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Article

Wild Emmer (*Triticum turgidum* ssp. *dicoccoides*) Diversity in Southern Turkey: Evaluation of SSR and Morphological Variations

Esra ÇAKIR [1,*], Ahmad ALSALEH [2], Harun BEKTAS [3] and Hakan ÖZKAN [1,*]

[1] Department of Field Crops, Faculty of Agriculture, Çukurova University, Adana, Turkey
[2] Department of Food and Agriculture, Institute of Hemp Research, Yozgat Bozok University, Yozgat, Turkey
[3] Department of Agricultural Biotechnology, Faculty of Agriculture, Siirt University, Siirt, Turkey
* Correspondence: ecakir@cu.edu.tr (E.Ç); hozkan@cu.edu.tr (H.Ö.)

Abstract: Wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) is the ancestral species of cultivated tetraploid wheat with BBAA genomes. Because of its full interfertility with domesticated emmer wheat, this wild species can serve as one of the most important genetic resources to improve durum as well as bread wheat. To clarify the magnitude of genetic diversity between and within populations of Turkish wild emmer wheat, 169 genotypes of ssp. *dicoccoides* selected from the 38 populations collected from the three sub-regions (East-1, West-1, and West-2) of the Southeast Anatolia Region of Turkey were molecularly and morphologically characterized. The populations showed significant variation in plant height, peduncle length, flag leaf length and area, length of the uppermost awn in the spikelet, length of the lowermost awn in the spikelet, width and height of the glume, length and width of the anther, and 1000 seeds weight. According to the results of nuclear-SSR analysis, the populations collected from the sub-regions East-1 and West-2 were genetically most distant (0.539), while the populations collected from the sub-regions West-1 and West-2 were genetically most similar (0.788) populations. According to the results of AMOVA, there was a 84 % within the populations studied, while the variation between the populations of the three sub-regions was 16 %. In the dendrogram obtained by using nuclear-SSR data, the populations formed two main groups. Populations from the sub-region East-1 were in the first group, and the populations from the sub-regions West-1 and West-2 were in the second group. From the dendrogram, it appears that the populations from the sub-region East-1 were genetically distant from the populations from the sub-regions West-1 and West-2. The results highlight the potential diversity in the Southeast Anatolia for wild emmer discovery and utilization.

Keywords: wild emmer; *T. turgidum* ssp. *dicoccoides*; SSR; plant morphology; Turkey

1. Introduction

Crop wild relatives are the primary sources of novel genetic diversity that is needed for crop improvement in all aspects [1]. A comprehensive evaluation of *In-situ* and *Ex-situ* resources as well as common cultivars to mine new alleles is a continuously needed process for crop improvement and agricultural sustainability [2]. The utilization of genetic resources not only requires a clear understanding of the allelic constitution of such species [3] but also requires the successful introduction of this diversity into the domesticated gene pool [4].

Wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*), as one of the closest known wild relatives of the domesticated tetraploid wheat, holds significant potential for wheat improvement [5–7]. It is the most likely ancestral species of cultivated tetraploid wheat with a genome constitution of BBAA. The geographical distribution area of wild emmer covers the Fertile Crescent in South-west Asia from Israel, Jordan, Lebanon, Syria, southern Turkey, northern Iraq to south/southwest Iran [8,9]. Due to

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its full interfertility with domesticated emmer wheat (*T. turgidum* subsp. *dicoccum* (Schrank ex Schübl.) Thell.), it can serve as one of the most important genetic resources to improve durum (*Triticum turgidum* L. ssp. *durum* (Desf.) as well as bread wheat (*Triticum aestivum* L.). Wild emmer has been used for the allele mining for many needs of wheat breeding, including but not limited to, drought tolerance [10,11], salinity stress tolerance [5,12], and for the biotic stress factors, fusarium head blight [13,14], stripe rust [15,16], root-lesion nematodes [17], and powdery mildew [18–22].

