KSÜ Tarım ve Doğa Derg 21(6):846-853, 2018
KSU J. Agric Nat 21(6):846-853, 2018

Linkage Disequilibrium (LD) Analysis in Alfalfa (*Medicago sativa* L.) Populations Spreading in Different Geographies of the World

Doğan İLHAN
Kafkas Üniversitesi Fen Edebiyat Fakültesi Moleküler Biyoloji ve Genetik Bölümü, Kars, Türkiye
✉: ilhand83@gmail.com

ABSTRACT

Cultivated alfalfa (*Medicago sativa* L.) was derived from *Medicago sativa* species complex as a result of breeding efforts. New efforts have focused on to determine the DNA polymorphisms based on molecular markers and to link these polymorphisms with related phenotype recently. Especially, the relationships between genotypes and phenotypes are evaluated as Linkage Analysis and Association Mapping Studies. On the basis of information, in this study, Linkage Disequilibrium (LD) analysis was performed using 31 SSR markers for the 70 populations. For the LD analysis, 23 of the 31 markers identified the physical distances on the 8 chromosomes of the alfalfa. Distances of 103 loci on 8 chromosomes were determined based on *Medicago truncatula* genome. A significant ($p<0,0001$) LD value was not observed in these populations for the evaluated markers.

DOI:10.18016/ksutarimdoga.vi.452852

Article History
Received : 20.02.2018
Accepted : 19.04.2018

Keywords
Medicago sativa species complex,
physical distances,
SSR markers,
chromosome

Research Article

Dünyanın Farklı Coğrafyalarında Yayılış Gösteren Yonca (Medicago sativa L.) Populasyonlarında Bağlantı Eşitsizliği Analizi

ÖZET

Kültür yoncası (*Medicago sativa* L.), ıslah çalışmaları sonucunda *Medicago sativa* tür kompleksinden geliştirilmiştir. Son zamanlarda, yonca bitkisindeki çalışmaların moleküler markörler temelinde DNA polimorfizmlerini belirlemeye ve bu polimorfizmlerle ilişkili olan fenotipler arasında bağlantı kurmaya odaklandığı bilinmektedir. Özellikle genotipler ve fenotipler arasındaki ilişkiler, Bağlantı Analizleri ve İlişki Haritalama çalışmaları şeklinde değerlendirilmektedir. Bilgiler temelinde bu çalışmada; 70 populasyon için 31 SSR markörü kullanılarak Bağlantı Eşitsizliği (Linkage Disequilibrium, LD) analizi de gerçekleştirilmiştir. Bağlantı Eşitsizliği analizi için; 31 markörden 23 tanesinin yonca'nın 8 kromozomu üzerindeki fiziksel mesafeleri belirlenmiştir. 8 kromozom üzerinde 103 lokustan oluşan mesafeler *Medicago truncatula* genomu temel alınarak saptanmıştır. Değerlendirilen markörler için; bu populasyonlarda önemli ($p<0,0001$) bir Bağlantı Eşitsizliği (LD) değeri gözlenmemiştir.

Makale Tarihçesi
Geliş : 20.02.2018
Accepted : 19.04.2018

Anahtar Kelimeler
Medicago sativa tür kompleksi,
fiziksel mesafeler,
SSR markörler,
kromozom

Araştırma Makalesi

To cite: İlhan D 2018. Linkage Disequilibrium (LD) Analysis in Alfalfa (*Medicago sativa* L.) Populations Spreading in Different Geographies of the World. *KSÜ Tar Doğa Derg 21(6) : 846-853, DOI : 10.18016/ksutarimdoga.vi.452852*

INTRODUCTION

Alfalfa (*Medicago sativa* L.) plant is rather important to show high genomic similarity to *Medicago truncatula* which is a model organisms of legume forage and, thus alfalfa is preferred as talented reference forage crop in terms of molecular studies (Kir et al., 2008; Julier et al., 2003). It is known that both morphologic characters and ploidy levels are used to identify the alfalfa subspecies classification. (Lesins and Lesins, 1979). Recently, the classification of these taxa is supported with molecular marker studies (Sakiroglu et al., 2011; İlhan et al., 2016). Most of alfalfa cultivars are synthetic populations which are improved as a result of recurrent phenotypic selections. Especially, main agronomic traits, such as winter hardness, pathogen resistance and biomass,

