant breeders mainly working in melon population.

Materials and Methods

Source of plant materials: In this study, we used 235 F_2 plants that were obtained from the cross of two different parental lines (MR-1 and Topmark). Topmark was developed by USDA (Thomas, 1986), this parental line is slow growing, having small fruit that produces light green mesocarp with black stripes, while MR-1 has been characterized as fast growing plant, the fruit is produced in round shape with yellow color (mesocarp). The F_1 plants were allowed to self-pollinate and subsequently F_2 generation was produced.

Plastic house evaluation, experimental design and hand pollination:F_2 populations including parents were evaluated in plastic greenhouse during the summer season at Horticulture Experimental Station of Northeast Agricultural University, Harbin, China (44°04'N, E125°42') from mid-April to the end of August, 2014. Two parents (described above) and F_2 generation each were grown in three replications using randomized complete block design, and 12 plants were grown in each row consisting of 24 plants per plot in fertile soil block supplemented with drip irrigation. Standard cultivation practices were followed according to Horticulture Experimental Station, Northeast Agricultural University. The plant to plant, row to row and plot to plot distances were kept at 35, 43 and 77 cm, respectively. F_2 generation and parent's male and female flowers were covered with dunce cap paper. Surveillance from 2 pm to 4 pm was done on daily basis in order to find the damaged buds, and next morning (7.00 am to 10.00 am), the self-pollinated flowers from each plant were labeled with a dated tag. All F_2 melon fruits were evaluated, a single fruit per plant was harvested when abscission layer developed day after pollination (DAP). For phenotypic analysis, harvested fruits were cut lengthwise and photographed to evaluate the difference following melon traits, total soluble solids (TSS) measured as Brix with a handheld refractometer.

Measurement method: We applied 2-3 drops of melon flesh on prism surface and pressed the start key, thereafter value was measured that was displayed in 3 seconds. After every measurement of melon flesh, hand refractometer prism surface was washed with distilled water. Melon fruit length and fruit width was measured using plastic ruler with results expressed in centimeter.

DNA isolation, primers and PCR amplification: Genomic DNA was isolated from 4 to 6 weeks old leaves using a modified CTAB procedure (Doyle & Doyle, 1990). Total DNA was kept in sterile water, visualized after electrophoresis in 1% agarose gels in 1×TBE (Tris–borate- EDTA), and quantified by comparing with DNA standards (Lambda phage DNA digest with *Hind*III; Invitrogen Life Technologies). The primers were designed based on previous studies (Danin-Poleg *et al.,* 2002) and in a total 96 melon CAPS markers were used. PCR reaction prepared with 20 ng of plant genomic DNA, 8-10 pmol primers, 0.25mM DNTP, 10 ×Tag buffer and 1 unit of tag polymerase in a total volume of 10 µl. PCR was performed by preheating samples for 3 min at 94°C followed by 35 cycles of 60 s at 94°C for denaturation, 30s at 50°C for annealing, and 90°C s at 72°C for extension, and finishing with post –heating for 5 min at 72°C.CAPS markers amplification products were separated on 1% denaturing polyacrylamide gel electrophoresis and detected by silver staining. The reaction mixture was for enzyme digestion contained 5 µl PCR products, 9µl ddH2O and 0.3 µl restriction enzyme which were then incubated at 37°C for 2 hours. The enzyme digested products were examined by 1% agarose gel electrophoresis.

Map construction, QTL and statistical analysis: Total of 96 CAPS markers were applied on 235 F_2 melon plants and these 235 F_2 plants were used to construct genetic map using IciMapping V3.3 software (Institute of Crop Science Chinese Academy of Agricultural Sciences, Beijing, China). CAPS markers were grouped at a minimum LOD score of 5.1 and a maximum threshold value of 8.3. IciMapping V3.3 software was used for Kosambi mapping function to translate recombination frequency into genetic map distance. Order of markers in each linkage group was determined by method of maximum likelihood. The software used as linkage Map Chart 3.0 (Van Ooijen and Voorrips, 2001) to graphically represent the linkage groups in map. QTL analyses were performed using Icimapping V3.3 software. QTLs and their significance was calculated using interval mapping (IM) and multiple QTL model (MQM). Standard deviations and trait distribution were conducted with SPSS V 19.0 program.

