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Genetic Diversity of Elite and Exotic Oilseed Meadowfoam Germplasm using AFLP Markers

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ABSTRACT

Amplified fragment length polymorphism (AFLP) is a PCR-based marker, which is suitable for DNA fingerprinting. The AFLP fingerprinting has not been described in meadowfoam. This powerful method was utilized to access genetic diversity of 41 meadowfoam accessions belonging to the genus *Limnanthes.* The objectives were to estimate polymorphic information contents (PIC) for AFLP markers and genetic distance among germplasm, and to assess the pattern of genetic diversity in meadowfoam germplasm. One hundred and seventy six polymorphic AFLP markers were produced using 6 primer combinations across 41 accessions. The PIC value ranged from 0.0 to 0.5 and 42 % of germplasm showed high PIC scores in a range between 0.45 and 0.5. Genetic distance ranged from 0.14 to 0.55 with an average of 0.44. The UPGMA clustering phenogram based on the distance matrix was consistent with the known taxonomic classification. The first three principal coordinate analyses accounted for 37 % of total variation of genetic distance estimated. Cluster analysis and principal component analysis clearly separated *L. floccosa* from *L. alba*. Within *L. alba*, subspecies *alba* and *versicolor* were distinctly separated into two groups. The results suggested genetic diversity among meadowfoam germplasm was very high. This information is useful to layout framework for meadowfoam improvement thereby enhancing productivity and performance of cultivated meadowfoam.

Key words: meadowfoam, *Limnanthes sp.,* genetic diversity, DNA fingerprinting, AFLP

INTRODUCTION

Meadowfoam (*Limnanthes* sp.) is an annual oil seed crop, native to Southern Oregon and California (Mason, 1952). Seed oil of meadowfoam contains unique unsaturated very long chain fatty acids (C_{20} and C_{22}) with outstanding oxidative stability (Isbell, 1997). Cultivated meadowfoam which belongs to section Inflexae, family Limnanthaceae is based on *Limnanthes alba*. The section Inflexae comprises of 4 species, namely *L. alba*, *L. floccosa*, *L. gracilis* and *L. montana*. The primary gene pool of *L. alba* is composed of *L. alba* ssp. *alba* and *L. alba* ssp. *versicolor,* whereas *L. floccosa*, *L. gracilis*, and *L. montana* are identified as a secondary gene pool of *L. alba.*

Meadowfoam has been domesticated since 1973 (Jain, 1986). The *L. alba* was evaluated as the most promising species in this genus for its lower moisture requirements, adaptation to wide ranges of environments, and high seed yield (Gentry and Miller, 1965). The first non- shattering cultivar,

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Foamore, developed for commercial production was released in 1974 (Calhoun and Crane, 1975). Breeding and cultivars development is underway at the Oregon State University with the main goal of increasing the productivity of meadowfoam by developing superior cultivars, discovering and developing novel phenotypes, and advancing our understanding of the genetics of economically important traits (Knapp and Crane, 1999).

Knowledge of the genetic diversity and relationships among germplasm is essential to the improvement of meadowfoam. Generally, the genetic diversity of germplasm collections can be obtained from pedigree records, morphological traits, isozyme and DNA markers (Smith *et al*., 1990; Mumm and Dudley, 1994). However, a small number of polymorphic markers obtained from isozyme markers and unfavorable phenotypic expressions of some morphological traits due to the environmental effects are known as limitations of these markers (Smith *et al*., 1990). The advent of DNA markers opens the ways to solve this problem since DNA markers can reveal tremendous number of genetic loci and they are phenotypic neutral and not subjected to environmental effects. A variety of DNA markers have been applied to cultivar improvement and germplasm management. Owing to its capacity to reveal a large number of marker loci in a short period of time (Vos *et al*., 1995), AFLP (Amplified fragment length polymorphism) appears to be the leading DNA-based marker systems for DNA fingerprints. AFLP has shown to be a powerful tool for genetic diversity study in many plant species such as soybean (Maughan *et al*., 1996), barley (Ellis *et al*., 1997), and rice (Zhu *et al*., 1998).

The high throughout AFLP markers were employed to evaluate the genetic diversity among recent meadowfoam germplasm. The objectives were (1) to estimate polymorphic information contents (PIC) for AFLP markers and estimate genetic distance among inbreds, open-pollinated cultivars, wild population and all genotypes, (2) to assess the pattern of genetic diversity and relationships of meadowfoam germplasm using UPGMA cluster analysis and principal coordinate analysis.

MATERIAL AND METHODS

Plant materials

A total of 41 meadowfoam accessions representing nine inbred lines, eight open-pollinated cultivars, and 24 wild populations were included in this diversity study (Table1). The meadowfoam seeds were germinated and grown as described by Katengam *et al.* (2002). Leaves from 50 to 55 day-old plants were harvested, immediately frozen, and stored at -80°C prior to DNA extraction.