present genetic merits for plants in consequence of phenotypic evaluations (Brummer, 1999). It is essential to determine DNA polymorphisms, which cause phenotypic variations, and genetic variations, which are underlying genetic diversity in plant breeding (Lande and Thompson, 1990). In the historical process, separating populations, together with molecular markers, are used to detect relations between genotype and phenotypes as a result of controlled crossbreeding (Stuber et al., 1999). Linkage Disequilibrium (LD) value has a major effect on giving the meaning of relations among populations (Yu et al., 2006). LD can be explained as the non-random association among alleles at distinct loci in a breeding population. The degree of LD among loci is substantial for genetic analysis (Flint-Garcia et al., 2003). Indeed, LD distance in genome substantially is a significant parameter both in specifying the number of marker and building the mapping strategy (Rafalski and Morgante, 2004). The size of LD in populations was detected to be positively correlated with number of markers (Li et al., 2011b).

In heterozygous plants, when the pairs of alleles located on the same haplotype they have high values of LD but when the alleles located different haplotype, the amount of LD decreases. Self-fertilizing plants commonly have less LD decay than homozygous ones due to inefficient recombination. Although, LD decay is shown at short distance (100-1500bp) in some outcrossing species such as maize (Remington et al., 2001; Tenaillon et al., 2001), In Barley and other selfing crops, it is revealed as at large distance (up to 20cM) (Kraakman et al., 2004) and durum wheat (Maccaferri et al., 2005). On the contrary, in natural populations such as *Medicago truncatula* (Branca et al., 2011) and *Arabidopsis thaliana* (Nordborg et al., 2002), is shown much faster LD decay. Perennial species have less LD decay because of limited recombinations (Raboin et al., 2008). Comprehensive studies about LD were carried out in *Arabidopsis thaliana* (Nordborg et al., 2002; Kim et al., 2007), Maize (Yan et al., 2009; Van Inghelandt et al., 2011), alfalfa (Li et al., 2014) and other plants but it is limited on alfalfa.

It is clear that the sizes of LD in some alfalfa populations are not known. Therefore, LD analysis of 70 alfalfa populations that are distributing from various regions of world were performed using 31 SSR markers in this study.

MATERIAL and METHOD

Plant materials

Seventy different populations of 3 subspecies (*sativa*, *varia* and *falcata*) were used as plant materials. Each population was represented with four individual genotypes and totally 280 genotypes including 112 individual *sativa*, 120 individual *falcat*a and 48 individual *varia* genotypes were used (Table 1 and 2). All seeds in this study were selected from the USDA National Plant Germplasm System.

Table 1. Localities and germplasm information of plant materials.

Number	Chromosome Number	PI Number	Subspecies	Origin Country	Latitude	Longitude	Status
1	32	PI 173733	*sativa*	Turkey	37 deg. 16 min. 0 sec. N (37.26666667)	38 deg. 49 min. 0 sec. E (38.81666667)	Unknown
2	32	PI 179369	*sativa*	Turkey	39 deg. 0 min. 0 sec. N (39)	43 deg. 21 min. 0 sec. E (43.35)	Unknown
3	32	PI 180303	*sativa*	India	22 deg. 18 min. 0 sec. N (22.3)	70 deg. 53 min. E (70.88333333)	Unknown
4	32	PI 182240	*sativa*	Turkey	38 deg. 13 min. 0 sec. N (38.21666667)	37 deg. 12 min. 0 sec. E (37.2)	Unknown
5	32	PI 183262	*sativa*	Saudi Arabia	-	-	Unknown
6	32	PI 196225	*sativa*	India	-	-	Unknown
7	32	PI 198963	*sativa*	Cyprus	-	-	Unknown
8	32	PI 199273	*sativa*	Poland	-	-	Landrace
9	32	PI 201863	*sativa*	Iran	31 deg. 55 min. 0 sec. N (31.91666667)	54 deg. 22 min. 0 sec. E (54.36666667)	Unknown
10	32	PI 206576	*sativa*	Greece	-	-	Unknown
11	32	PI 206698	*sativa*	Turkey	38 deg. 36 min. 0 sec. N (38.6)	39 deg. 2 min. E (39.03333333)	Unknown
12	32	PI 208683	*sativa*	Algeria	-	-	Unknown
13	32	PI 210763	*sativa*	Spain	-	-	Unknown
14	32	PI 214218	*falcata*	Denmark	-	-	Wild
15	32	PI 217648	*sativa*	Iraq	-	-	Unknown

