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QTL MAPPING FOR SIGNIFICANT SEED TRAITS OF WATERMELON (*CITRULLUS LANATUS* SCHRAD.)

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Abstract

With the ever-increasing demand of healthy vegetable oil for domestic and industrial use, there is the need to seek for alternative sources through molecular breeding to meet this demand. Recently, the Cleaved Amplified Polymorphism Sequence (CAPS) markers were emerged as a powerful source for molecular breeding experiments. Two watermelon accessions named as P_1 = W1-1 (low oil percentage), P_2 = PI-186490 (high oil percentage) and with their F_1 and $F_{2:3}$ generation was used for measuring seed oil percentage, also other seed phenotypic traits (seed length, seed thickness, seed width, thickness of seed coat, 100 seed weight). Mean of seed oil percentage (SOP) in PI-186490 (mean = 22.25%) was significantly higher than that of W1-1 (mean = 14.75%). The $F_{2:3}$ seeds had seed oil percentage ranging from 9% to 32%. Means of other seed traits in P_1 and P_2 were: seed length (8.81 mm and 14.71 mm), seed width (5.96 mm and 10.53 mm), seed thickness (2.06 mm and 2.65 mm), thickness of seed coat (0.33 mm and 0.15 mm) 100 seed weight (4.70 g and 18.20 g). Overall, 145 CAPS markers with six restriction endonucleases (*Bam*HI, *Eco*RI, *Hin*dII, *Hin*dIII, *Hin*fI, *Pst*I) were applied to the F_2 population for molecular genotyping. The linkage map spanned a distance of 3000.19 cM covering the whole genome with an average distance of 20.01 cM. Total of 11 QTLs were detected as follows: seed length (2 QTLs), seed width (1 QTLs), seed thickness (1 QTLs), thickness of seed coat (4 QTLs), 100 seed weight (2 QTLs), seed oil percentage (1 QTLs). QTLs for Seed Length, Seed Width, Thickness of Seed Coat and 100 Seed Weight (*qSL*-6-1, *qSW*-6-1, *qTSC*-6-1, *q100SWT*-6-1) were all located on chromosome 6 within a less than 5 cM of genetic distance indicating colocalization. The data on seed oil percentage and detected QTLs presented by this study will provide very practical information for future breeding and genetic schemes in watermelon.

Key words: Watermelon, Seed oil percentage, CAPS markers, QTL mapping, Linkage map.

Introduction

Watermelon belongs to Cucurbitaceae family which includes many economically important domesticated species (Sandlin *et al.*, 2012). The plant family Cucurbitaceae is a large one that produces edible seeds or seeds used for oil production in some African and Middle East countries (Jarret & Levy, 2012). Watermelon fruit has not only been a source of refreshment in hot summer weather conditions but also provides good source of nutrition. Watermelon seed oil is known as medically to be useful in the prevention of health issues and other disease (Guner & Wehner, 2004). In West Africa, a region where soups play important role in the food culture, seeds of watermelon with a unique seed characteristic locally referred to as *egusi* are the major soup ingredient. When the seed are coarsely ground up, they serve as thickeners in stews. Egusi seed meal when compacted into patties is served as a meat substitute in African dishes (Oluba *et al*., 2008). Analysis have shown that, watermelon seed averagely consist of 31.90% protein, 4.40% carbohydrates, 57.10% fat, 8.20% fibre, 6.20% ash, 130 mg calcium, 456 mg phosphorus, 7.5 mg Iron as well as other necessary amino acids such as Leucine, Isoleucine, Tryptophan and Valine (Razavi & Milani, 2006). The global sources for vegetable oils consumed annually in million tons units are mainly dominated by soybean (31.6), palm (30.5), rapeseed (15.5), and sunflower (8.6) (Stevenson *et al*., 2007). The ever-increasing demands for domestic and industrial sectors cannot be met by only these conventional sources, therefore the need exists to explore other non-conventional sources to meet the demand. (Idouraine *et al*., 1996). Oil percentage is a key factor for increasing oil production in seed oil crops (Wang *et al*., 2013). Some species from the Cucurbitaceae family produces seeds that can potentially be edible oil sources to supplement the ever growing demands for vegetable oil (Esuoso *et al*., 1998). Considerable studies have been undertaken to measure the seed oil percentage of watermelon seeds with varying results: 45.5% (Dhingra & Biswas, 1945), 19% (T-Sao & Potts, 1952), 50% (Olaofe *et al.*, 1994), 44-53% (Achu *et al.*, 2005), 20.14%-40.60% (Prothro *et al.*, 2012a). This study seeks to measure the seed oil percentage (SOP) and through QTL mapping identify the major loci for SOP and other significant seed traits in watermelon (*Citrullus lanatus*). Conversely, in the present time of molecular breeding studies, Cleaved Amplified Polymorphism Sequence (CAPS) markers have been applied for breeding programs in plant and animal genome association studies (Kole & Abbott, 2008). CAPS markers are known for its high marker resolution in trait identification of crops especially for important fruit traits in melon (Amanullah *et al*., 2018; Baloch *et al*., 2016). According to the importance of watermelon seed characters, we developed a molecular study for identifying the seed characters QTLs in inbred watermelon (*Citrullus lanatus*) lines by usage of CAPS markers.