AFLP fingerprints

Genomic DNA was extracted from frozen tissue using a protocol similar to Lodhi *et al.* (1994) with minor modification. AFLP analysis was carried out essentially as developed by Keygene (Waeningen, NL) with the minor modification that the selection of a subset of fragments on steptavidin beads was omitted (Vos *et al*., 1995). AFLP fingerprints were produced using six *Mse*I-*Eco*RI primer pairs with three selective nucleotides (Table 2).

Data analysis

Gene diversity was used to describe the relative value of AFLP marker with respect to the degree of polymorphism exhibited for each polymorphic locus. Thus,

$$\text{Gene diversity} = \sum_{i=1}^{k} p_i^2$$

where p_i is the frequency of i^{th} allele and k is the number of alleles (Ott, 1991). Anderson *et. al.* (1993) indicated that gene diversity was essentially the same as the polymorphic information content (PIC) as used by Botstein *et al.* (1980). This parameter is sometimes called heterozygosity. Due

Table 1 Meadowfoam germplasm (41 accessions) for AFLP fingerprinting.

Accessions	Description
1. OMF63 S_5	Self-pollinated inbred line (Selected from OMF159)
2. OMF64 S_5	Self-pollinated inbred line (Selected from OMF160)
3. OMF66 S_5	Self-pollinated inbred line (Selected from OMF66)
4. OMF109-1	Self-pollinated inbred line (Selected from Mermaid x OMF62/ OMF64)
5. OMF109-2	Self-pollinated inbred line (Selected from Mermaid x OMF62/ OMF64)
6. OMF109-3	Self-pollinated inbred line (Selected from Mermaid x OMF62/ OMF64)
7. OMF40-11 (Mermaid S_5)	Insect-pollinated *L. alba* ssp. *alba* inbred line (Selected from PI 283703)
8. LAG109 F_4	Self-pollinated Mermaid x *L. gracilis* ssp. *parishii* inbred line
9. LAG111 F_4	Self-pollinated Mermaid x *L. gracilis* ssp. *parishii* inbred line
10. OMF66 (Redding)	Wild *L. alba* ssp. *versicolor* population
11. OMF 158	Wild *L. alba* ssp. *versicolor* population (Recollected PI 283705)
12. OMF159	Wild *L. alba* ssp. *versicolor* population (Recollected PI 374791)
13. OMF160	Wild *L. alba* ssp. *versicolor* population (Recollected PI 374801)
14. OMF161	Wild *L. alba* ssp. *versicolor* population (Recollected PI 374802)
15. OMF57	Wild *L. alba* ssp. *versicolor* population (UC328 or UC457)
16. OMF52	Wild *L. alba* ssp. *alba* population (UC- Calaveras)
17. OMF53	Wild *L. alba* ssp. *alba* population (UC-Sonoma)
18. PI 374793	Wild *L. alba* ssp. *alba* population (Placer county)
19. PI 374794	Wild *L. alba* ssp. *alba* population (Placer county)
20. PI 374795	Wild *L. alba* ssp. *alba* population (Placer county)
21. PI 374796	Wild *L. alba* ssp. *alba* population (Butte county)
22. PI 374797	Wild *L. alba* ssp. *alba* population (Butte county)
23. PI 374798	Wild *L. alba* ssp. *alba* population (Butte county)
24. PI 367900	Wild *L. alba* ssp. *alba* population (Sacremento county)
25. PI 374792	Wild *L. alba* ssp. *alba* population (Shasta county)
26. Foamore	Open-pollinated cultivar (Selected from PI 283704)
27. Mermaid	Open-pollinated cultivar (Selected from PI 283703)
28. Floral	Open-pollinated cultivar (Mermaid x *L. floccosa* ssp. *grandiflora*)
29. Knowles (OMF69)	Open-pollinated cultivar (Selected from bulk of *L. alba* ssp. *alba*)
30. OMF86	Open-pollinated cultivar (Selected from Knowles)
31. OMF78	Open-pollinated cultivar (Selected from intermating between *L. alba* ssp.*alba* and ssp. *versicolor*)
32. OMF87	High oil open-pollinated population (Selected from OMF62)
33. PI 283724	*L. gracilis* ssp. *parishii* (Wild species)
34. PI 420137	*L. gracilis* ssp. *gracilis* (Wild species)
35. PI 283720	*L. floccosa* ssp. *bellingeriana* (Wild species)
36. PI 420133	*L. floccosa* ssp. *grandiflora* (Wild species)
37. PI 283719	*L. floccosa* ssp. *floccosa* (Wild species)
38. OSU-LF-4	*L. floccosa* ssp. *californica* (Wild species)
39. PI 283721	*L. floccosa* ssp. *pumila* (Wild species)
40. PI 283725	*L. montana* (Wild species)
41. OMF62-92	High oil open-pollinated population (*L. alba* ssp. *alba*)

to a bi-allelic feature, the PIC value for AFLP markers therefore ranges from 0.0 (monomorphic) to 0.5 (polymorphic).