Number	Chromosome Number	PI Number	Subspecies	Origin Country	Latitude	Longitude	Status
16	32	PI 220531	*sativa*	Afghanistan	34 deg. 20 min. N (34.33333333)	62 deg. 12 min. 0 sec. E (62.2)	Unknown
17	32	PI 222198	*falcata*	Afghanistan	33 deg. 42 min. 36 sec. N (33.71)	69 deg. 10 min. 12 sec. E (69.17)	Wild
18	32	PI 239954	*sativa*	Algeria	35 deg. 33 min. 0 sec. N (35.55)	5 deg. 10 min. 12 sec. E (5.17)	Unknown
19	32	PI 244317	*sativa*	Spain	-	-	Unknown
20	32	PI 256337	*sativa*	Pakistan	-	-	Unknown
21	32	PI 262544	*sativa*	İsrael	-	-	Unknown
22	32	PI 299053	*sativa*	USSR	-	-	Unknown
23	32	PI 315484	*sativa*	USSR	-	-	Wild
24	32	PI 399551	*sativa*	Romania	-	-	Unknown
25	32	PI 420400	*sativa*	Spain	41 deg. 38 min. N (41.63333333)	0 deg. 53 min. W (-.88333333)	Unknown
26	32	PI 440517	*sativa*	Kazakhistan	-	-	Wild
27	32	PI 442877	*sativa*	China	-	-	Unknown
28	32	PI 464801	*varia*	Turkey	-	-	Wild
29	32	PI 464813	*varia*	Turkey	-	-	Wild
30	32	PI 476393	*varia*	Ukraine	-	-	Cultivar
31	32	PI 486202	*varia*	Ukraine	-	-	Wild
32	32	PI 486210	*varia*	USSR	-	-	Wild
33	32	PI 491407	*falcata*	China	-	-	Unknown
34	32	PI 494661	*falcata*	Romania	46 deg. 54 min. 36 sec. N (46.91)	23 deg. 25 min. 12 sec. E (23.42)	Wild
35	32	PI 499548	*falcata*	China	43 deg. 58 min. 0 sec. N (43.96666667)	116 deg. 2 min. E (116.03333333)	Wild
36	32	PI 499664	*falcata*	China	44 deg. 5 min. 24 sec. N (44.09)	88 deg. 30 min. 36 sec. E (88.51)	Wild
37	32	PI 499665	*falcata*	China	43 deg. 34 min. 12 sec. N (43.57)	87 deg. 2 min. 24 sec. E (87.04)	Wild
38	32	PI 502441	*falcata*	Russia	46 deg. 11 min. 24 sec. N (46.19)	43 deg. 53 min. 24 sec. E (43.89)	Wild
39	32	PI 502446	*falcata*	Russia	-	-	Wild
40	32	PI 502459	*sativa*	Kazakhistan	-	-	Wild
41	32	PI 502474	*sativa*	Armenia	-	-	Wild
42	32	PI 502514	*varia*	USSR	-	-	Cultivar
43	32	PI 502521	*varia*	USSR	-	-	Cultivar
44	32	PI 502529	*varia*	USSR	-	-	Cultivar
45	32	PI 502532	*varia*	USSR	-	-	Cultivar
46	32	PI 502533	*varia*	USSR	-	-	Cultivar
47	32	PI 502540	*varia*	USSR	-	-	Cultivar
48	32	PI 503867	*varia*	Romania	45 deg. 2 min. N (45.03333333)	29 deg. 10 min. 0 sec. E (29.16666667)	Unknown
49	32	PI 516902	*sativa*	Morocco	31 deg. 38 min. 24 sec. N (31.64)	7 deg. 43 min. 48 sec. W (-7.73)	Wild
50	32	PI 538983	*falcata*	Ukraine	-	-	Wild
51	32	PI 631573	*falcata*	Italy	45 deg. 38 min. N (45.63333333)	13 deg. 46 min. 0 sec. E (13.76666667)	Wild
52	32	PI 631579	*falcata*	Italy	45 deg. 52 min. 0 sec. N (45.86666667)	13 deg. 29 min. E (13.48333333)	Wild
53	32	PI 631582	*falcata*	Turkey	39 deg. 45 min. 0 sec. N (39.75)	37 deg. 2 min. E (37.03333333)	Wild
54	32	PI 631585	*falcata*	Italy	45 deg. 39 min. 0 sec. N (45.65)	13 deg. 47 min. E (13.78333333)	Wild
55	32	PI 631592	*falcata*	Italy	45 deg. 39 min. 0 sec. N (45.65)	13 deg. 47 min. E (13.78333333)	Wild
56	32	PI 631796	*falcata*	Czech Republic	49 deg. 12 min. 0 sec. N (49.2)	16 deg. 38 min. E (16.63333333)	Wild