Materials and Methods

W1-1 (China watermelon line) with a nearly round shape, high Brix level and seeds with low oil content was the female parent (P_1). The male parent (P_2) was an inbred watermelon line, PI-186490 was late-maturing, white flesh colour, bitter in taste with a densify fruit skin and large seeds which are high in oil content. These two parents as shown (Fig. 1) below were used to develop the F_1 and $F_{2:3}$ populations for DNA and seed oil extraction and other seed characteristics. The cross between the two watermelons parents were raised at Xiangyang Agricultural Experiment Station Harbin, China. Watermelon seeds from each line at their maturity phase were hand-collected and washed up with tap water, then sun-dried until the total moisture was removed. All necessary phenotypic data were recorded on the seed traits as well.

DNA extraction and polymerase chain reaction (PCR): Genomic DNA was extracted from fresh leaves using the cetyl trimethylammonium bromide (CTAB) method as described earlier (Luan *et al*., 2008). The DNA quality and quantity was verified through 1.0% agarose gel. Primer Premier Version 6.0 software; was used for primer design and analysis as described earlier by Amanullah *et al*., (2018). The design of the primers conformed to the criteria described earlier by Amanullah *et al.,* (2018). The PCR mixture preparation, amplification, restriction enzyme digestion, gel-electrophoresis and image analysis followed the protocol described by Amanullah *et al.*, (2018). Markers that exhibited polymorphic co-dominance among PI-189460, W1-1, and their F_1 generation (Fig. 2) were utilized for the construction of linkage map.

Extraction of seed oils: The two parents, F_1 and $F_{2:3}$ watermelon seeds were used for seed oil extraction using the soxhlet extraction method with n-Hexane as the solvent. 4 g (four) of intact seeds were grinned into fine seed powder using a normal kitchen blender. The extraction duration for each sample was 4 hours. After extraction, the total extracted oil from the seed was weighed and calculated as a percentage of 4 g watermelon seed.

Statistical analysis

Statistical analysis was performed using SPSS version 23.0 and Microsoft Excel 2013.

Linkage map construction: A total of 149 polymorphic primer pairs from 6-restriction endonuclease (*Bam*HI, *Eco*RI, *Hin*dII, *Hin*dIII, *Hin*fI, *P*stI,) were used for linkage mapping of the F_2 population. A high-density linkage map construction with a total of 149 polymorphic markers by use of Ici Mapping Version (4.0) software (Meng *et al.*, 2015). Recombination frequency translation into genetic map distance was performed by Kosambi mapping function. Based on re-sequencing data of plant genomic on chromosome locations of CAPS markers, 11 watermelon linkage groups were named from chromosomes 1 to 11. The linkage map covered the entire 11 chromosomes. The 149 CAPS markers were scattered across the 11 LGs. Chromosomes 10 had the highest number of 19 markers covering a genetic distance of 397.73 cM length with average distance of 20.93 cM. Chromosome 4 had its share although it had the least number of markers of 9 covering a distance of 217.99 cM with average distance of 24.22 cM. The whole genome was covered by the 149 markers with a total genetic distance of 3000.19 cM and average distance of 20.14 cM.