Table 1. The analysis of fruits in parents, F_1 and F_2 generation.

Traits name	MR-1	Topmark	F_1	F_2	F_2,Max	F_2,Min
Edge part Brix	5.76	8.21	7.51	6.76	1.78	0.57
Central part Brix	6.67	7.65	7.98	7.50	2.00	1.01
Fruit length (cm)	9.59	14.26	18.77	15.10	2.24	1.03
Fruit width (cm)	9.98	8.08	10.41	10.17	3.00	1.10
Fruit shape index	3.0	2.0	1.0	0.73	14.22	1.13

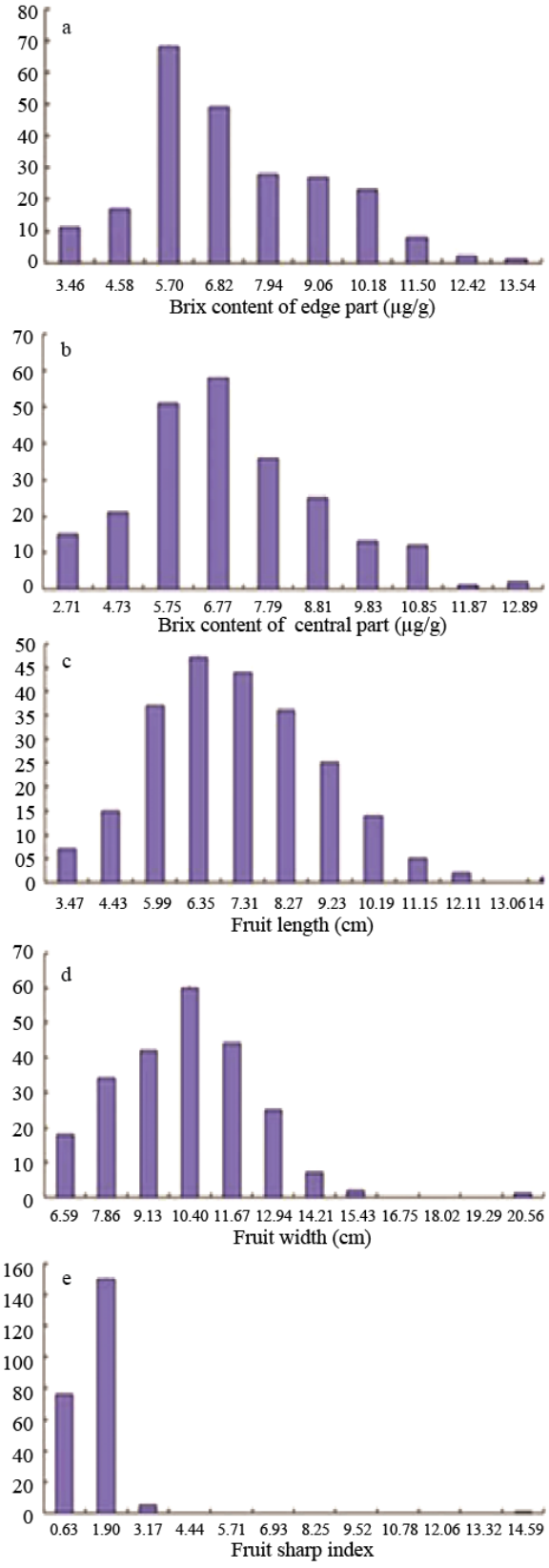

Fig. 1. Distribution of F_2 population fruits as Brix content edge part, Brix content as central parts of melon flesh mesocarp, Fruit length (cm), Fruit width (cm) and fruit shape index (FSI).