Number	Chromosome Number	PI Number	Subspecies	Origin Country	Latitude	Longitude	Status
57	32	PI 631845	*falcata*	Sweden	-	-	Wild
58	32	PI 631855	*falcata*	Sweden	57 deg. 11 min. N (57.18333333)	12 deg. 20 min. E (12.33333333)	Wild
59	32	PI 631859	*falcata*	Sweden	-	-	Wild
60	32	PI 641381	*falcata*	Russia	56 deg. 5 min. N (56.08333333)	92 deg. 46 min. 0 sec. E (92.76666667)	Wild
61	32	PI 641383	*falcata*	Russia	55 deg. 27 min. 0 sec. N (55.45)	78 deg. 18 min. 0 sec. E (78.3)	Wild
62	32	PI 641400	*falcata*	Russia	-	-	Wild
63	32	PI 641545	*falcata*	Mongolia	49 deg. 59 min. 7 sec. N (49.98527778)	107 deg. 13 min. 37 sec. E (107.22694444)	Wild
64	32	PI 641546	*falcata*	Mongolia	50 deg. 17 min. 59 sec. N (50.29972222)	104 deg. 58 min. 51 sec. E (104.98083333)	Wild
65	32	PI 641548	*falcata*	Mongolia	50 deg. 1 min. 43 sec. N (50.02861111)	105 deg. 17 min. 27 sec. E (105.29083333)	Wild
66	32	PI 641581	*falcata*	Kazakhistan	49 deg. 26 min. 24 sec. N (49.44)	58 deg. 37 min. 14 sec. E (58.62055556)	Wild
67	32	PI 641582	*falcata*	Kazakhistan	49 deg. 27 min. 6 sec. N (49.45166667)	58 deg. 37 min. 14 sec. E (58.62055556)	Wild
68	32	PI 641585	*falcata*	Kazahkistan	49 deg. 33 min. 51 sec. N (49.56416667)	58 deg. 55 min. 0 sec. E (58.91666667)	Wild
69	32	PI 641588	*falcata*	Kazakhistan	49 deg. 18 min. 51 sec. N (49.31416667)	59 deg. 3 min. 34 sec. E (59.05944444)	Wild
70	32	PI 641599	*falcata*	Kazakhistan	48 deg. 34 min. 19 sec. N (48.57194444)	57 deg. 19 min. 6 sec. E (57.31833333)	Wild

Table 2. The Numbers of Used Individuals and Accessions Numbers for Plant Materials

Subspecies	The Number of Used Accessions	The Number of Used Individuals
M. sativa ssp. *sativa*	28	112
M. sativa ssp. *falcata*	30	120
M. sativa ssp. *varia*	12	48
Total Numbers	70	280

Plant growing and DNA isolation

Plant seeds were sown in plastic pots containing soil under sterile conditions. Plants were grown under greenhouse conditions (25±2 ^{0}C, 8/16-h photoperiod) and organized with 4 replicates at Kafkas University of Kars city for 3 months. DNA isolation was achieved using CTAB method and leaves (Doyle and Doyle, 1990). DNAs were diluted to10 ng/µl so that PCR reactions can be set up.

PCR reactions and data scoring with SSR markers

We selected 31 SSR markers (Table 3), which were used in alfalfa studies (Diwan et al., 2000; Julier et al., 2003; Robins et al., 2007). PCR reactions were conducted with M13 protocol (Schuelke, 2000). SSR markers were amplified by independent PCR reactions (Julier et al., 2003; Sledge et al., 2005). PCR products were visualized with ABI3730 sequencer at the institute of The Samuel Robert Noble Foundation in the USA. Allele scoring was carried out using GENEMARKER software (SoftGenetics, State College, PA). Scoring was accomplished as presence or absence of each individual allele in this study.

Linkage Disequilibrium (LD) analysis

LD tests were carried out among SSR loci using POWERMARKER v3.23 (Liu, 2002) software. Since many tests were performed, experimental error ratio increased in the study.