Binary data representing the presence (1) and absence (0) of specific AFLP marker was generated. Only unambiguous polymorphic bands were scored and entered into a binary matrix as input for the genetic distance analysis. The genetic distance of Roger as modified by Wright (1978) was estimated among all genotypes using NTSYS-pc, version 1.8 (Rohlf, 1993). A dendrogram was subsequently generated by cluster analysis based on the unweighted pair group method on the basis of arithmetic averages (UPGMA) using a genetic distance matrix. Goodness of fit of a cluster analysis was tested using cophenetic correlation (r) value from MXCOMP program in NTSYS, which allowed direct comparison between the original dissimilarity matrix that was clustered and the cophenetic value matrix. Principal coordinate analysis based on genetic distance matrix was carried out using the PROC PRINCOMP procedure of SAS (1996) (SAS Institute, Inc., Cary, NC) to visualize the dispersion of individuals in relation to the first three principal axes of variation.

RESULTS

AFLP fingerprinting

The AFLP fingerprinting of 41 accessions including nine inbred lines, eight open-pollinated cultivars, and 24 wild populations of four species (10 taxa) was performed using six *Mse*I-*Eco*RI primer combinations (Table 2). These primer combinations were chosen based on previous information of polymorphism level from screening parents for AFLP meadowfoam mapping study (Katengam *et al*., 2002). A total of 176 AFLP markers were revealed from six primer combinations, which were polymorphic between two or more accessions across the 41 germplasm (Table 3). The polymorphic markers from each primer combination varied from 18 to 40 markers

Table 2 Oligonucleotide adapters and primers used for AFLP fingerprinting.

Adaptors:		
*Eco*RI adaptors*	91M35	5'-CTCGTAGACTGCGTACC-3'
	91M36	3'-CTGACGCATGGTTAA-5'
*Mse*I adaptors*	92A18	5'-GACGATGAGTCCTGAG-3'
	92A19	3'-TACTCAGGACTCAT-5'
AFLP primers		
*Eco*RI +1**	92R11	5'-AGACTGCGTACCAATTC / A-3'
*Mse*I +1**	92H20	5'-GACGATGAGTCCTGAGTAA / C-3'
*Eco*RI +3	92SO5	5'-GACTGCGTACCAATTC / ACA-3'
*Mse*I +3	92G23	5'-GATGAGTCCTGAGTAA / CAG-3'
	92G24	5'-GATGAGTCCTGAGTAA / CAT-3'
	92G29	5'-GATGAGTCCTGAGTAA / CTG-3'
	92G30	5'-GATGAGTCCTGAGTAA / CTC-3'
	92F10	5'-GATGAGTCCTGAGTAA / CAC-3'
	92F41	5'-GATGAGTCCTGAGTAA / CAA-3'

* = *Eco*RI and *Mse*I adaptors were ligated onto the ends of genomic restriction fragments.

** = *Eco*RI+1 and *Mse*I+1 primers were used in the preamplification of template DNA. The AFLP markers were generated using pairs of *Eco*RI+3 and *Mse*I +3 primers.

with an average of 29 markers per primer pair. The sizes of these markers ranged from 50 to 250 bp. Out of 176 AFLP markers, 142 and 138 AFLP markers showed polymorphism in at least two inbred lines and two cultivars, respectively, whereas 175 AFLP markers revealed polymorphism in at least two wild populations of meadowfoam.

Gene diversity (Polymorphic information content, PIC)

Estimation of gene diversity in meadowfoam germplasm was represented by polymorphic information content (PIC) value which showed the probability of polymorphism between two randomly lines. The PIC scores for 176 AFLP markers ranged from 0.0 to 0.5 (Figure1). Mean PIC scores was 0.31 for inbred lines, 0.30 for open-pollinated cultivars, 0.40 for wild populations, and 0.39 for all genotypes based on 142, 138, 175, and 176 polymorphic AFLP markers respectively. The distribution of PIC score was dramatically increased from 0.0 to 0.5. Nearly half of markers (42.61%) showed maximum PIC scores with a range of 0.45 - 0.50, indicating a high genetic diversity in meadowfoam germplasm.