Due to its possible domestication in or near the Levant [6,9], there has been a high level of reports from Israel and vicinities in the Jordan Valley [23–25]. There were only a few studies on the accessions of wild emmer wheat from some locations out of Turkey [9,26–28], but there is not any comprehensive evaluation of *In-situ* or *Ex-situ* gene-pools from Southeast Anatolia. On the other hand, since the early 20th century, there has been a significant number of reports on the diversity and distribution of wild emmer wheat from the Levant and Jordan Valley, mostly Israel and Lebanon [23,24,29–39]. This concentrated evaluation of a specific region for wild emmer did continue in the following years [7,11,20,40–53]. Evaluation of the natural populations in the central Fertile Crescent, especially the Karacadağ region of Southeast Anatolia, is neglected. Even though this region is recently emphasized for its role in wheat domestication [9,54], there is no comprehensive report of the natural wild emmer diversity in this region and Turkey. Therefore, the purpose of this study was to document the phenotypic and genotypic diversity between and within 169 accessions of 38 *In-situ* populations of wild emmer wheat collected from the Karacadağ region of Southeast Anatolia.

2. Materials and Methods

In this study, 169 accessions of 38 wild emmer wheat populations recently collected from Southeast Anatolia were used (Table 1). The study was conducted in Adana, Turkey during 2020-2021 growing season. The seeds from each population were grown at the experimental area of Çukurova University, Adana, Turkey, for studying genotypic and phenotypic variation. Initially, ten seeds from each genotype were germinated in Petri dishes. Once the seedlings developed, they were transferred to small pots. Each seedling was then transplanted into a separate row, maintaining a spacing of 30 cm between and within the rows. To prevent damage from spike breakage and to ensure complete self-pollination while preserving seed purity, the main spikes of all wild emmer plants (*Triticum turgidum* subsp. *dicoccoides*) were bagged for self-pollination.

Total genomic DNA was isolated from young leaves according to cetyltrimethylammonium bromide (CTAB) protocol [55] with some modification of Ozkan, et al. [56]. The extracted DNA was evaluated qualitatively in addition to quantity, measured by 0.8% agarose gel electrophoresis. Before using DNA for molecular analysis, the DNA diluted to the required concentration for 10 ng/ml for SSR applications. Initially, 100 SSR primers mapped to the A and B genomes were first screened on eight wild emmer genotypes not only to detect their polymorphism level but also PCR amplification. After screening, 16 SSR primer was select for further work (Table 2). M13 tailed-primer PCR amplification of SSRs according to [57] was performed in a 12 µL PCR mix containing 1X buffer, 0.125 mM dNTPs, 0.4 pmol M13 sequences tailed forward primer, 0.3 pmol reverse primers, 3.0 pmol universal M13 primer labeled with one of four (6-FAM, VIC, NED or PET) fluorescent dyes, 0.12U *Taq* DNA polymerase, and approximately 50ng genomic DNA. PCR amplification was performed with an initial denaturation at 94°C for 5 min; 30 cycles of 94 °C for 1 min, 55 to 67 °C (annealing temperature depending on primers) for 1 min, 72 °C for 1 min; followed by 8 cycles of 94°C for 30 s, 53°C for 45 s, and 72°C for 45 s; and a final extension at 72°C for 10 min. A set of four PCR products (1 µl each) labeled with a different dye was combined with 0.25 µL GeneScan-400 LIZ® size standards (Applied Biosystems) and 9.86µL Hi-Di™ Formamide (Applied Biosystems), denatured at 94°C for 5min, chilled on ice, and separated on an ABI 3130xl Genetic Analyzer (Applied Biosystems). The SSR fragments were scored and checked twice using the Gene Mapper software v3.7 (Applied Biosystems) as described in the user manual.

The SSR was scored as binary data (1/0), indicating the presence or absence of a marker in the genomic representation of each sample. Several genetic diversity parameters were calculated for each

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locus and population using the GENALEX6.5 program [58]. Principal coordinate analysis (PCoA) used to explore multivariate relationships among inter-individual genetic distances within and among populations was also performed with the GENALEX6.5 program.

Table 1. Collection site information about 38 wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) populations.