Table 3. 31 SSR Primers and Allele Sizes

Marker	Forward Primer	Reverse Primer	Allele Sizes
al369471	AACCAGTGAGTGGATGTGGTC	GTGAAAACCCTTAGCACCGA	155-222
aw373	TATCATCCTGGTTCGTTCCTCT	GGTTGAGCTTGAGAAAATCTGA	118-152
mtic332	CCCTGGGTTTTTGATCCAG	GGTCATACGAGCTCCTCCAT	119-170
bg648700	GCTTTTCACACCTCCACTCC	ACGGGAAAGACTCCCACTCT	208-273
aw282	CGACCAAATCACTCTTCTTCAA	AATCCAAGACCATTCACCTGAG	208-308
bf650422	ACAACAACGATGGACAACGA	CAGGCATTGGTGGAAACAGT	265-319
aw774443	ATTCGCAGTGAGCTGATCCT	GACATTTGCAGACCACCATT	215-239
aw394	AGGATGATGTGGAAGGAAGAAA	TTGCTAGAGCCTTAAACCCTGT	233-275
aw319	AAAAGGTTTCTAACACCAAGCA	TTCCTGACTTTCCATGATCCTT	216-246
aw586158	GATCAATTCGTGCAGAAGCA	ATTCATCCTTGCTCGTTTCG	202-236
b21e13	GCCGATGGTACTAATGTAGG	AAATCTTGCTTGCTTCTCAG	133-192
aw690263	TTACCATATTAACCCCCGCA	CGCATATCACCTCCCAGAAT	245-265
aw379	GTCTCTCTCTATTCTCTTCCCTTTTC	TTCTCGAAATCTTCTGCTCTCG	208-262
aw691701	CACCACAAAACGCAAACAAC	ACCCTATTGTCTCCCCATCC	107-162
aw387	GAACACTCTCCGAAACAAGGAC	ATAAGCCATTCTCAGCACCGTA	194-226
aw688546	GGTGAATTTTCTCCACTTCCA	TCGGCTCAGTTTAGGCTTCT	292-350
al367160	CCCCATTGACGCATTCTTAC	TCCTCAACCAACCACTTCCT	246-330
aw559239	TTCTCTTCCCCAATGGACAG	TCTCTGATACCCATTTGCCC	239-389
bg448975	TCGGATCTGACACGATTTTG	TTGGTTAAAAGATGAAGATGAACG	207-256
aw685868	AAGCAAGTTCTGTTGATGGAGA	TTGTGAAAGCCAAAACACCA	271-310
be100	GCATTAGCACCCTCATTCATATC	TGCAGAGACTTTTGAACACCTT	273-311
aw295	CAACATTCTTCCATTTCCTTCC	TCTTCATCTTCGTCGTCTTCAA	216-277
aw348	GCAACCATCTAAACCCAACAA	AGGCTAATCGACGGGAAAAT	206-255
bg647796	GCAAGAAAGCATAGGCTGAGA	GTGAAGCTGCACGAATTTCA	260-307
mtic14	CAAACAAACAACACAAACATGG	CCCATTGATTGGTCAAGGTT	121-139
aw256	ACCACTACTGCGTTTGTTTGTG	TAAGGAGTTTGGAATGGGAAGA	212-240
aw343	GGTTCGTGTATTTGTTCGATCC	AATCTCCAAGGTTCCATCTTCA	205-243
afct45	TAAAAAACGGAAAGAGTTGGTTAG	GCCATCTTTTCTTTTGCTTC	135-179
aa660573	TTCCGCCCATAGTCTTTGAC	TAAATGTGTCCTGCGTCTGG	294-363
bg454744	TCACAAAGCGAAAAATGTGA	CCAGGATCAAGGTAAGCCAA	365-403
aw695813	AACAGAATGCATTGCACGAA	TTCGTTGAACGTTGGATTGA	265-727

We calculated Exact-p values to prevent this inconvenience. After using QValue 1.36.0 software (Dabney and Storey, 2007), Q values, which are appropriate experimental error ratio for P values, were obtained. Subsequently, we determined physical locations of 23 SSR alleles by means of *M. truncatula* genome sequence build program (version of 3.5.1). Finally, LD values were calculated comparing with physical distances.

