Construction of Preliminary QTL Mapping of Seed-related Traits based on SLAF Linkage Map of Muskmelon

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Background

Melon (*Cucumis melo L.*) is one of the important cash crops of Cucurbitaceae, with a wide cultivated area, strong heterosis and great economic value (Liu J P et al., 2000). Seed size is an important agricultural character, and seed germination is the beginning of plant growth. Seed size directly affects plant survival and is very important for plant evolution. Large seeds can accumulate enough nutrients for germination and have better tolerance to abiotic stress, while small seeds are beneficial to disperse and reproduce a large number of offspring (WestobyM, etal., 2002; Moles AT, etal., 2005). The results showed that seed size and 100-seed weight were directly related to germination rate, germination cycle, seedling activity, survival rate and fecundity, and seed size was also an important part of seed yield. Seed-related traits are important agronomic traits of crops, which are of great significance to melon genetics and breeding and related molecular biology research. Therefore, the genetic research of melon seed characters has always been the goal of common concern of breeding experts. At present, there are few in-depth studies on muskmelon seed characters, so on this basis, this study uses Specific-Locus Amplified Fragment Sequencing (SLAF-seq) technology to select muskmelon materials with different seed sizes. The QTL controlling seed coat color, seed shape, seed length, seed width and 100-seed weight of muskmelon were detected and analyzed in order to provide a basis for the isolation and cloning of genes related to muskmelon seed traits.

Methods

Specific-Locus Amplified Fragment Sequencing (SLAF-seq) is a simplified genome deep sequencing technology developed based on high-throughput sequencing technology, which carries out double-terminal sequencing of specific restriction fragments and has high-resolution SNP site recognition and typing. The markers developed by this technology have high density, good consistency and many effective markers, and the development cost of high-throughput molecular markers is lower than that of conventional markers. Since the emergence of SLAF-seq technology, high-density genetic maps have been constructed by using cucumber (Wei, et al.,2014), soybean (Li, 2014), kiwifruit (Huang et al.,2013) and melon (Wang Guichao, 2021), and used to study the mapping of related genes. In this study, thin-skinned melon P5 (small seed, length is 4.73±0.15mm, width is 2.73±0.10, 100-seed weight is 0.66±0.05) was selected as female parent, thick-skinned muskmelon P10 (large seed, length is 7.70±0.93, width is 3.74±0.19, 100-seed weight

is 1.35±0.42) was selected as male parent, were used to make crossing and constructed $F_{2:3}$ families, using 450 F_2 individual plants to carry out the relevant trait of genetic analysis.The 127 F_2 individual plants were used to carry out specific locus amplification fragment sequencing (Specific-Locus Amplified Fragment Sequencing, SLAF-seq) technique and construct a genetic map of high saturation muskmelon, and preliminary QTL analysis of seed-related characters was carried out.

Results

The results indicated that the seed shape and seed color accorded with the segregation ratio of 3:1 (X2 = 0.59 and 0.66) and the segregation ratio of single gene. It was a quality trait controlled by a pair of alleles. The results of F_2 population distribution showed that seed length, seed width and 100-seed weight showed unimodal normal distribution, which were quantitative traits.(Fig.1)

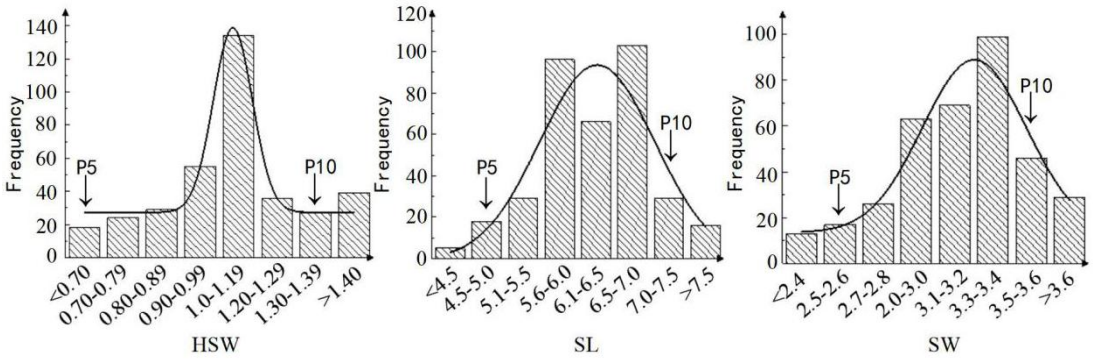

Fig.1 Frequency distribution in F_2 population for 100-seed weight，seed length and seed diameter

