Supplementary Table 1 Diploid and tetraploid mapping populations evaluated using high-resolution melting curve assay

Population	Parent 1	Species	Parent 2	Species	Ploid level	Population type	Progeny evaluated
DIP1	MS-13	*M. sativa* subsp. *caerulea*	MS-186	*M. sativa* subsp. *caerulea*	Diploid	F_1	10
DIP2	P1-4	*M. sativa* subsp. *caerulea*	P6-1	*M. prostrata*	Diploid	F_1	12
CHBC	Chilean	*M. sativa* subsp. *sativa*	F1-192	F_1 from Chilean × Wisfal	Tetraploid	Backcross	16
MFBC	Wisfal	*M. sativa* subsp. *falcata*	F1-192	F_1 from Chilean × Wisfal	Tetraploid	Backcross	16
AL608	Altet-4	*M. sativa* subsp. *sativa*	NECS-141	*M. sativa* subsp. *sativa*	Tetraploid	F_1	16
ALNECS	Altet-4	*M. sativa* subsp. *sativa*	95-608	*M. sativa* subsp. *sativa*	Tetraploid	F_1	16

Supplementary Table 2 Source, sequence and amplicon size of the primers used for high-resolution melting curve analysis

Primer Name	Forward	Reverse	Amplicon Size	LG in the F_1 linkage map	LG in the BC linkage map	Type of Marker	Matching chromosome or BAC ID in Mt V3.5	MT 3.5 gene ID
MTTF063	CACCTCCTTATATGCGCTGG	CACGTATAGATGGTGCACGG	111	NA	2	Simplex	chr02	Medtr2g038590.1
MTTF509	CATTGTGACAGAGCCTGGG	TTGTCACCTTGGTAGGGCAT	149	3	NA	Duplex	chr03	Medtr3g151360.1
MTTF562	AGGTTGGAGTGGACATATAACCC	CCACCAAGGACCTCTGTCAT	146	NA	NA	Simplex	chr03	Medtr3g104390.1
MSCWSNP0049	CAGAATGGAACTTGGAGTTGG	CATCCATCGCATGACTTCTC	100	NA	2	Simplex	chr02	IMGA|Medtr2g097470.1
MSCWSNP0212	CGAGAGAGAAGATTGGATTTGC	GAAGAATGGCGGGTTACAGA	100	NA	2	Simplex	chr02	IMGA|Medtr2g075950.1
MSCWSNP0222	TGTATGGTTCATCAAGGCTCA	AAGCACTTGCTCCAACAGGTA	51	NA	3	Simplex	chr03	IMGA|Medtr3g070100.1
MSCWSNP0353	CACAAGCTGAACAGCACCAT	CACTGGGACCTCCTTTGTTG	80	NA	6	Simplex	chr06	IMGA|Medtr6g023320.1
MSCWSNP0392	GCTTGGGTTGATGAGAAACAG	CACACAACACTAGCACATCCAA	128	8	8	Simplex	chr08	IMGA|Medtr4g128750.1
MSCWSNP0402	TTTGTGTCCTTCCTCTGTTTCA	TGGCATCAAGAGAATGGTAGC	89	2	2	Duplex	AC233655.2	IMGA|AC233655_7.1
MSCWSNP0406	AACAGGACTGTGTTGCACGTA	CTGCTTCTGCTGATGGACAA	86	7	NA	Simplex	chr07	IMGA|Medtr7g083570.1
MSCWSNP0407	CCCACTGAGGGTACTCATGC	AGCTGCAACAACTCCTCCAT	105	1	1	Simplex	chr01	IMGA|Medtr1g075610.1
MSCWSNP0413	GGCAGCTGAGATGAAAGCAT	CCAGGGACACCTTCAACAAT	95	3	NA	Simplex	chr03	IMGA|Medtr3g118390.1
MSCWSNP0414	GGAAATTCTGGTCTTGCCACT	CAATACCATCCTCTGACGACTCT	55	2	2	Simplex	chr02	IMGA|Medtr2g029850.1
MSCWSNP0416	TTGGTCACTCTGAAGCAAACC	GCGGACCACCAAATAACCT	110	2	NA	Simplex	chr02	IMGA|Medtr2g029800.2
MSCWSNP0420	ACATTCTTCTGCTCGCCAGT	GCTGAAGGTGCGAGTTTACC	101	4	4	Simplex	chr04	IMGA|Medtr4g036260.1
MSCWSNP0427	AACAACGTCGCCGTAGAGAC	TTCACCACCAGCGTAAACAA	102	5	5	Simplex	chr05	IMGA|Medtr5g091060.1
MSCWSNP0429	CACTACTTGGGTGCAAGCCTA	GCTTCTACACCACCTGTTCCA	101	NA	7	Simplex	chr07	IMGA|Medtr7g111240.1
MSCWSNP0430	TGGAGGGATAAAGGAGACTCAA	TGGTTTCAGCGTATTTCCAA	170	5	5	Simplex	chr05	IMGA|Medtr5g031310.1
MSCWSNP0460	GGAAATCCCGATCCAACTCT	CAAATCGGTGAGGGTGCTAC	93	2	NA	Simplex	AC234842.1	IMGA|Medtr2g029850.1
MSCWSNP0466	TGGTGGCAGCATTTAGTTCA	AACCCAGAGGGACCAATAGC	124	2	2	Simplex	chr02	IMGA|Medtr2g042130.1