RESULTS AND DISCUSSION

Linkage Disequilibrium (LD) analysis

Because of absent and present, in order to achieve LD analysis of SSR markers, only allele tests were implemented in the study. *M. truncatula* genetic maps were used to find locations on chromosomes of markers. Compatibly, aw774443 and AW981317 markers constituted group of Linkage 1 localized on chromosome 1, AW691788, aw586158, B21E13 markers, group of Linkage 2 localized on chromosome 2, aw690263, AW776398, aw691701, AW980858 markers, group of Linkage 3 localized on chromosome 3, aw688546, al367160, aw559239, bg448975 markers, group of Linkage 4 localized on chromosome 4, BE317308, AW689203, AW695900 markers, group of Linkage 5 localized on chromosome 5, AW686906, AW694962, afct45 markers, group of Linkage 7 localized chromosome 7 and aa660573, aw695813, bg647796 markers, group of Linkage 8 localized on chromosome 8. LD was not calculated for chromosome 6 owing to the fact that only mtic14 marker localized on this chromosome.

Consequently, physical distances of 23 markers that consisted of 103 loci were detected all on 8 chromosomes of the *M. truncatula* and LD analysis was achieved. P values derived from LD analysis were converted into firstly –log (P value) and then –log (Q value) for easy visualization. P values were 0.0001 or lower [(-log (Q value) ≥ 3] when the logarithmical Q

values were compared with physical distances which are size of Mega Base of markers on chromosomes. It is concluded that there was no significant (p<0,0001) LD in these populations (Figure 1).

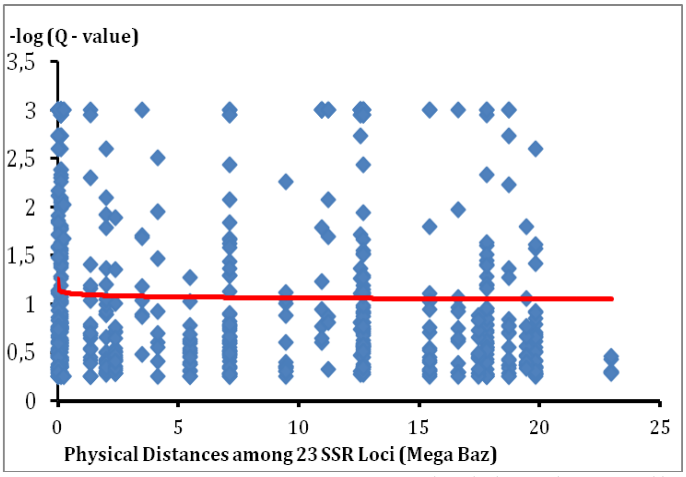

Figure 1. Linkage Disequilibrium (LD) (-log (Q value)) plot based on 8 chromosomes of *Medicago truncatula* for 70 alfalfa populations

Linkage Disequilibrium values in plants ranged increasingly from hundreds of base pairs to thousands according to species or population (Alm et al., 2003; Hyten et al., 2007; Liu and Burke, 2006; Mather et al., 2007; Morrell et al., 2005; Simko et al., 2006). It is known that prime biologic factors such as selection, mutation, genetic drift, recombination rate and population structure effect LD value (Flint-Garcia et al., 2003). Especially, on the ground that autogamic species have effective recombination rate, autogamic species have lower level LD values than allogamic species have (Nordborg, 2000). It seems that confirmed varieties generally have more LD values than wild populations. Sunflower (*Helianthus annuus* L.) (Liu and Burke, 2006) and Barley (*Hordeum vulgare* L.) (Caldwell et al., 2006) can be exemplified for this event. Previous studies show that in wild diploid alfalfa collections within candidate genes relation with lignine biosynthesis (Sakiroglu et al., 2012) and within some genes which is responsible for flowering time of certain tetraploid varieties (Herrmann et al., 2010), there is a substantially LD degradation. We concluded that there was no significant (P<0,0001) LD in these populations. Here, we used only 31 markers and 280 genotypes. This number of marker may not be effective for evaluating LD. Another possibility may be wild alfalfa populations because in alfalfa used in breeding population SSR markers were practised and found 0.5 Mbp (P<0.01) of LD value (Li et al., 2011b). Used wild alfalfa subspecies, which were collected broad and diversity regions in the world in this study, confirm the expectations with lower LD value.

CONCLUSIONS

The size of LD is an important theoretical genomic parameter that has implications for genome mapping efforts. The genome mapping is a crucial breeding tool to pinpoint the polymorphism that controls phenotype of interest. Effective detection of the genomic regions corresponding to the target trait is one of the ways to accelerate the breeding efforts. Therefore, determining LD is an important breeding goal. However, LD estimates could be different among various marker systems owing to the evolutionary pattern of each marker system and ploidy levels of genotype panel. Since SSR markers are much more recent, they are supposed to indicate more LD than other marker systems (Şakiroğlu et al., 2012) provided that the genome is sufficiently covered. The results provided here reveals that the number of markers is expected to be more to establish a robust LD estimate when a tetraploid panel is used.