Distance analysis

Genetic distance among 41 accessions based on 176 AFLP markers was estimated using Rogers genetic distance as modified by Wright (1978), ranging from 0.14 to 0.55, with an average of 0.44 (Table 4). The distances estimated among nine inbred lines varied from 0.14 (between OMF109-1 and OMF109-3) to 0.47 (between LAG109F_4 and OMF109-1, OMF109-2, and OMF109-3) with an average of 0.39. OMF109-1, OMF109-2 and OMF109-3 were related as they were developed from the same cross (Mermaid x OMF62/ OMF64), but were selected for different fatty acid concentration profiles. OMF109-2 was

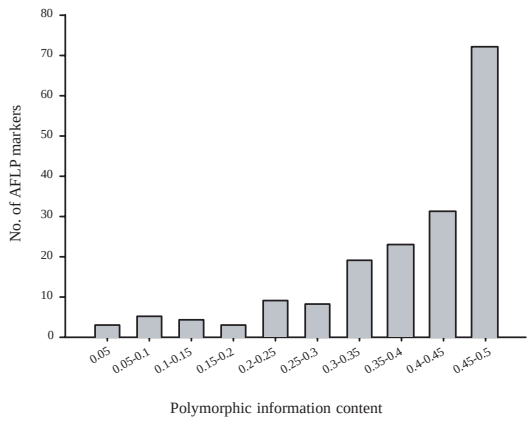

Figure 1 Distribution of polymorphic information content (PIC) scored for 176 AFLP markers among 41 meadowfoam accessions.

Table 3 Total numbers of informative AFLP marker detected with six primer combinations (one *Eco*RI+3 primers (ACA) and six *Mse*I+3 primers) used in diversity study.

Primers combinations (*Eco*RI+3 / *Mse*I+3)	Total polymorphic AFLP markers
ACACTC	28
ACACAG	38
ACACTG	40
ACACAC	35
ACACAA	18
ACACAT	20
Total	176
Average	29.33