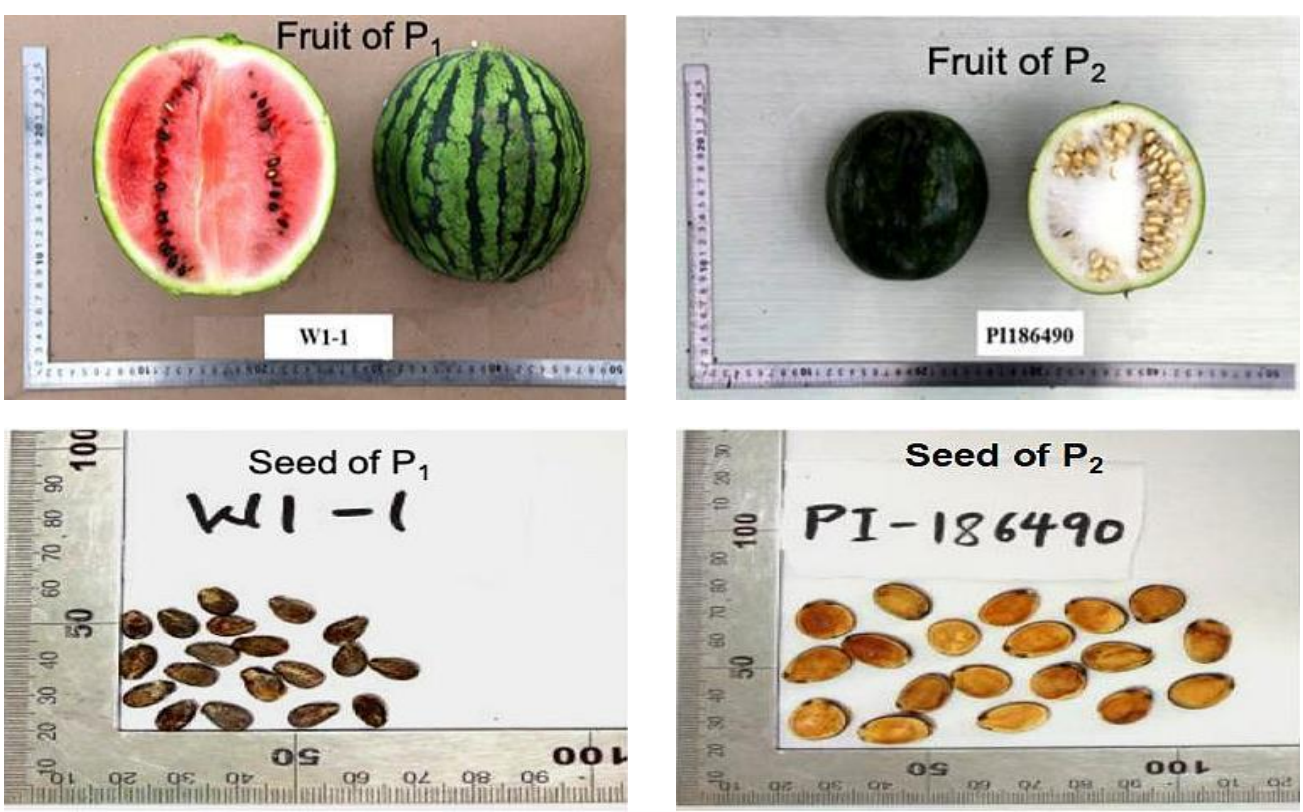

Fig. 1. Fruit and seed characteristics of the two watermelon parental lines.

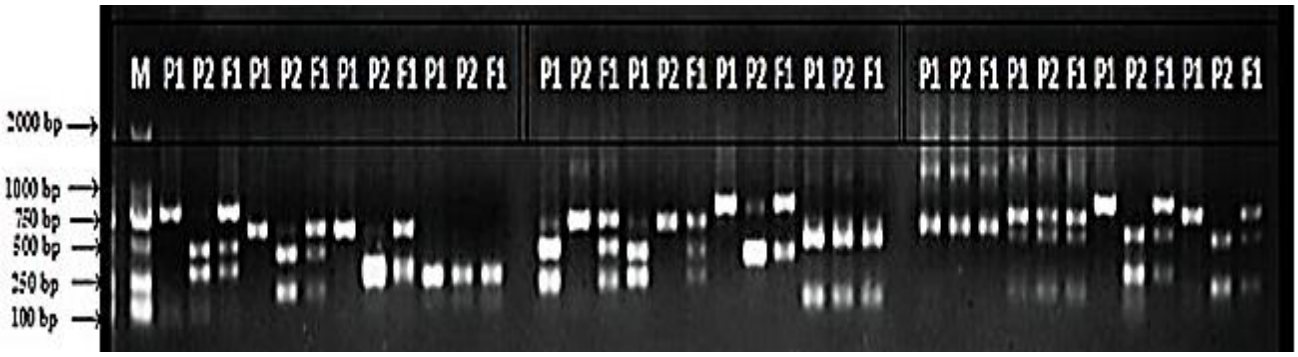

Fig. 2. CAPS polymorphic markers by use of 6-endonuclease restriction enzymes (*Bam*HI, *Eco*RI, *Hin*dII, *Hin*dIII, *Hin*fI, *Pst*I,) in W1-1 (P_1), PI-186490 (P_2) and F_1.

Phenotypic traits: Phenotypic traits data and frequency distribution were recorded in parental lines (PI-186490 and W1-1), F_1 and $F_{2:3}$ populations (Table 1 and Fig. 3). Pearson's correlation also calculated (at $p \leq 0.01$) for the seed-related traits. Strong correlation was observed among the phenotypic traits (Table 3).

Seed length: There were variations in the seed length values for the two parental lines. The mean values for W1-1 and PI-186490 were 8.81 ± 0.07 mm and 14.71 ± 0.64 mm respectively. The F_1 value was 11.93 ± 0.07 which was a little closer to that of PI-186490 compered to W1-1. On the other hand, within the $F_{2:3}$ values ranged from a minimum of 8.75 mm and a maximum of 15.46 mm. The parental material with the highest value score is shown to have a dominance effect.

Seed width: The Seed Width of the two parents had no observed similarity between themselves. The W1-1 and PI-186490 mean values were 5.96 ± 0.03 mm and 10.53 ± 0.81 mm respectively. The recorded Mean for F_1 was 6.96 ± 0.27 mm which was closer to that of W1-1. The $F_{2:3}$ had a minimum and a maximum values of 5.88 mm and 10.28 mm respectively.

Seed thickness: The mean for seed thickness for P_1 and P_2 were 2.06 ± 0.08 mm and 2.65 ± 0.08 mm respectively. The mean value for F_1 population was 2.19 ± 0.40 mm while the mean for $F_{2:3}$ was 2.63 ± 0.44 mm. The minimum and maximum range for the $F_{2:3}$ was 1.00 mm and 4.00 mm respectively.