ACKNOWLEDGEMENT

This study was supported by Scientific Research Projects of Kafkas University in Turkey with Project No: 2012-FEF-29. We thank The Samuel Roberts Noble Foundation, Ardmore, OK, USA for supports with their laboratories and facilities.

REFERENCES

Alm V, Fang C, Busso CS, Devos KM, Vollan K, Grieg Z, Rognli OA 2003. A linkage map of meadow fescue (*Festuca pratensis* Huds.) and comparative mapping with other Poaceae species. Theor. Appl. Genet. 108:25–40.

Branca A, Paape TD, Zhou P, Briskine R, Farmer AD, Mudge J, Bharti AK, Woodward JE, May GD, Gentzbittel L, Ben C, Denny R, Sadowsky MJ, Ronfort J, Bataillon T, Young ND, Tif- fin P 2011. Whole-genome nucleotide diversity, recombination and linkage disequilibrium in the model legume *Medicago trun-catula*. Proc Natl Acad Sci USA 108:E864–E870.

Brummer EC 1999. Capturing heterosis in forage crop cultivar development. Crop Sci 39: 943–954.

Caldwell KS, Russell J, Langridge P, Powell W 2006. Extreme population-dependent linkage disequilibrium detected in an inbreeding plant species, *Hordeum vulgare*. Genetics 172:557–567.

Dabney AR, Storey JD 2007. A new approach to intensity-dependent normalization of two-channel microarrays. Biostatistics 8: 128-139.

Doyle JJ, Doyle JL 1990. Isolation of plant DNA from fresh tissue. Focus 12:13–15.

Flint-Garcia SA, Thornsberry JM, Buckler ES 2003. Structure of linkage disequilibrium in plants. Annu. Rev. Plant Biol. 54:357–374.

Herrmann D, Barre P, Santoni S, Julier B 2010. Association of a CONSTANS-LIKE gene to

flowering and height in autotetraploit alfalfa. Theor Appl Genet., 121: 865-876.

Hyten DL, Choi IY, Song Q, Shoemaker RC, Nelson RL, Costa JM, Specht JE, Cregan PB 2007. Highly variable patterns of linkage disequilibrium in multiple soybean populations. Genetics 175:1937–1944.

İlhan D, Li X, Brummer EC, Sakiroglu M 2016. Genetic Diversity and Population Structure of Tetraploid Accessions of the *Medicago sativa – falcata* Complex. Crop Science 56: 1-11.

Julier B, Flajoulot S, Barre P, Cardinet G, Santoni S, Huguet T, Huyghe C 2003. Construction of two genetic linkage maps in cultivated tetraploit alfalfa (*Medicago sativa*) using microsatellite and AFLP markers. BMC Plant Biol., 3:9.

Kim S, Plagnol V, Hu TT, Toomajian C, Clark RM, Ossowski S, Ecker JR, Weigel D, Nordborg M 2007. Recombination and linkage disequilibrium in *Arabidopsis thaliana*. Nat Genet 39:1151–1155.

Kir B, Soya H 2008. The investigation on some yield and quality characteristics of some pasture type alfalfa cultivars. Ege Univ. Journal of Agriculture Faculty, 45 (1): 11-19. (in Turkish).

Kraakman ATW, Niks RE, Van den Berg PMMM, Stam P, Van Eeu- wijk FA 2004. Linkage disequilibrium mapping of yield and yield stability in modern spring barley cultivars. Genetics 168:435–446.

Lande R, Thompson R 1990. Efficiency of marker-assisted selection in improvement of quantitative traits. Genetics, 124: 743-756.

Lesins KA Lesins I 1979. Genus *Medicago* (Leguminosae), A taxogenetic study. Dr. W. Junk Publishers, The Hague.

Li X, Wei Y, Moore KJ, Michaud R, Viands DR et al. 2011b. Association mapping of biomass yield and stem composition in a tetraploid alfalfa breeding population. Plant Genome 4: 24–35.

Li X, Han Y, Wei Y, Acharya A, Farmer AD, Ho J, Monteros MJ, Brummer EC 2014. Development of an alfalfa SNP array and its use to evaluate patterns of population structure and linkage disequilibrium. PLoS One 9:e84329.