Table 4 Genetic distance matrix estimated by Roger-W from AFLP fingerprints of 41 meadowfoam accessions.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. OMF63S_5	0																			
2. OMF64S_5	0.31	0																		
3. OMF66S_5	0.33	0.44	0																	
4 OMF109-1	0.37	0.39	0.46	0																
5 OMF109-2	0.37	0.38	0.46	0.25	0															
6 OMF109-3	0.36	0.39	0.45	0.14	0.27	0														
7 OMF40-11	0.35	0.43	0.42	0.42	0.43	0.43	0													
8 LAG109F_4	0.41	0.44	0.45	0.47	0.47	0.47	0.38	0												
9 LAG111F_4	0.35	0.42	0.40	0.44	0.44	0.44	0.42	0.30	0											
10 OMF66	0.31	0.41	0.32	0.42	0.43	0.43	0.41	0.45	0.42	0										
11 OMF158	0.30	0.38	0.35	0.42	0.42	0.42	0.41	0.43	0.38	0.32	0									
12 OMF159	0.33	0.41	0.39	0.42	0.42	0.42	0.40	0.47	0.41	0.37	0.35	0								
13 OMF160	0.32	0.38	0.38	0.41	0.42	0.41	0.41	0.46	0.42	0.35	0.37	0.34	0							
14 OMF161	0.32	0.38	0.38	0.37	0.37	0.36	0.44	0.47	0.41	0.34	0.34	0.38	0.38	0						
15 OMF57	0.43	0.49	0.47	0.47	0.47	0.49	0.43	0.44	0.44	0.47	0.45	0.46	0.47	0.46	0					
16 OMF52	0.43	0.49	0.45	0.49	0.48	0.50	0.42	0.47	0.44	0.47	0.47	0.48	0.48	0.48	0.42	0				
17 OMF53	0.38	0.44	0.43	0.46	0.46	0.46	0.40	0.47	0.44	0.42	0.43	0.44	0.42	0.43	0.47	0.44	0			
18 PI 374793	0.38	0.46	0.39	0.45	0.46	0.45	0.33	0.41	0.38	0.41	0.41	0.43	0.38	0.41	0.44	0.44	0.41	0		
19 PI 374794	0.39	0.49	0.39	0.45	0.44	0.46	0.42	0.47	0.43	0.41	0.43	0.45	0.43	0.43	0.47	0.44	0.44	0.36	0	
20 PI 374795	0.40	0.47	0.45	0.50	0.46	0.49	0.41	0.45	0.45	0.44	0.47	0.46	0.43	0.44	0.49	0.47	0.46	0.42	0.40	0
21 PI 374796	0.38	0.46	0.41	0.47	0.45	0.48	0.41	0.46	0.42	0.40	0.41	0.43	0.41	0.43	0.45	0.48	0.46	0.34	0.38	0.39
22 PI 374797	0.37	0.43	0.40	0.46	0.44	0.47	0.40	0.45	0.41	0.38	0.40	0.42	0.39	0.43	0.45	0.46	0.40	0.38	0.38	0.43
23 PI 374798	0.37	0.40	0.42	0.46	0.45	0.46	0.42	0.47	0.44	0.40	0.40	0.40	0.39	0.43	0.49	0.46	0.43	0.40	0.42	0.42
24 PI 367900	0.38	0.47	0.43	0.46	0.45	0.47	0.43	0.48	0.45	0.44	0.44	0.46	0.44	0.44	0.47	0.43	0.40	0.40	0.41	0.45
25 PI 374792	0.38	0.42	0.40	0.42	0.43	0.42	0.41	0.46	0.42	0.40	0.38	0.36	0.37	0.39	0.46	0.43	0.43	0.41	0.41	0.47
26 Foamore	0.37	0.45	0.40	0.46	0.46	0.45	0.43	0.49	0.43	0.44	0.41	0.41	0.38	0.45	0.49	0.44	0.44	0.38	0.40	0.42
27 Mermaid	0.40	0.46	0.43	0.49	0.48	0.49	0.37	0.48	0.42	0.46	0.43	0.44	0.42	0.46	0.46	0.45	0.43	0.40	0.41	0.44
28 Floral	0.38	0.43	0.44	0.45	0.43	0.46	0.37	0.41	0.38	0.42	0.42	0.40	0.39	0.45	0.46	0.42	0.42	0.40	0.45	0.44
29 Knowles	0.40	0.39	0.44	0.44	0.43	0.45	0.40	0.47	0.43	0.44	0.43	0.44	0.40	0.43	0.46	0.43	0.43	0.40	0.44	0.42
30 OMF86	0.37	0.39	0.42	0.41	0.43	0.42	0.38	0.46	0.45	0.41	0.41	0.42	0.37	0.42	0.47	0.47	0.40	0.40	0.46	0.44
31 OMF78	0.35	0.40	0.43	0.39	0.41	0.40	0.40	0.49	0.45	0.41	0.40	0.40	0.41	0.40	0.46	0.46	0.42	0.39	0.43	0.47
32 OMF87	0.36	0.41	0.43	0.42	0.42	0.42	0.39	0.47	0.43	0.40	0.41	0.41	0.41	0.43	0.49	0.46	0.43	0.41	0.44	0.44
33 PI 283724	0.47	0.51	0.49	0.49	0.48	0.50	0.47	0.46	0.44	0.52	0.48	0.49	0.50	0.49	0.45	0.48	0.45	0.47	0.47	0.51
34 PI 420137	0.38	0.47	0.44	0.46	0.47	0.46	0.41	0.45	0.41	0.46	0.43	0.42	0.43	0.43	0.49	0.47	0.44	0.38	0.46	0.44
35 PI 283720	0.47	0.52	0.49	0.49	0.50	0.50	0.48	0.48	0.46	0.53	0.51	0.54	0.52	0.52	0.47	0.46	0.47	0.49	0.45	0.49
36 PI 420133	0.46	0.52	0.51	0.50	0.52	0.51	0.47	0.50	0.46	0.51	0.49	0.52	0.50	0.53	0.48	0.48	0.49	0.51	0.47	0.49
37 PI 283719	0.46	0.52	0.51	0.50	0.51	0.49	0.49	0.46	0.45	0.49	0.49	0.55	0.51	0.52	0.48	0.48	0.50	0.49	0.47	0.49
38 OSU-LF_4	0.47	0.52	0.50	0.48	0.49	0.48	0.48	0.51	0.48	0.52	0.52	0.52	0.51	0.52	0.48	0.49	0.51	0.49	0.46	0.51
39 PI 283721	0.45	0.49	0.46	0.46	0.46	0.46	0.46	0.49	0.45	0.47	0.46	0.49	0.47	0.47	0.47	0.45	0.51	0.48	0.45	0.49
40 PI 283725	0.38	0.41	0.43	0.46	0.45	0.45	0.43	0.45	0.40	0.46	0.39	0.38	0.44	0.40	0.46	0.47	0.41	0.42	0.46	0.44
41 OMF6229	0.39	0.42	0.44	0.43	0.42	0.44	0.39	0.45	0.42	0.46	0.47	0.45	0.45	0.46	0.47	0.44	0.44	0.45	0.45	0.47

Table 4 Continued.