Thickness of seed coat: In reference to thickness of seed coat, 0.33 ± 0.05 mm and 0.15 ± 0.04 mm were the mean thickness of seed coat values for W1-1 and PI-186490 respectively. For F_1 population, the mean was 0.35 ± 0.16 mm and for the $F_{2:3}$ the minimum (0.05 mm) and maximum (0.64 mm) values were recorded.

100 seed weight: Seed weight of 100 seeds from the two parents, F_1 and the $F_{2:3}$ were measured. For P_1 and P_2, their individual means were 4.70 ± 0.27 g and 18.20 ± 0.45 g respectively. The F_1 weight was 11.30 ± 0.29 g which was between the two parental lines. Minimum of 6.00 g and maximum of 21.00 g was observed in the $F_{2:3}$. There was a normal distribution with regard to this trait.

Seed oil percentage: The Seed oil percentage (SOP) of the two parents, F_1 and $F_{2:3}$ seeds were analysed and shown that SOP value for PI-186490 (22.25 ± 0.45) which was higher than W1-1 (14.75 ± 1.11). For the F_1 line, the mean value was 11.30 ± 0.29%. Seed oil percentage in $F_{2:3}$ seeds ranged from 9.00% to 32.00%.

Detection of QTLs: Cleaved Amplified Polymorphism Sequence based linkage map was constructed using the F_2 population for the detection of QTLs. Eleven (11) QTLs were detected on the linkage map (Fig. 4 and Table 4) for the following traits; seed length, seed width, seed thickness, thickness of seed coat, 100 seed weight and seed oil percentage. Seed length 2 QTLs, Seed width 1 QTL, seed thickness 1 QTL Thickness of seed coat 4 QTLs, 100 seed weight 2 QTLs, Seed oil percentage 1 QTL.

Seed length QTLs: Two QTLs (*qSL*-6-1, *qSL*-10-1) for seed length were detected on chromosomes 6 and 10 respectively (Fig. 4 and Table 4). The QTL (*qSL*-6-1) was located on chromosomes 6 at 153 cM position and at a very short distance of 1cM away from seed width QTL (*qSW*-6-1) and 2 cM from QTLs (*q100SWT*-6-1) for 100 Seed weight. The second QTL (*qSL*-10-1) located at 5 cM position on chromosome 10 was found in a distance of 1 cM away from 100 seed weight QTL (*q100SWT*-10-1). The LOD for the two detected QTLs (*qSL*-6-1, *qSL*-10-1) was (22.85 and 2.60) respectively. Additive effects for QTLs (*qSL*-6-1, *qSL*-10-1) were (1.55, -0.43) respectively. PVE% values with individual effects were (47.55, 3.80) and the combined effect was 51.35%. The QTL (*qSL*-10-1) had a negative additive effect which accounted for short seed length, whiles QTL (*qSL*-6-1) with a positive additive effect accounted for long seed length.

Seed width QTLs: Only one QTL was detected for seed width trait (*qSW*-6-1) which was located on chromosome 6 at 152 cM just 1 cM away from (*qSL*-6-1) of seed length and Thickness of seed coat (*qTSC*-6-1) (Fig. 4 and Table 4). The other QTL detected in close proximity to seed width trait (*qSW*-6-1) was (*q100SWT*-6-1). The LOD score was (21.91), PVE% values (45.91) and a positive additive effect of 1.02 which contributed to the high seed width.

Table 1. Phenotypic results for traits of two the parents, F_1 and $F_{2:3}$ population.

Seed traits	W1-1	PI-186490	F_1	$F_{2:3}$	$F_{2:3}$ Range	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Min	Max
Seed Length (mm)	8.81 ± 0.07	14.71 ± 0.64	11.93 ± 0.07	11.64 ± 1.51	8.75	15.46
Seed Width (mm)	5.96 ± 0.03	10.53 ± 0.81	6.96 ± 0.27	7.70 ± 0.99	5.88	10.28
Seed Thickness (mm)	2.06 ± 0.08	2.65 ± 0.08	2.19 ± 0.40	2.63 ± 0.44	1.00	4.00
Thickness of Seed coat (mm)	0.33 ± 0.05	0.15 ± 0.04	0.35 ± 0.16	0.24 ± 0.11	0.05	0.64
100 seed weight (g)	4.70 ± 0.27	18.20 ± 0.45	11.30 ± 0.29	11.36 ± 3.32	6.00	21.00
Seed Oil Percentage (%)	14.75 ± 1.11	22.25 ± 0.45	21.75 ± 1.13	20.83 ± 4.08	9.00	32.00