A muskmelon genetic map containing 12 linkage groups was constructed, and 3716 markers were obtained, the total map distance was 1356.49cM, and the average genetic distance between markers was 0.37cM (Table1). The loci *SS3.1* controlling melon Seed Shape (SS) was located between 31470843 and 31659961 on muskmelon chromosome 3, and the linkage markers at both ends of the distance were Marker555072 and Marker555263, and the genetic distances were 83.91cM and 84.34cM, respectively, with a contribution rate of 26.45%. The loci *SC12.1* controlling muskmelon Seed Color (SC) was located between 12785226 and 15029452 of muskmelon chromosome 12. The linkage markers at both ends of the distance were Marker1467315 and Marker1479935, and the genetic distances were 62.60cM and 63.44cM, respectively. The contribution rate was 14.07%. Two QTL loci (*HSW6.1* and *HSW7.1*) were detected to control the a Hundred of Seed Weight (HSW) of muskmelon seeds. *HSW6.1* was located between 1114486 and 1131014 of muskmelon chromosome 6. The linkage markers at both ends of the distance were Marker795334 and Marker79526, and the genetic distances were 18.51cM and 18.51cM, respectively. The contribution rate was 13.97%. *HSW7.1* was located between 458518 and 458554 of muskmelon chromosome 7. The linkage markers at both ends of the distance were Marker854935 and Marker854936. The genetic

distances were 3.17cM and 3.17cM, respectively. The contribution rate was 9.76%. A QTL loci (*SL3.1*) was detected to control the Seed Length (SL) of muskmelon, *SL3.1* was located between 31430517 and 31553644 of muskmelon chromosome 3. The linkage markers at both ends of the distance were Marker554875 and Marker554952, and the genetic distances were 82.65cM and 83.49cM, respectively. The contribution rate was 27.43%. Two QTL loci (*SW3.1* and *SW12.1*) controlling the Seed Width (SW) of melon were detected. *SW3.1* was located between 31447562 and 31470545 on chromosome 3 of melon. The linkage markers at both ends of the distance were Marker554875 and Marker554924, and the genetic distances were 82.65cM and 83.07cM, respectively. The contribution rate was 16.03%. *SW12.1* was located between 1295536 and 1295580 on chromosome 12 of muskmelon. The genetic distances of Marker1411669 and Marker1411668 were 29.83cM and 29.83cM, respectively, and the contribution rate was 12.88%. The results provide a theoretical basis for further cloning gene mining and functional analysis of muskmelon fruit related traits.

Table1 Statistics of basic genetic map in formation

Linkage Group ID	Markers number	Length of map/cM	Average Distance/cM	Gaps<=5	Spearman
chr01	217	128.65	0.60	99.07%	0.9978
chr02	391	126.23	0.32	100.00%	0.9995
chr03	142	84.34	0.60	99.29%	0.9900
chr04	194	104.22	0.54	100.00%	0.9960
chr05	324	120.15	0.37	98.76%	0.9992
chr06	233	106.51	0.46	99.57%	0.9986
chr07	346	130.19	0.38	99.71%	0.9977
chr08	659	130.23	0.20	100.00%	0.9989
chr09	237	104.38	0.44	100.00%	0.9983
chr10	161	85.64	0.54	99.38%	0.9981
chr11	391	116.61	0.30	99.23%	0.9976
chr12	421	119.34	0.28	99.76%	0.9678
Total	3716	1356.49	0.37	99.56%	0.9950

Conclusion

In this study, a muskmelon genetic map containing 12 linkage groups and 3716 markers was constructed by using F_2 segregation population. the total length of the linkage map was 1356.49cM and the average genetic distance between markers was 0.37cM. Based on the preliminary mapping of melon seed shape and seed coat color, the genes controlling melon seed shape were located in the third linkage group, and the genes controlling melon seed coat color were located in the 12th linkage group. QTL analysis of 100-seed weight, seed length and width showed that five QTL; muskmelon 100-seed weight QTL loci were located in linkage groups 6 and 7,

muskmelon seed length QTL was located in the third linkage group, and muskmelon seed width QTL loci were located in the third and 12th linkage groups. The contribution rate of the three characters is 9.76%-27.43%. The results of this study laid a theoretical foundation for fine mapping and functional analysis of key genes of muskmelon seed size in the future.

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References

1. Eriksson O. Game theory provides no explanation for seed sizevariation in grasslands [J]. Oecologia，2005，144(1)： 98-105.
2. Huang S, Ding J, Deng D, et al. Draft genome of the kiwifruit actinidia c hinensis. Nature Communications, 2013, 4: 2640.
3. Klinkhamer B G，Jong T J. Plangt size and seed production in themonocar pic pcrennial Cynoglossum officinale L [J]. New Phytol ，1987，106: 773-783.
4. Li B, Tian L, Zhang J Y, et al. Construction of a high-density genetic ma p based on large-scale markers developed by specific length amplified frag ment sequencing (SLAF-seq) and its application to QTL analysis for is of lavone content in Glycine max. BMC Genomics, 2014, 15: 1086.
5. Liu J P, Yu Z W, M Y. 2000. Review on the development of wat ermelon and muskmelon in China[J]. China Watermelon and Musk melon, (01):4-8.
6. Moles A T, Ackerly D D, Webb C O, et al. A brief history of seed size [J].Science, 2005, 307（5709）: 576-580.
7. Wang G C. QTL Mapping and Molecular Marker Development of flowering traits in muskmelon [D]. Heilongjiang Bayi Agricultural Reclamation University, 2020.
8. Wei Q Z, Wang Y Z, Qin X D, et al. An SNP-based saturated genetic ma p and QTL analysis of fruit-related traits in cucumber using specific-length amplified fragment (SLAF) sequencing. BMC Genomics, 2014, 15: 1158.
9. Westoby M, Falster D S, Angela T M, et al. Plant ecological strategies: so me leading dimensions of variation between species[J]. Annual Review of Ecology and Systematics, 2002, 33（1）: 125-159.