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Evaluation of SSR Markers Associated with High Oleic Acid in Sunflower

Atitaya Singchai, Nooduan Muangsan, Thitiporn Machikowa

***Abstract*—**Sunflower oil with high oleic acid content is most desirable because of its high oxidative stability. Screening sunflower of high oleic acid using conventional method is laborious and time consuming. Therefore, the use of molecular markers as a screening tool is promising. The objective of this research was to evaluate SSR primers for high oleic acid content in sunflower. Two sunflower lines, 5A and PI 649855 were used as the representative of low and high oleic acid sunflowers, respectively, and thirty seven SSR markers were used to identify oleic acid content trait. The results revealing 10 SSR primers showed polymorphic between high and low oleic acid lines and thus were informative. With these primers, therefore, it is possible to identify the genetic markers associated with high oleic acid trait in sunflower genotypes.

***Keywords*—**Microsatellite, *Helianthus annuus* L., fatty acid composition, molecular markers.

I. Introduction

SUNFLOWER (*Helianthus annuus* L.) is one of the most important oilseed crops. Its oil is a high quality vegetable oil which contains high level of unsaturated fatty acid (88%) i.e. linoleic acid and oleic acid. Oil with high proportion of oleic acid is great demand due to its high oxidative stability. Oleic acid content in sunflower oil is a quantitative trait controlled by polygenes [1], [2] and environmental effect implicated with the expression of this trait. The variation of this trait is continuous distribution which is difficult to select by phenotype. The standard method to determine oleic acid content is Gas Chromatography (GC) which produces accurate result but is expensive, time consuming and involves hazardous chemicals [3]. Molecular marker associated with high oleic acid trait is a useful tool to facilitate sunflower breeding program [4]. Simple sequence repeats (SSRs) or microsatellites are widely used as molecular markers. The advantages of microsatellites are locus-specific, co-dominant, highly polymorphic and easily verified by PCR technique. Nagarathna et al. [4] used microsatellite marker to screen high oleic acid sunflower and found polymorphic between high and low oleic acid genotypes. Grandon et al. [5] reported 82 markers that can validate high oleic acid sunflower and 35 of 82 markers localize on 16 sunflower linkage groups. Therefore, this study was conducted with an aim to evaluate SSR primers for high oleic acid content in sunflower genotypes.

A. Singchai is with the Institute of Agricultural Technology, Suranaree University of Technology, NakhonRatchasima, 30000, Thailand (e-mail: zagura_japan@hotmail.com).

N. Muangsan is with the Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand (e-mail: nooduan@sut.ac.th).

T. Machikowa is with Institute of Agricultural Technology, Nakhon Ratchasima 30000, Thailand (phone: 66-4422-4579; fax: 66-4422-4281; e-mail: machiko@sut.ac.th).

II. Materials and Methods

A. Plant Materials and Oleic Acid Content Determination

A low oleic acid content (22%) inbred line (5A) developed by Institute of Agricultural Technology, Suranaree University of Technology, Thailand, and a high oleic acid content (83%) obtained from US Department of Agriculture (USDA) germplasm accession PI 649855 (www.ars-grin.gov) were used in this study. These lines were used as the representative of low and high oleic acid sunflowers and planted in the experimental field of Suranaree University of Technology, Thailand in September 2012. At maturity, plants were harvested and seeds were dried. Then, the seeds from both lines were sampled and analyzed for oleic acid content by gas chromatography [6]. Three categories according to oleic acid content were identified as low (≤50%), intermediate (50–65%) and high (>65%) [7].

B. DNA Extraction

Thirty days after planting, fully expanded leaves of 5A and PI 649855 were collected for DNA extraction. DNA was extracted from 100mg leaf tissue using a CTAB protocol as modified by Rogers and Bendich [8]. Concentration of DNA was measured at 260nm in a nano spectrometer (NanoDrop Technology, USA) and the quality of DNA was checked by running 5µl of genomic DNA on 1.2% agarose gels with ethidium bromide.

C. SSR Primers Screening

Thirty seven SSR primers (Table I) including 34 primers of ORS set [9], 2 primers of ha set [10]and N1-3F primer [4]were selected and used to identify DNA samples from two lines (high and low oleic acid contents). PCR amplifications were carried out using Top Taq Master Mix Kit (Qiagen, USA) in 25µl reaction scale with 10ng/µl of template DNA and 0.2µM of each primer. The touchdown PCR was used for the amplification of DNA. Amplified PCR products were separated by gel electrophoresis on 3% agarose gels with ethidium bromide. The size of the SSR fragments was determined by comparison with a 100-bp ladder.