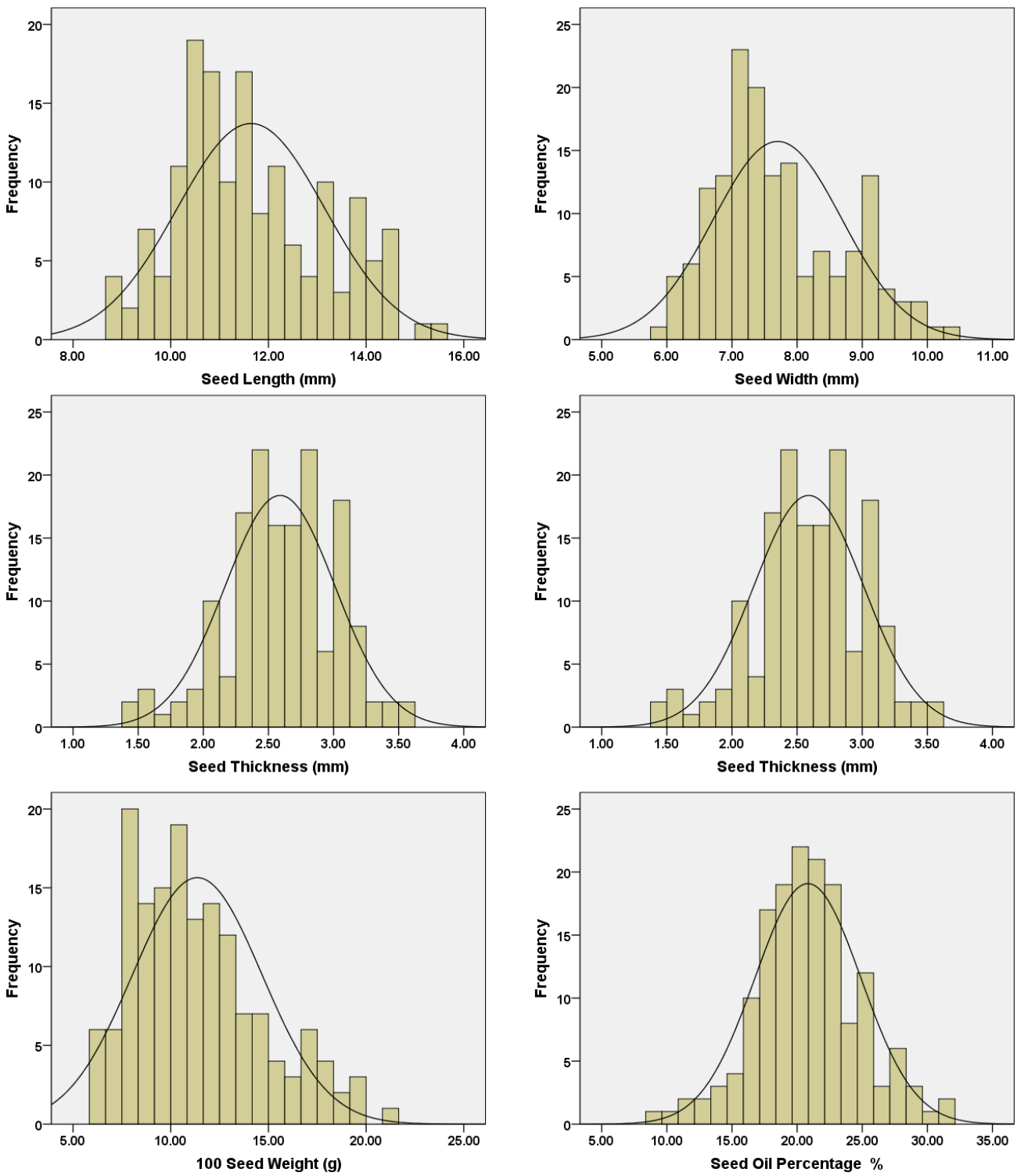

Fig. 3. Frequency distribution for seed traits (Seed length, seed width, seed thickness, thickness of seed coat, 100 seed weight and seed oil percentage in the two parents, F_1 and $F_{2:3}$.