Results

Variation in traits: Significant variations for various traits were found between parents and its F_2 population. Brix is a phenotypic quantitative trait and it is controlled by several genes that are influenced by environmental factors (Gusmini and Wehner, 2005). The range of Brix content is different in all generation's fruits, therefore, Brix contents of edge part and central part were determined (Table 1). The mean values for the traits including fruit length (FL), fruit width (FW) and fruit shape (FS) of the both parents (MR-1 and Topmark) were determined. The significant differences were found among FL, FW and Brix content edge and central part, and fruit shape of F_2 population was recorded (Table 1). Trait variations in histogram of F_2 population are also shown in Fig. 1. Both parents were different from each other in Brix content, MR-1 (parent1) showed 7.4 and 8.9% in central part and edge part, respectively, while in Topmark (parent 2) the Brix content of central part was found to be 8.9% and edge part was observed as 8.3%. Fruit length and width of MR-1 was found to be 9 and 11 cm, respectively. However, the fruit length of P_2 was recorded as 15cm and width was 7 cm. The fruit shapein MR-1(P_1) was round and color was yellow while fruits with oval shape having light green color were found in Topmark (P_2), yet fruit was smaller than (P_1). In F_1 hybrids, the Brix content in central part was 8.8% and edge part was 8.5%, the fruit length and width was 17.2 and 11.2 cm, respectively. The fruit shape of F_1 was oval. F_1 population was very close to F_2 population in Brix content, fruit length, fruit width and fruit shape. All these traits were different from each other and can be controlled through different genes and molecular markers.

Linkage map of melon: We used 96 CAPS co-dominant markers for the construction of linkage map (Fig. 2). The Joinmap4.0 software was used to construct the genetic map. In previous studies, a total distance of 2,384 cM and 1,390 cM in melon map was recorded (Baudracco-Arnas & Pitrat, 1996). However, in our study, the total length of linkage map was found to be 4984.1 cM with an average of 51.9177 cM between the genetic markers. Based on re-sequencing data of plant genomic on chromosome locations of CAPS markers, 12 melon linkage groups were named from chromosome 1 to chromosome 12.

QTL analysis: The Table 2 provided the list of different QTLs between different genetic markers, QTLs position is showed on right side in linkage map between two markers which are shown in different color bars (Fig. 2). We detected total of 10 QTLs and amongst, 9 were minor and only one QTL was found to be major. Brix 1, 2 and FS indicated Brix content in edge part, Brix content in central part and fruit shape, respectively. Chromosome numbers 5, 7, 10, 11 and 12 which contained Brix 1 content of QTLs are represented in blue bars located between two CAPS markers; chromosome numbers 1, 11 and 12 contained Brix 2 content QTLs are indicated in red bars, while the chromosomes number 4 and 12 that contained FS QTLs is indicated in purple bars. The chromosome number 5 contained the only major QTL as Brix 1 content, and its phenotypic variation explained (PVE) remained 16.32%. Additive effect and dominate effect were found to be -1.7739 and -1.6444, respectively (Table 2). The minors QTLs are located at chromosome numbers 7, 10, 11 and 12 as Brix 2 content, and chromosome number 4 and 12 are shown as FS.