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
21 PI 374796	0																				
22 PI 374797	0.35	0																			
23 PI 374798	0.37	0.39	0																		
24 PI 367900	0.43	0.41	0.39	0																	
25 PI 374792	0.42	0.41	0.40	0.40	0																
26 Foamore	0.40	0.40	0.35	0.40	0.40	0															
27 Mermaid	0.43	0.42	0.43	0.44	0.42	0.41	0														
28 Floral	0.43	0.42	0.41	0.42	0.42	0.41	0.41	0													
29 Knowles	0.43	0.42	0.39	0.43	0.38	0.41	0.39	0.38	0												
30 OMF86	0.44	0.41	0.38	0.42	0.40	0.44	0.38	0.39	0.34	0											
31 OMF78	0.43	0.43	0.39	0.43	0.41	0.40	0.41	0.36	0.40	0.34	0										
32 OMF87	0.43	0.40	0.35	0.42	0.40	0.42	0.40	0.41	0.40	0.38	0.37	0									
33 PI 283724	0.47	0.49	0.48	0.45	0.46	0.46	0.46	0.47	0.48	0.47	0.49	0.49	0								
34 PI 420137	0.43	0.43	0.39	0.42	0.42	0.38	0.41	0.42	0.40	0.43	0.41	0.40	0.47	0							
35 PI 283720	0.51	0.49	0.49	0.46	0.49	0.52	0.49	0.51	0.50	0.48	0.52	0.50	0.44	0.49	0						
36 PI 420133	0.49	0.49	0.49	0.49	0.49	0.49	0.45	0.50	0.51	0.50	0.51	0.48	0.48	0.50	0.40	0					
37 PI 283719	0.50	0.48	0.51	0.51	0.50	0.51	0.48	0.52	0.51	0.50	0.51	0.49	0.48	0.51	0.32	0.41	0				
38 OSU-LF_4	0.52	0.52	0.50	0.49	0.48	0.51	0.48	0.53	0.49	0.50	0.52	0.49	0.49	0.48	0.39	0.34	0.41	0			
39 PI 283721	0.47	0.46	0.48	0.48	0.44	0.47	0.46	0.48	0.47	0.49	0.49	0.45	0.49	0.49	0.44	0.33	0.43	0.33	0		
40 PI 283725	0.44	0.42	0.41	0.44	0.39	0.41	0.40	0.43	0.40	0.40	0.42	0.38	0.46	0.39	0.52	0.51	0.52	0.49	0.48	0	
41 OMF6229	0.49	0.46	0.45	0.46	0.44	0.46	0.44	0.41	0.42	0.43	0.42	0.40	0.47	0.47	0.49	0.49	0.49	0.47	0.40	0.43	0

selected for *L. alba* ssp. *versicolor* fatty acid profile, which had high dienoic (22:2 Δ5Δ13) and low erucic acid (22:1Δ13) content while OMF 109-3 was selected based on *L. alba* ssp. *alba* fatty acid profile, which had high erucic acid but low dienoic acid content. OMF109-1 was selected based on heterozygote progeny, which had fatty acid profiles between these two subspecies. In contrast to OMF109 inbred lines, LAG109F_4 was derived from an interspecific cross between *L. alba* (Mermaid) and *L. gracilis* ssp. *parishii*, therefore it showed the greatest distance to those derived from intersubspecific (*L. alba*) crosses.

Amongst the eight open-pollinated cultivars, the distance estimated varied from 0.34 to 0.46 with an average of 0.40. The greatest distance was found between Foamore and OMF62-29, whereas the least distance or close relationship was found between OMF86 and Knowles and OMF86 and OMF78.

Among 24 wild meadowfoam populations, the greatest distance (0.55) was found between OMF159 (*L. alba* ssp. *versicolor*) and PI 283719 (*L. floccosa* ssp. *floccosa*). The least distance (0.32) was found between two wild populations of *L. alba* ssp. *versicolor*. The average genetic distance among wild population was 0.45 indicating high genetic diversity in these wild populations.

Cluster analysis

Cluster analysis using UPGMA (unweighted pair group method based on arithmetic mean) was performed to examine genetic relationships among meadowfoam germplasms. A phenogram was produced from the UPGMA cluster analysis of genetic distance matrix for 41 accessions based on mean AFLP data from each accession (Figure 2). There were three major diverse clusters. The first cluster (I) was comprised of *L. alba* ssp. *versicolor*. Wild populations of *L. alba* ssp. *versicolor* and inbred lines derived from them tended to group together in this cluster. The second and largest cluster (II) was primarily comprised of *L. alba* ssp. *alba* with two distinct subclusters. The first subcluster included wild populations of *L. alba* ssp. *alba* and the other consisted of all elite germplasm (open-pollinated cultivars) and wild populations of *L. alba* ssp. *alba* (PI 374798), *L. gracilis* spp. *gracilis (*PI 420137), and *L. montana* (PI 283725). The third cluster (III) was composed of five taxa of *L. floccos*a including subspecies *bellingeriana, floccosa, grandiflora, california and pumila* (PI 283720, PI 283719, PI 420133, OSU-LF_4 and PI 283721, respectively).

Three inbred lines (OMF62-29, LAG109-F_4 and LAG111-F_4), separately formed a small cluster far from the others (cluster IV). OMF62-29 was high oil content enhanced germplasm derived from *L. alba* ssp. *alba*, while LAG109-F_4 and LAG111-F_4 were inbred lines derived from interspecific cross between *L. alba* ssp. *alba* (Mermaid) and *L. gracilis* ssp. *parishii.* This phenogram showed that these two inbred lines were in between Mermaid and *L. gracilis* ssp. *parishii* indicating that they were equally related to their parents. The remaining two small clusters (V and VI) consisted of two wild populations of *L. alba* ssp. *alba*, OMF53 and PI 367900 and two wild populations of *L. alba alba* (OMF52) and *L. alba versicolor* (OMF57), respectively. The latter was distantly related to their groups (Figure 2).