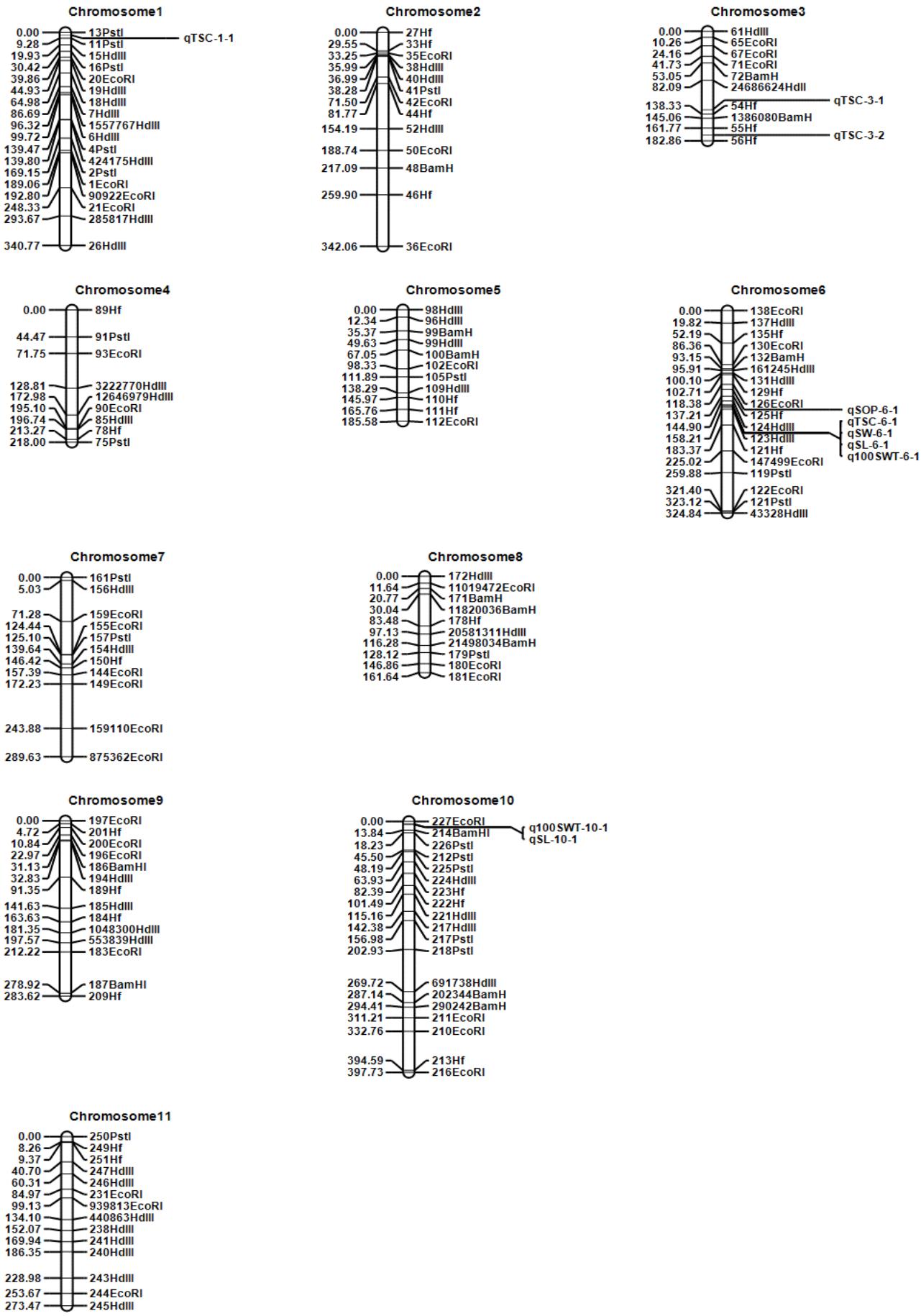

Fig. 4. Linkage map of watermelon based on F_2 generation from parents (W1-1 and PI-186490). QTLs abbreviations; Seed length (*qSL*), Seed width (*qSW*), Seed Thickness (*qST*), Thickness of seed coat (*qTSC*), 100 seed weight (*q100SWT*) and Seed Oil Percentage (*qSOP*).

Table 2. Summary of watermelon F_2 population based Linkage map.

Chr. name	No. markers	Genetic distance cM	Average distance cM
Chr1	18	340.77	18.93
Chr2	13	342.06	26.31
Chr3	10	182.86	18.29
Chr4	9	217.99	24.22
Chr5	13	185.58	14.28
Chr6	18	324.84	18.05
Chr7	11	289.63	26.33
Chr8	10	161.64	16.16
Chr9	14	283.62	20.26
Chr10	19	397.73	20.93
Chr11	14	273.47	19.53
Total	**149**	**3000.19**	**20.14**

Seed thickness QTL: The seed thickness QTL (*qST*-4-1) was detected on Chromosome 4 at a position of 79 cM (Fig. 4 and Table 4). This was the only QTL detected on this chromosome in the study. LOD of 2.85, PVE% value of 7.88 and additive effect of -0.06 were recorded for the QTL. This negative additive effect indicates the small seed thickness size.

Thickness of seed coat QTL: Four QTLs (*qTSC*-1-1, *qTSC*-3-1, *qTSC*-3-2, *qTSC*-6-1) for thickness of seed coat were mapped to chromosomes (1, 3 and 6) respectively (Fig. 4 and Table 4). The first QTL (*qTSC-1-1*) was located at 4 cM on chromosome 1. The next two QTLs (*qTSC*-3-1, *qTSC*-3-2) were both located on chromosome 3 at positions 131 and 173 cM respectively. The last QTL was located on chromosome 6 at 151 cM which is in very close proximity of less than 5cM from these other QTLs (*qSW*-6-1, *qSL*-6-1, *and q100SWT*-6-1) on the same chromosome 6. The additive effect of the discovered QTLs was (0.02, 0.02, 0.02 and -0.09). The thickness of seed coat with negative additive effects indicated low level of thickness of seed coat. On the other hand, positive effect indicated a high thickness of seed coat. LOD (3.87, 2.86 2.52 and 36.60), PVE% (4.28, 4.23, 3.11, 47.21) was with the sum effect of variation on this trait of 58.83%.

100 seed weight QTL: Chromosomes 6 and 10 hosted the two respective QTLs (*q100SWT*-6-1, *q100SWT*-10-1) for 100 seed weight traits (Fig. 4 and Table 4). The first QTL (*q100SWT*-6-1) was located at 155 cM. The second QTLs was located at 4 cM chromosome 10. LOD for the 100 seed weight QTLs (14.20, 2.51), PVE% (34.56, 5.20) and a cumulative effect of variation (39.76), Additive effects (2.67, -0.94). The negative additive for (*q100SWT*-10-1) contributed to low 100 seed weight but on the other hand the positive 2.67 for (*q100SWT*-6-1) contributed to high 100 seed weight.