Liu J 2002. POWERMARKER-a powerful software for marker data analysis. Raleigh, NC: North Carolina State University, Bioinformatics Research Center. http://www.POWERMARKER.net

Liu A, Burke JM 2006. Patterns of nucleotide diversity in wild and cultivated sunflower. Genetics 173:321–330.

Maccaferri M, Sanguineti MC, Noli E, Tuberosa R 2005. Population structure and long-range linkage disequilibrium in a durum wheat elite collection. Mol Breed 15:271–290.

Mather KA, Caicedo AL, Polato NR, Olsen KM, McCouch S, Purugganan MD 2007. The extent of linkage disequilibrium in rice (*Oryza sativa* L.). Genetics 177:2223–2232.

Morrell PL, Toleno DM, Lundy KE, Clegg MT 2005. Low levels of linkage disequilibrium in wild barley (*Hordeum vulgare* ssp. *spon- taneum*) despite high rates of self-fertilization. Proc. Natl. Acad. Sci. USA 102:2442–2447.

Nordborg M 2000. Linkage disequilibrium, gene trees and selfing: An ancestral recombination graph with partial self-fertilization. Genetics 154:923–929.

Nordborg M, Borevitz JO, Bergelson J, Berry CC, Chory J, Hagen- blad J, Kreitman M, Maloof JN, Noyes T, Oefner PJ, Stahl EA, Weigel D 2002. The extent of linkage disequilibrium in *Arabi- dopsis thaliana*. Nat Genet 30:190–193.

Raboin L-M, Pauquet J, Butterfield M, D'Hont A, Glaszmann J-C 2008. Analysis of genome-wide linkage disequilibrium in the highly polyploid sugarcane. Theor Appl Genet 116:701–714.

Rafalski A, Morgante M 2004. Corn and Humans: recombination and linkage disequilibrium in two genomes of similar size. Trends Genet., 20:103-111.

Remington DL, Thornsberry JM, Matsuoka Y, Wilson LM, Whitt SR, Doebley J, Kresovich S, Goodman MM, Buckler ES 2001. Structure of linkage disequilibrium and phenotypic associations in the maize genome. Proc Natl Acad Sci USA 98:11479–11484.

Robins JG, Luth D, Campbell IA, Bauchan GR, He CL et al. 2007. Genetic mapping of biomass production in tetraploid alfalfa. Crop Sci 47: 1–10.

Schuelke M 2000. An economic method for the fluorescent labelling of PCR fragments. Nat Biotechnol 18:233–234

Sledge MK, Ray IM, Jiang G 2005. An expressed sequence tag SSR map of tetraploid alfalfa (*Medicago sativa* L.) Theor Appl Genet, 111: 980-992.

Stuber CW, Polacco M, Senior ML 1999. Synergy of empirical breeding, marker-assisted selection, and genomicsto increase crop yield potential. Crop Sci., 39: 1571-1583.

Sakiroglu M, İlhan D, Kaya MM, Demirozogul O, Ulucay O, Eren B 2011. In the Light of The Current State of The Molecular Complex of (*M. sativa* L.). Kafkas Univ. Journal of Science Instit. 4(1):32-42. (in Turkish).

Sakiroglu M, Sherman-Broyles S, Story A, Moore KJ, Doyle JJ and Brummer EC 2012. Patterns of linkage disequilibrium and association mapping in diploid alfalfa (*M. sativa* L.). Theoretical and Applied Genetics, 125(3):577-590.

Sakiroglu M, Brummer EC 2012. Presence of phylogeographic structure among wild diploid alfalfa populations (*Medicago sativa* subsp. *microcarpa* Urb.) with evidence of the center of origin. Genet Resour Crop Evo., 60:23-31.

Tenaillon MI, Sawkins MC, Long AD, Gaut RL, Doebley JF, Gaut BS 2001. Patterns of DNA

sequence polymorphism along chromo- some 1 of maize (*Zea mays* ssp. mays L.). Proc Natl Acad Sci USA 98:9161–9166.

Van Inghelandt D, Reif JC, Dhillon BS, Flament P, Melchinger AE 2011. Extent and genome-wide distribution of linkage disequilibrium in commercial maize germplasm. Theor Appl Genet 123:11–20.

Yan J, Shah T, Warburton ML, Buckler ES, McMullen MD, Crouch J 2009. Genetic characterization and linkage disequilibrium estimation of a global maize collection using SNP markers. PloS One 4:e8451.

Yu J, Pressoir G, Briggs WH, Vroh BI, Yamasaki M et al. 2006. A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat Genet 38: 203–208.