The goodness of fit of this UPGMA cluster analysis was performed based on the cophenetic correlation (r) value between the cophenetic value matrix and the original distance matrix. The cophenetic correlation was high (r = 0.85) indicating a good fit of the UPGMA cluster analysis performed.

Principal coordinate analysis (PCA)

A two-dimensional presentation of genetic distance produced by principal coordinate analysis is shown in Figure 3. The first three principal coordinates accounted for 37 % of the total variation in AFLP-based genetic distance (the first, second, and the third eigenvalues were 0.22, 0.09, and

0.06, respectively). The first and the second as well as the first and the third coordinate clearly separated the wild populations of *L. floccosa* from the other populations. Within wild populations of *L. alba*, *L. alba* ssp. versicolor were clustered and separated from the *L. alba* ssp. *alba*.

DISCUSSION

In this study, 41 accessions of meadowfoam was fingerprinted including nine inbred lines, eight open-pollinated cultivars and 24 wild populations. One hundred seventy six polymorphic AFLP makers were produced from six primer combinations in 41 accessions whereas 141 and 138 AFLP markers were polymorphic among inbred lines and open-pollinated cultivars, respectively). Abundant of AFLP markers providing an efficient mean to UPGMA cluster analysis (Rohlf, 1993) with the cophenetic correlation (r) of 0.84 suggested that a good fit of cluster analysis was performed. The dendrogram produced from UPGMA cluster analysis showed concordance with the taxonomic classification (Mason, 1952) and previous systematic and phylogeny studies using morphological traits and allozyme markers (McNeill and Jain, 1983).

The principal component analysis provided three-dimensional presentation of estimated genetic distance and supported the results of the

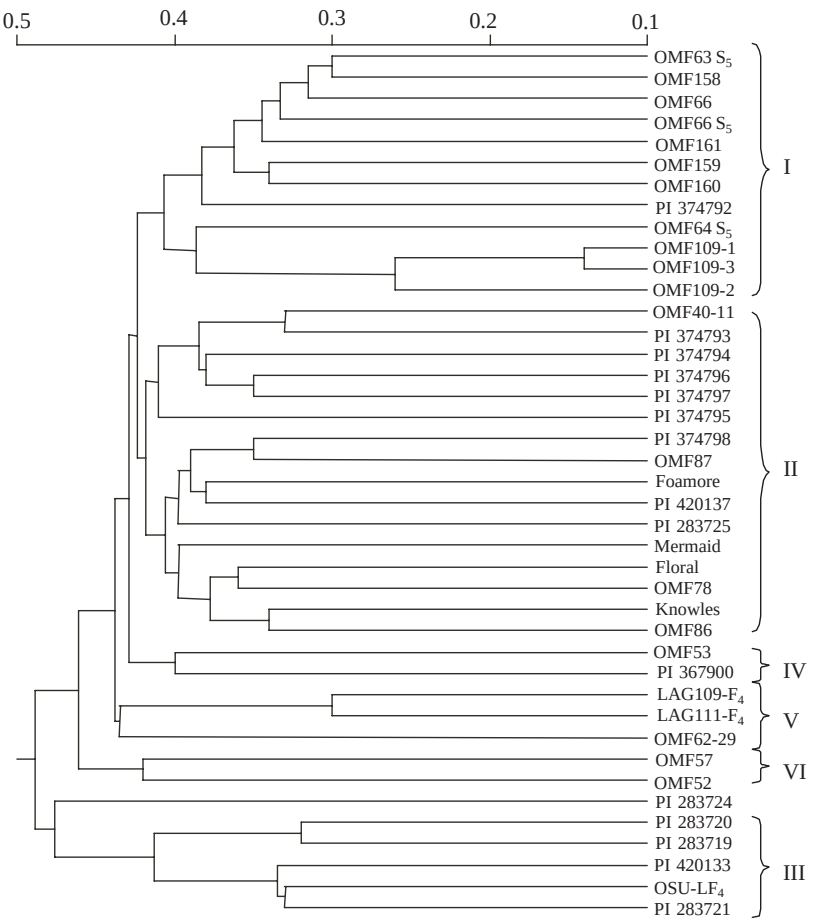

Figure 2 A dendrogram produced by UPGMA clustering of Roger-W genetic distance based on AFLP data among 41 meadowfoam accessions.

UPGMA cluster analysis. *L. floccosa* subspecies were distinctly separated from the others. Within *L. floccosa*, two subgroups were clearly distinguished in which *L. floccosa* subspecies *floccosa* and *bellingeriana* were closely related with genetic distance estimated at 0.32 (Table 4), while the other members of this species, *grandiflora*, *californica*, and *pumila* formed a more distantly related groups. This result is not only in agreement with previous studies of allozyme markers and morphological traits (McNeill and Jain, 1983) but agreed with morphological or taxonomical classification of this species as described by Mason (1952) and Arroyo (1973). The subspecies *floccosa* and *bellingeriana* were classified as fully autogamous, producing cleistogamous flowers, while the remaining three subspecies, *grandiflora*, *californica*, and *pumila*, were assigned as semi-autogamous due to their relatively more chasmogamous flowers and the presence of a small degree of protandry.