Seed oil percentage QTL: The QTLs for seed oil percentage trait (*qSOP*-6-1) was located on chromosome 6 at 126 cM positions. LOD of (5.30), PVE% (14.41), Additive effect (2.53) (Fig. 4 and Table 4). The positive additive effect contributed for the high seed oil percentage.

Discussion

Linkage map construction with CAPS markers: In plant breeding, the effective approach to analyse quantitative traits in plants is through quantitative trait loci (QTL) mapping. Genetic linkage maps are very important in the identification of economically important traits in crops which then can be utilized in marker-assisted selection (MAS). In genetic linkage map construction and QTL mapping, there is the need to apply sufficient molecular markers since this will likely provide an accurate genetic linkage map that will serves as the basis for QTL detection and analysis. 6-restriction endonuclease (*Bam*HI, *Eco*RI, *Hin*dII, *Hin*dIII, *Hin*fI, *Pst*I) were applied to 149 CAPS marker pairs depicting polymorphism between PI-186490, W1-1 and its F_1 generation. Total of 149 makers were applied on the F_2 segregated population for linkage map construction covering all the 11 chromosomes (Table 2). The CAPS markers were not evenly distributed on all the chromosomes and there were some large genetic distances between some makers on some chromosomes. This drawback can easily be addressed if more markers are added to these locations.

Detection of watermelon seed traits QTLs: Genes controlling significant seed related traits in watermelon have been identified in studies undertaken by (Poole *et al.*, 1941; Tanaka *et al.*, 1995; Li *et al.*, 2018; Meru & McGregor, 2013; Kang *et al.*, 2000). In fruit sold for consumption small seeds are preferred but large seeds preferred for planting and edible seed production.

Total of 11 QTLs were detected for watermelon seed traits (Seed Length, Seed Width, Seed Thickness, Thickness of Seed coat, 100 Seed weight and Seed Oil Percentage) during this study (Table 4). Out of the 11 chromosomes, chromosomes (2, 5, 7, 8, 9, and 11) had no QTL positioned on them. 6 QTLs were considered as major (LOD value > 3) and the other 5 QTLs as minor (LOD value <3).

With reference to seed length, two genes (s) and (l) controlling short and long seed length size in watermelon were detected by Poole *et al.*, (1941). According to Wehner (2008), seed length gene (ts) was found to be shorter than the short-seed genotype ll ss. In this study, two seed length QTLs *qSL*-6-1 (major), *qSL*-10-1 (minor) were identified on chromosomes 6 and 10 respectively. The earlier identified QTLs for seed length in watermelon were on chromosomes 6 and 5, this attest to our finding in the study and adding up the number of QTLs since a novel QTL was discovered on chromosome 10 through our studies.

Seed weight which is normally measured in hundred (100) or thousand (1000) is an important yield component of most seed oil crops since it generally correlates positively with seed yield. Despite the its significance environmental conditions easily affect seed weight trait and reports showed that it is controlled by many genes thereby resulting in a large number of QTLs (Hyeun-Kyeung *et al*., 2010; Brummer *et al.*, 1997; Lee *et al.*, 1996). In this present study, one major QTLs (*q100SWT-6-1*) on chromosome 6 and one minor (*q100SWT*-10-1) on chromosome 10 in regard to 100 Seed weight were detected. 100 seed weight QTL on chromosome 6 and linkage groups 4 and 9 have been reported earlier by (Meru & McGregor, 2013; Prothro *et al*., 2012b). Two other QTLs for 1000 seed weight (TSW) in watermelon have also been reported on linkage groups 4 and 6 by (Li *et al*., 2018).

Seed width is an important trait that is mostly used as the standard for seed size. Watermelon seed size is very diverse and are characterized into six categories (YongJae *et al*., 2009). Through breeding techniques, there is an achieved increase with respect to seed width of 10 mm (<1,500 kg/ha) to 11 mm (2,250 kg/ha) (Zhang, 1996). The recorded value of seed width of PI 186490 (10.53 ± 0.81 mm) which is similar to that stated above. The only QTL detected for seed width was located on chromosome 6 explained 45.91% of the phenotypic variation. This QTL was discovered on the same chromosome 6 with 13.6 LOD and 34.4% PVE in earlier study by Meru & McGregor (2013) suggesting that this location may be associated with seed length in watermelon.

Four thickness of seed coat QTLs (*qTSC*-1-1, *qTSC*-3-1, *qTSC*-3-2, *qTSC*-6-1) with phenotypic variations of 4.28%, 4.23%, 3.11%, 47.21% respectively were detected in this study. These results suggested additional genetic factors that might be contributing to the seed trait. Seed coat thickness QTLs (*qsdp*2, *qsdp*10.1 and *qsdp*10.2) identified in cowpea (*V. unguiculata*) were detected on LG2 and LG10 (Andargie *et al.*, 2014). Kernel Percentage is known to be highly correlated with SOP (Jarret & Levy, 2012).