NA indicates the marker was not mapped in this population due to lack of polymorphism or segregation pattern suitable for mapping.

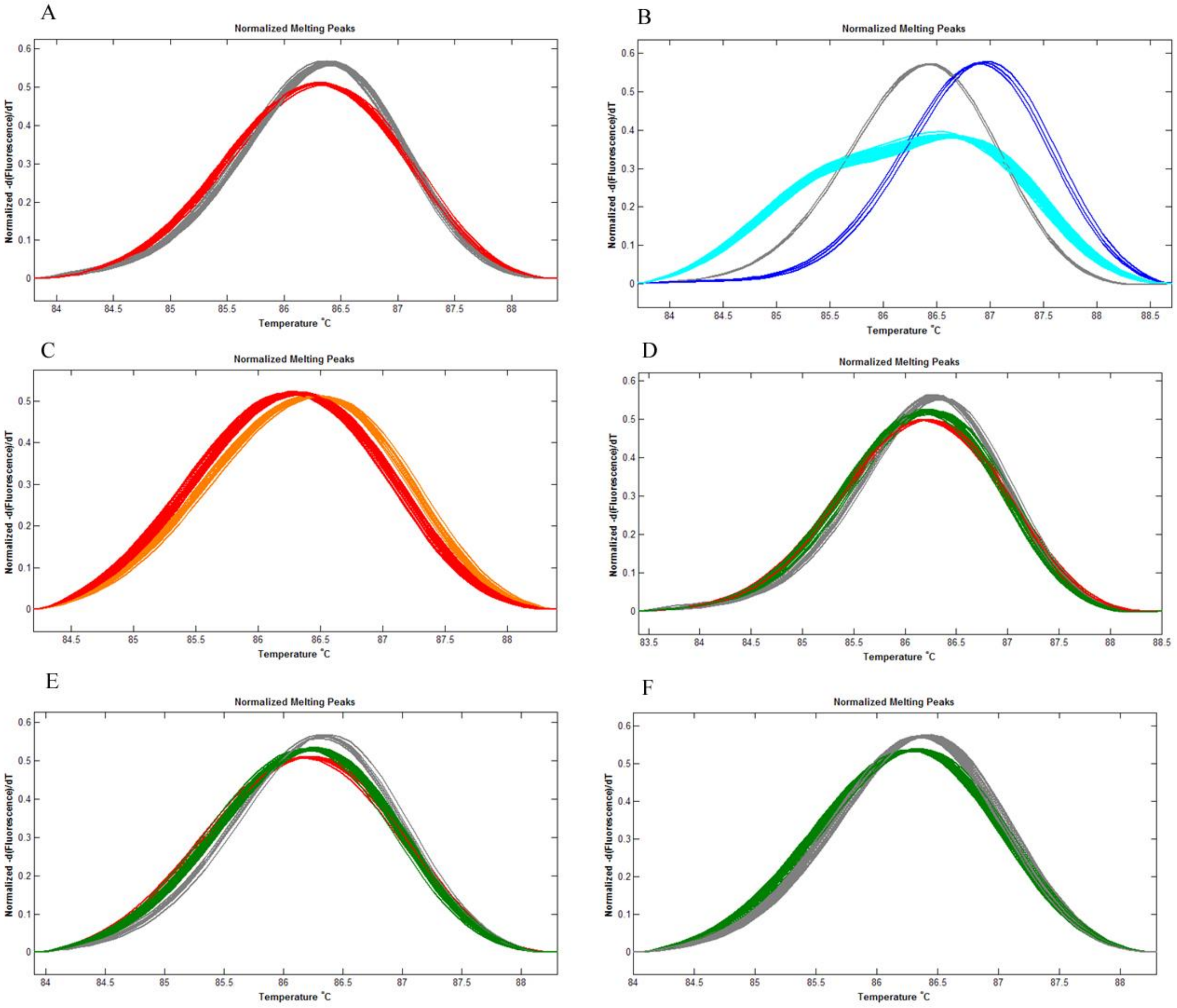
Supplementary Table 3 Genotypes of six tetraploid alfalfa mapping parents amplified by primer MTTF562