III. Results and Discussion

The oleic acid content by GC analysis showed that 5A plants had low oleic acid content (20–23%), whereas PI 649855 plants had high oleic acid content (65–72%). Out of

the 37 SSR primers screened for polymorphism between low oleic acid and high oleic acid plants, 10 SSR primers (27.03%) including N1-3F, ORS 296, ORS 311, ORS 321, ORS 333, ORS 339, ORS 371, ORS 488, ORS 1088 and ha 4149 (Figs. 1 (a)-(e)) generated differentiating bands between the high and low oleic content lines. Some primers produced single polymorphic bands, but some primers showed the amplification of multiple band patterns as shown in Figs. 1 (a)-(f). The result that N1-3F primer could distinguish between the two parental lines is consistent with the previous report by Nagarathra et al. [4]. With the 10 SSR markers above, it is possible to identify the genetic markers linked to high oleic acid trait in the F_2 mapping population which may be useful for further sunflower breeding program.

TABLE I
PRIMER SEQUENCE OF SSR PRIMERS USED FOR EVALUATION HIGH AND LOW OLEIC ACID SUNFLOWER LINES

No.	Primer name	Forward sequence	Reverse sequence
1	ha 2879	CATACCGTTCTTGTTC	CAACCTCCTAGGTCA
2	ha 4149	CAAAAACCTCTCTCCGTTGGC	GACTCCAAAGTCCACCAAATC
3	N1-3F	GAGAAGAGGGAGGTGTGAAG	AGCGGTTATGGTGAGGTCAG
4	ORS 16	GAGGAAATAAATCTCCGATTCA	GCAAGGACTGCAATTTAGGG
5	ORS 160	TCCCTTCCTTTCATCGTCTGCT	TGGCAATTTGCCAAGGACC
6	ORS 188	CTTCGTAGCCAACTCCCACC	CAATGGTTGACAATGGGTTTGC
7	ORS 287	CGGATTCACTGCTTTCCAAT	GCATAGTTGCCCATCAGAGTAA
8	ORS 290	TCTTTACTTCCACGGTGCACTA	GCATTCACAACAAACATCATCA
9	ORS 296	CCTTGCACTTAGCCCA	GCATTCACAACAAACATCATCA
10	ORS 300	GAATGCGGAGACAAAGGCT	ATAAGTGTGGCGGTGGAAGA
11	ORS 301	CGTGACCTGTGAAACACCAA	CGATAACCGTGTGAAATCGTG
12	ORS 309	CATTTGGATGGAGCCACTTT	GATGAAGATGGGGAATTTGTG
13	ORS 310	AATTCCCACGCAAACTTCAA	GGGTAAATGGGGCAACCTAT
14	ORS 311	TCCCGAATTAGCCAAAGAAC	GGTGTGGGTGTTGCAGCTAT
15	ORS 315	GCCGTGAATAATGGGATTGA	GATTGGGTCAGCTTGTGTGA
16	ORS 316	TGGCGTCTTCATAGCATCAG	GAGATTTGAGCTTCGTGTTGC
17	ORS 318	TCCATGAGTTGGTCGTATGC	CCGCATATTGAAACTGCATC
18	ORS 319	TCATCAATCCAAGCACCAAA	TTGGGCCGTAAACCCTTAAC
19	ORS 321	TGTCGAAGAGTTGTCGGAAC	GGGAAGGTGAAACCCTAACC
20	ORS 322	TGCACCACTTGGAACTTGAC	GCATTCATCCATAGTCATCAAGA
21	ORS 323	CGGGAAACTAGGATCAGAGG	GCCGGAGGATTAGAGGAGTT
22	ORS 324	CACTTCTACTCCATCTTCTTCATCAA	ATGATGCTCCGCAACAGTTT
23	ORS 331	TGAAGAAGGGTTGTTGATTACAAG	GCATTGGGTTCACCATTTCT
24	ORS 332	GACCAGCCGCATATTTCAA	AAACCGGCCTCTTATTTGGT
25	ORS 333	CGGTTAAGATGGTTCAGTTGG	ATATTAAGTTTTGGTTTTAGCCAGAA
26	ORS 337	TTGGTTCATTCATCCTTGGTC	GGGTTGGTGGTTAATTCGTC
27	ORS 339	CCCTCTTCCTCTCCCTTACTTT	AAATCCGCACTCCAATATGC
28	ORS 371	GGTGCCTTCTCTTCCTTGTG	CACACCACCAAACATCAACC
29	ORS 488	CCCATTCACTCCTGTTTCCA	CTCCGGTGAGGATTTGGATT
30	ORS 598	CCAAATGTGAGGTGGGAGAA	ATAGTCCCTGACGTGGATGG
31	ORS 822	CAATGCCATCTGTCATCAGCTAC	AAACAAACCTTTGGACGAAACTC
32	ORS 878	TGCAAGGTATCCATATTCCACAA	TATACGCACCGGAAAGAAAGTC
33	ORS 899	GCCACGTATAACTGACTATGACCA	CGAATACAGACTCGATAAACGACA
34	ORS 920	CGTTGGACGAAGAACTTGATTT	ACTTCCGTTTGTTCCGAGCTT
35	ORS 988	TTGATTTGGTGAAAGTGTGAAGC	CGAACATTATTTACATCGCTTTGTC
36	ORS 1068	AATTTGTCGACGGTGACGATAG	TTTTGTCATTTCATTACCCAAGG
37	ORS 1088	ACTATCGAACCTCCCTCCAAAC	GGATTTCTTTCATCTTTGTGGTG