Seed thickness is among the most important traits that defines the size of seeds in many seed crops including watermelon. Prothro *et al*., (2012b) detected five QTLs for seed length in watermelon on linkage groups 2, 4, 9 and 11A with LOD range of 3.7 to 39.6 and PVE% range of 5.3 to 69.2%. A genome wide QTL scanning detected 2 QTLs (*SL*4, *SL*6) with *SL*4 increasing the seed length in the elite male parent (14CB11) by 0.06 cm and *SL6* increasing the elite female (ZXG01478) seed length by 0.30 cm (Li *et al*., 2018). The QTL (*qST-4-1*) for seed thickness in this study was located on chromosome 4 with 2.85 and 7.88 for LOD and PVE% respectively. This QTL was considered a minor QTL for the seed thickness trait in this study.

QTL associated with seed size was mapped on linkage group 2 in watermelon suggesting that seed size played a role in SOP (Prothro, 2010). A very stable major QTL on linkage group that control seed size have been identified in watermelon (Prothro *et al*., 2012b). There are significant reports on seed oil QTL in peanut, soybean and other oil crops (Pandey *et al.*, 2014; Eskandari *et al*., 2013; Sarvamangala *et al*., 2011). In sunflower, seed oil content showed negative correlation with 100 Seed Weight, Seed Length, and Seed Width (Tang *et al*., 2006). In watermelon, some studies have been undertaken to study the variations in Seed Oil Percentage (SOP) of the egusi (similar to PI-186490) and normal (W1-1) seed types respectively: (35.6% and 23.2%; Jarret & Levy, 2012), (30.30% to 40.60% and 20.14% to 26.55%; Prothro *et al*., 2012a). In this particular study, the major QTL detected for Seed Oil Percentage (*qSOP-6-1*) was located on chromosome 6 with LOD and PVE of 5.30 and 14.41% respectively.

QTLs for Seed Length, Seed Width, Thickness of Seed Coat and 100 Seed Weight (*qSL*-6-1, *qSW*-6-1, *qTSC*-6-1, and *q100SWT*-6-1) were all located on chromosome 6 within a 5cM of genetic distance. Similar colocalization were observed in some seed related traits in sunflower and watermelon (Meru & McGregor, 2013; Tang *et al.*, 2006). Co-localization of QTL for different traits at same chromosomal locations might be an indication of a single gene with pleiotropic effect or different genes tightly linked (Meru & McGregor, 2013).

Table 3. Pearson's correlation for phenotypic traits in two the parents and F_2 population.

Traits	Seed length	Seed width	Seed thickness	Thickness of seed coat	100 Seed weight	Seed oil percentage
Seed length	1					
Seed width	0.912** 0.000	1				
Seed thickness	0.121 0.134	0.100 0.215	1			
Thickness of seed coat	-0.565** 0.000	-0.587** 0.000	0.119 0.138	1		
100 Seed weight	0.814** 0.000	0.824** 0.000	0.292** 0.000	-0.434** 0.000	1	
Seed oil percentage	0.383** 0.000	0.400** 0.000	-0.159 0.048	-0.516** 0.000	0.337** 0.000	1

** $p<0.01$

Table 4. QTLs identified for watermelon seed traits on interval mapping basis of markers in the two parents and F_2 population; (Chr= Chromosome, PVE%= Phenotypic variance, LOD= Log-likelihood, ADD= Additive effect).

Trait Name	QTLs	Chr	Position (cM)	Flanking markers	LOD	PVE (%)	ADD	Dom
Seed length	*qSL-6-1*	6	153.0	124*Hin*dIII / 123*Hin*dIII	22.85	47.55	1.55	-0.65
	qSL-10-1	10	5.0	227*Eco*RI / 214BamHI	2.60	3.80	-0.43	0.38
Seed width	*qSW-6-1*	6	152.0	124*Hin*dIII / 123*Hin*dIII	21.91	45.91	1.02	-0.41
Seed thickness	*qST-4-1*	4	79.0	93*Eco*RI / 13222770*Hin*dIII	2.85	7.88	-0.06	-0.28
Thickness of seed coat	*qTSC-1-1*	1	4.0	13*Pst*I / 11*Pst*I	3.87	4.28	0.02	-0.05
	qTSC-3-1	3	131	24686624*Hin*dII / 54*Hin*fI	2.86	4.23	0.02	-0.05
	qTSC-3-2	3	173	55*Hin*fI / 56*Hin*fI	2.52	3.11	0.02	-0.04
	qTSC-6-1	6	151	124*Hin*dIII / 123*Hin*dIII	36.60	47.21	-0.09	0.13
100 Seed weight	*q100SWT-6-1*	6	155	124*Hin*dIII / 123*Hin*dIII	14.20	34.56	2.67	-0.79
	q100SWT-10-1	10	4.0	227*Eco*RI / 214*Bam*HI	2.51	5.20	-0.94	1.06
Seed oil percentage	*qSOP-6-1*	6	126	126*Eco*RI / 125*Hin*fI	5.30	14.41	2.53	-1.33

Conclusion

This study revealed that QTLs control the different seed traits and also gave a deep insight about whole genetic background. Eleven (11) QTLs were detected in this study, six were considered major QTLs and five (5) were minor QTLs. The data on seed oil percentage and detected QTLs presented by this study will provide useful information for future breeding and genetic schemes in watermelon.

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