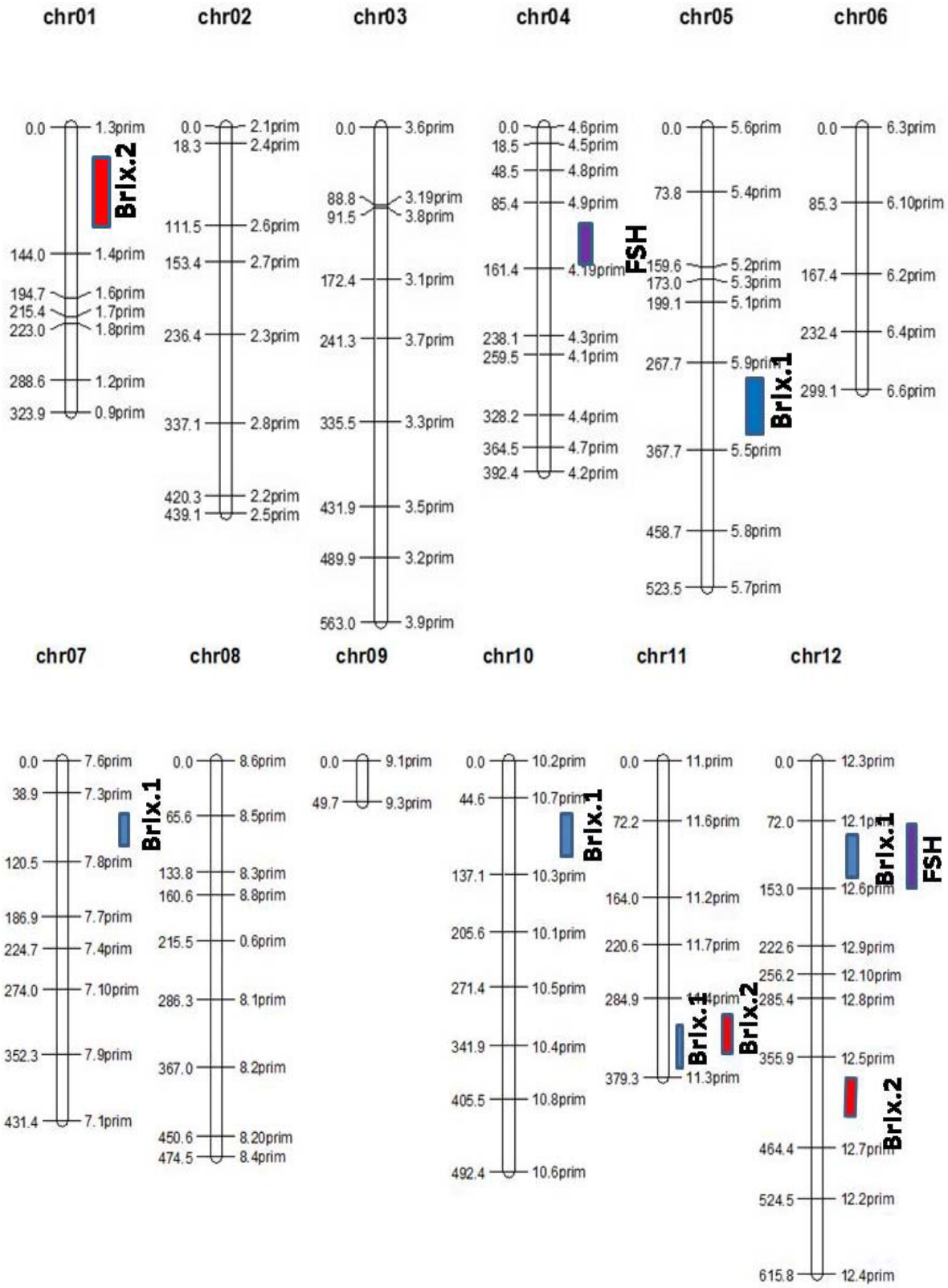

Fig. 2. Linkage map construction on based of F_2 population derived from MR-1 and Topmark parents by using CAPS markers that are shown with numbers at the right side of linkage group. Codes in form of prim as CAPS markers and (CentiMorgan) distances are indicated at left side of linkage groups. QTLs mapping locations are indicated in red, blue and purple colors bars on the right of linkage groups. Brix content of edge, central part and fruit shape (FSH).

Table 2. QTLs trait, number of chromosome, position (cM), flanking marker, LOD score, additive effect, dominate effect and PVE (%).

QTLs	Chromosomes	Position (cM)	Flanking marker	LOD score	Additive effect	Dominate effect/	PVE (%)
Brix.1	5	1187	5.9prim, 5.5prim	5.1745	-1.7739	-1.6444	16.32%
Brix.1	7	80	7.3prim, 7.8prim	3.7937	-1.4159	-2.1697	5.21%
Brix.1	10	87	10.7prim, 10.3prim	4.343	-1.6072	-1.6491	3.16%
Brix.1	11	326	1.4prim, 11.3prim	6.9037	-1.6637	-1.7244	2.56%
Brix.1	12	108	12.1prim, 12.6prim	3.8451	-1.6039	-1.5592	2.58%
Brix.2	1	63	1.3prim, 1.4prim	3.415	-1.5276	-1.7686	9.85%
Brix.2	11	331	11.4prim, 11.3prim	3.2227	-1.4421	-1.3394	4.35%
Brix.2	12	408	12.5prim, 12.7prim	3.0157	-1.4202	-1.957	2.36%
FSH	4	125	12.5prim, 12.7prim	8.3508	-0.4345	-0.6751	7.95%
FSH	12	104	12.1prim, 12.6prim	4.2838	-0.0077	-0.8021	5.06%