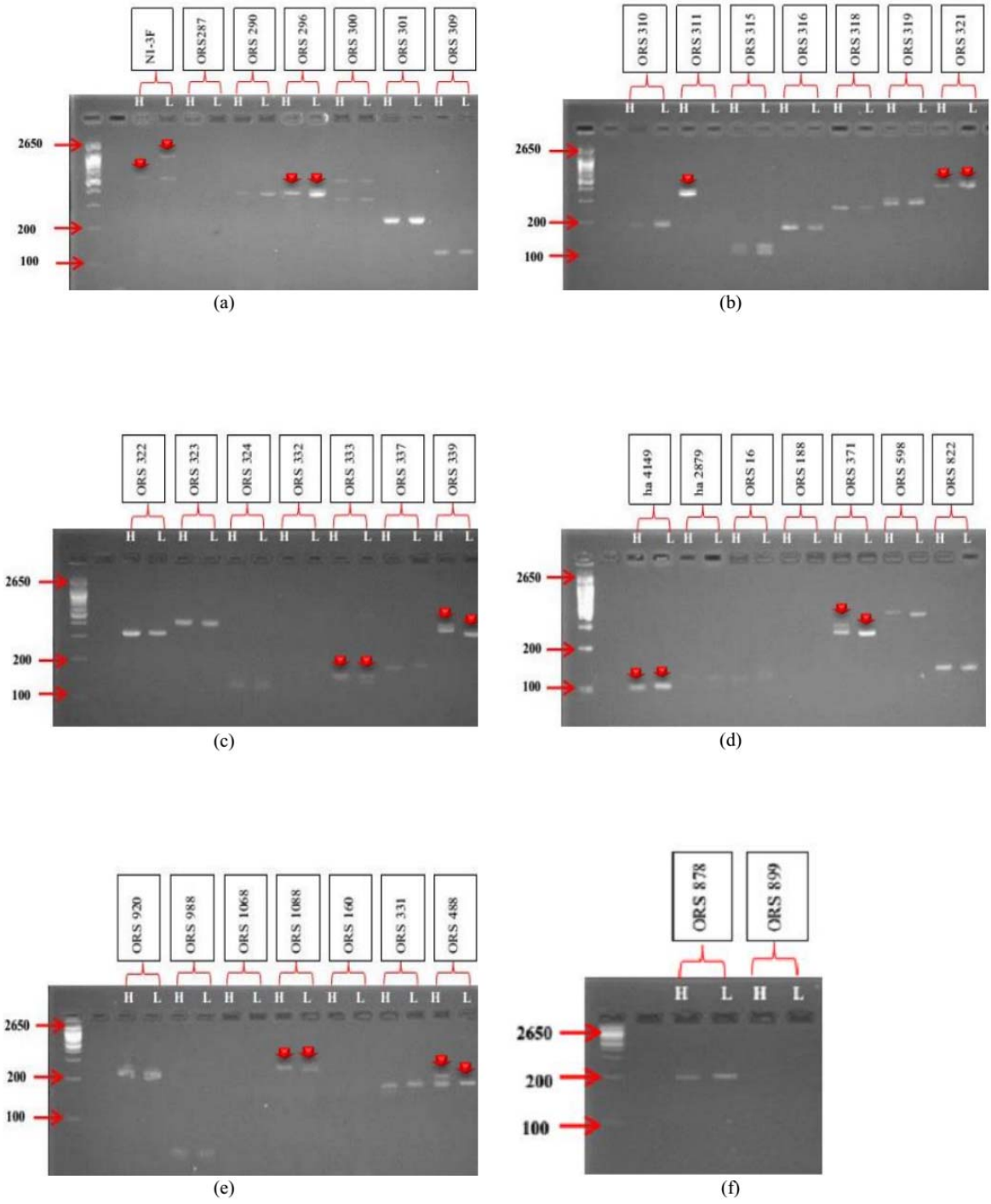

Fig. 1 (a)-(f) Polymorphic SSR primers between high (H) and low (L) oleic acid content lines

IV. Conclusion

Sunflower cultivar with high oleic acid content is a target trait in breeding programs. However, the screening of oleic acid content by conventional method was laborious, time consuming and influenced by environmental conditions. The results of this study indicated that it is possible to use these 10 SSR markers for screening sunflower with high or low oleic acid contents because they illustrated highly polymorphism. However, these markers need to be further validated in different sunflower populations in order to confirm their capability to identify high and low oleic acid contents.

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