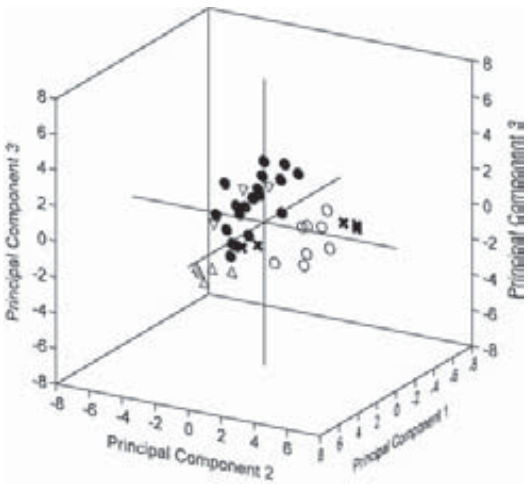

Figure 3 Principal coordinate plots of 41 meadowfoam germplasm for the first, second and third principal coordinates estimated with 176 AFLP markers, using Roger-W distance matrix. ●, *L. alba ssp. alba*; ○, *L. alba ssp. versicolor*; Δ, *L. floccosa*; ∇, *L. glacillis* and *L. montana*; ×, Interspecific hybrid.

Limnanthes species contains a wide range of mating systems from cleistogamy involving full autogamy in *L. floccosa* through intermediate stage in *L. gracilis* ssp. *parishii*, *L. graclilis* ssp. *gracilis*, and *L. montana*, to *L. alba* with dominantly protandous, showy, insect-pollinated flower and with the lowest autofertility in the section Inflexae (Mason, 1952; Arroyo, 1973). *L. alba* Benth. has been domesticated since 1971, and several open-pollinated cultivars have been developed for commercial production. These results revealed two distinct clusters including wild populations of *L. alba* ssp. *alba* and *L. alba* spp *versicolor* (Figure 2) which was consistent with taxonomic classification. Commercial open-pollinated cultivars formed a subgroup within *L.alba* ssp. *alba* cluster. Foamore was the first meadowfoam cultivar developed (Calhoun and Crane, 1975), followed by Mermaid and Floral (Calhoun and Crane, 1984). All cultivars were developed by mass selection. Knowles and OMF86 were closely related since they were derived from OMF58 by one and two cycles of recurrent half-sib family selection, respectively. OMF78 was developed by one cycle of recurrent half-sib family selection in OMF59. The result from genetic distance showed that all three cultivars were closely related.

L. alba was addressed as an outcrossing species and primarily consisted of two subspecies, *alba* and *versicolor* (Arroyo, 1973; Brown *et al.*, 1979). The mating systems of this species and the other species in section Inflexae have been widely investigated (Arroyo, 1975; McNeill and Jain, 1983). Several studies reported the presence of self-pollinated progeny in wild populations of *L. alba* (Arroyo, 1975). Knapp and Crane (1997) screened 26 accessions of *L. alba* for self-pollinated phenotypes and found that six populations of *L. alba* ssp. *versicolor* produced seed in a high percentage of flowers, which indicated that these geographically isolated populations seemed to have allelic diversity for self-pollination. *L. alba* ssp. *versicolor* is distributed from ~37 to 41°N and ~

120 to 123 ∞W in central and northern California (Brown *et al*., 1979; McNeill and Jain, 1983). Self- pollination seems to be concentrated in populations originating near Redding California (40.5 °N, 122.4 °W), and OMF66 (Redding) was found to be a source of self-pollinated phenotypes (Knapp and Crane, 1997). Self-pollinated inbred lines have been developed from OMF66 and two other wild populations of *L. alba* ssp. *versicolor* (OMF159 and OMF160) (Table 1). The self-pollinated inbred lines developed from these species provide useful resources for developing elite meadowfoam cultivars (Table 1).

CONCLUSION

AFLP fingerprinting was proven to be a promising approach for evaluating genetic diversity in addition to constructing a genetic linkage map in meadowform. AFLPs revealed the great diversity among meadowfoam germplasm, which broadened the opportunity for meadowfoam improvement. Wild species primarily contained sources of many desirable genes underlying important agronomic and quality traits. Interspecific and intersubspecific hybridization among meadowfoam germplasm provided an opportunity to broaden the genetic base of meadowfoam cultivars. Molecular breeding and genome mapping underlying economically important traits, particularly fatty acid concentration (low erucic acid content) and self-pollination traits, are underway in the laboratory, and this information will be used in marker-assisted selection for meadowfoam cultivar improvement.

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