Discussion

The human population is increasing, hence more food is required and agriculture sector faces several problems, irrigation is becoming short and the crops yield is decreasing by insect pest and plant diseases. These aforementioned problems can be addressed through molecular genetics and breeding (Jannink *et al.,* 2010). Molecular markers are particular components of DNA, and are the future tools for major and minor crops species through which the improvement can be done in crops species (Scarano and Rao, 2014). Cleaved amplified polymorphic sequences (CAPS) are latest molecular markers based on PCR and are widely used in plant research and also quite useful for plant genotyping. In the present study, we used 96 CAPS markers and every CAPS marker has different locations in linkage map between linkage group (Fig. 1). We converted various primers and named them CAPS marker, after analyzing the genotypic data, CAPS markers were placed in genetic map. Our results show that melon linkage map spans over 4984.1 cM, on an average, 51.9177 cM between genetic markers. For the first time QTLs mapping was done in melon that involved flower and fruit shape by using recombinant inbred population (Prin *et al.*,2002). Research has already been conducted relating to fruits shape in other crops such as tomato (Chaim *et al.,* 2006), pepper (Naegele & Hausbeck, 2014) and cucurbit crop melon (Eduardo *et al.,* 2007). According to previous melon studies, the fruit size of melon ranges from elongated to oblong, weighing from some grams to several kilograms and from sweet to bitter in taste. However in present experiment, the fruit shape was round in parent 1 (MR-1), whereas the fruit shape in parent 2 (Topmark) was oval. The F_1 population's fruits were little longer, while the fruit of F_2 population were not similar in FW, FL and FS, and some fruit were found as round, long, oval and elongated, and these results were presumably due to segregation of genes.

Brix is common measuring method in many vegetable crops and it predicts the TSS or content in vegetable crop and sugar accumulation in watermelon (Zhang *et al.,* 2006). Harel-Beja *et al.,* (2010) detected six QTLs in sugar content of the melon fruit and suggested that these QTLs interacted additive in manner to accounting for nearly different in the sugar accumulation between two parental lines. We detected five QTLs in melon fruit Brix, one major QTL detected in edge part of melon fruit and other four minor QTLs in central part of melon fruit. The edge and central parts of melon did not show the similar Brix content, whereas in green mesocarp (flesh) of melon was higher than yellow mesocarp (flesh) as Brix content in the fruits harvested from 45 to 60 days after pollination (DAP). One major QTL was located at chromosome number 5 between two markers (5.9 prima, 5.5prim), and other four QTLs of Brix content are shown in Table 2. We further detected two minor QTLs in fruit shape (FS), and these two QTLs were located at chromosome number 4 and 12. In chromosome number 4, the QTLs were located between two markers (4.9 prim and 4.19 prim) while in chromosome number 12 (12.1prim and 12.6prim).We detected more QTLs in edge and central parts of melon; however it must be noted that healthy melon fruit should be harvested from 45 to 60 days (DAP), due to the fact that it has higher percentage of Brix.

Conclusion

QTLs control different fruit quality traits and also give a deep knowledge about whole genetic background. The current study revealed a total of 10 QTLs, in which one was major, while remaining was found as minor. Five QTLs were detected in the edge part of melon fruit and three QTLs were identified in central parts of melon and all were considered as Brix content. Two QTLs were concerned with fruit shape. The obtained results of QTLs mapping will be proved helpful in plant breeding programs for the advancement of economically important horticultural traits.

Acknowledgments

This research was supported by the National Nature Science Foundation of China (31272186). And China Agriculture Research System (CARS-26-02)

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(Received for publication 17 April 2015)