	SNP position (bp)						
Name	SNP 43	SNP 44	SNP 66	SNP 74	SNP 79	SNP 87	SNP 93
Chilean	AAAA	CCTT	AAAA	TTTT	TTTT	AAAA	AAGG
Wisfal	AATT	CCCC	AAGG	TTTT	AACT	AAGG	AAGG
F1-192	AATT	CCCT	AAAG	TTTT	ACTT	AAAG	AAGG
Altet-4	AAAA	CCCC	AAAA	TTTT	TTTT	AAAA	AAAA
NECS-141	AATT	CCCC	AAAG	TTTT	ATTT	AAAG	AAGG
95-608	AATT	CCTT	AAGG	CTTT	AATT	AAGG	GGGG

Supplementary Table 4 SNP base positions, genotypes and derived haplotypes of MFBC parents and 16 tetraploid progeny with primer MTTF562

Parents	---------- SNP position (bp) ----------						Genotype based on the SNP haplotype
	SNP 43	SNP 44	SNP 66	SNP 79	SNP 87	SNP 93	
Wisfal	AATT	CCCC	AAGG	AACT	AAGG	AAGG	I, II, IV, V
F1-192	AATT	CCCT	AAAG	ACTT	AAAG	AAGG	I, III, IV, V
Number of progeny							
4	AATT	CCCC	AAAG	ACTT	AAAG	AAAG	I, I, IV, V
3	AATT	CCCT	AAAG	ACTT	AAAG	AAGG	I, III, IV, V
3	AATT	CCCC	AAGG	AACT	AAGG	AAGG	I, II, IV, V
2	AATT	CCCT	AAGG	AACT	AAGG	AGGG	II, III, IV, V
2	AATT	CCCC	AAGG	AATT	AAGG	AAGG	I, I, V, V
1	AATT	CCCT	AAGG	AATT	AAGG	AGGG	I, III, V, V
1	AATT	CCCT	AAAG	ACCT	AAAG	AAGG	II, III, IV, IV
Haplotype							
I	A	C	A	T	A	A	
II	A	C	G	A	G	G	
III	A	T	A	T	A	G	
IV	T	C	A	C	A	A	
V	T	C	G	A	G	G	

Supplementary Fig. 1 Normalized melting peaks of ten parents and progeny of six alfalfa mapping populations using primer MTTF509 with three replicates. The amplicon is 145 bp long and covers two SNP sites. Each color represents a different SNP genotype. At MTTF509 SNP 123, AA (2X) and AAAA (4X) = gray, AAAG = green, AG (2X) and AAGG (4X) = red, AGGG = orange, GGGG = blue. At SNP 79, CC (P6-1) and CCCC = blue, and TTTT = all other colors. **A.** MS-13 (gray) × MS-186 (red) and ten progeny (four red and six gray). **B.** P1-4 (gray) × P6-1 (blue) and 12 progeny (all aqua). **C**. Wisfal (orange) × F1-192 (red) and 16 progeny (five orange and eleven red). **D**. Chilean (gray) × F1-192 (red) and 16 progeny (five gray, three red and nine green). **E**. Altet-4 (gray) × NECS-141 (red) and 16 progeny (two red, two gray and twelve green). **F**. Altet-4 (gray) × 95-608 (green) and 16 progeny (seven gray and nine green)


A
Normalized Melting Peaks
Normalized -d(Fluorescence)/dT
Temperature °C
B
Normalized Melting Peaks
Normalized -d(Fluorescence)/dT
Temperature °C
C
Normalized Melting Peaks
Normalized -d(Fluorescence)/dT
Temperature °C
D
Normalized Melting Peaks
Normalized -d(Fluorescence)/dT
Temperature °C
E
Normalized Melting Peaks
Normalized -d(Fluorescence)/dT
Temperature °C
F
Normalized Melting Peaks
Normalized -d(Fluorescence)/dT
Temperature °C

Supplementary Fig. 2 SNP markers mapped to linkage group 2 in two tetraploid populations (BC and F_1 maps) and the position of their corresponding sequences in the *M. truncatula* physical map V3.5. SNP markers are shown in red and SSR markers are shown in black. Map construction was performed by JoinMap® 3.0 software and map distances calculated using the Kosambi mapping function