Collection No	Collection Locality	Zone	Altitude (m)	Latitude (°N)	Longitude (°E)
1	24.5 km SW from Diyarbakır to Ovadag	East1	780	37°47′38″	40°12′14″
2	12.9 km NW from Ovadag to Pirinçlik	East1	1007	37°47′31″	39°57′18″
3	18.5 km NW from Ovadag to Pirinçlik	East1	920	37°49′17″	39°59′34″
4	20.1 km SW from Pirinçlik	East1	1080	37°52′02″	39°51′05″
5	20 km SW from Pirinçlik	East1	1260	37°50′40″	39°47′58″
6	2.9 km NE from Karabahçe to Pirinçlik	East1	1300	37°49′12″	39°46′29″
7	41.2 km SW from Pirinçlik	East1	1250	37°46′42″	39°44′50″
8	6.3 km N from Karabahçe (42.9 km W from Diyarbakır to Siverek)	East1	1070	37°50′21″	39°43′23″
9	4.6 km SW from Karabahçe	East1	1180	37°46′19″	39°44′03″
10	17.9 km SW from Karabahçe	East1	1160	37°44′29″	39°42′50″
11	21.7 km SW from Karabahçe	East1	1235	37°42′51″	39°44′03″
12	37.9 km SW from Karabahçe	East1	1170	37°39′49″	39°42′49″
13	37.9 km SW from Karabahçe	East1	1180	37°36′27″	39°43′41″
14	41.6 km SW from Karabahçe	East1	1170	37°35′08″	39°44′36″
15	48.7 km SW from Karabahçe	East1	1030	37°33′09″	39°42′06″
16	27.6 km SW from Karacadag (69.6 km SW from Karabahçe)	East1	950	37°37′40″	39°33′40″
17	30.2 km SW from Çermik to Siverek	East1	800	38°00′56″	39°22′11″
18	Siverek Karakeçi Road Azemi Village	East1	733	37°36′51″	39°20′12″
19	Karakeçi road	East1	737	37°33′27″	39°20′35″
20	Karakeçi grassland	East1	758	37°32′22″	39°21′52″
21	5km from Siverek to Siverek Hilvan Road	East1	645	37°42′23″	39°16′34″
22	72 km SE from Turkoglu SE (W of Karadag)	West1	800(853)	37°19″46″	37°16′29″
23	72 km SE from Turkoglu SE (W of Karadag)	West1	800(853)	37°19″46″	37°16′29″
24	34 km ESE from Narlı (WSW of Karadag)	West1	840 (877)	37°18′53″	37°15′41″
25	34 km ESE from Narlı (WSW of Karadag)	West1	780 (813)	37°20′12″	37°17′53″
26	39 km ESE from Narlı (SW of Karadag)	West1	760 (793)	37°17′06″	37°17′39″
27	39 km ESE from Narlı (SW of Karadag)	West1	760 (793)	37°17′06″	37°17′39″
28	Between Kahramanmaraş Kelleş village and Yiğitce village	West1	791	37°20′25″	37°17′54″
29	Between Gaziantep Tekirsin village and Dundarlı village	West1	882	37°15′20″	37°23′26″
30	37 km NE from Kilis to Gaziantep	West2	830	37°20′19″	37°16′50″
31	39 km NE from Kilis to Gaziantep	West2	920	37°19′50″	37°18′51″
32	41 km NE from Kilis to Gaziantep	West2	770	37°24′23″	37°25′47″
33	42 km NE from Kilis to Gaziantep	West2	750	37°24′58″	37°24′50″
34	58 km NE from Kilis to Gaziantep	West2	720	37°16′01″	37°30′52″
35	59 km NE from Kilis to Gaziantep	West2	770	37°15′33″	37°29′03″
36	21 km NE from Kilis to Gaziantep	West2	620	36°45′52″	37°15′04″
37	24 km NE from Kilis to Gaziantep	West2	700	36°52′20″	37°12′12″
38	25 km NE from Kilis to Gaziantep	West2	830	36°33′25″	37°11′57″

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Table 2. List of SSR primers used in the study with respective repeat motif and chromosome location.

Name	Ch	Motif	Forward Primer sequence	Reverse Primer sequence
cfa2219	1A	(GT)21	TCTGCCGAGTCACTTCATTG	GACAAGGCCAGTCCAAAAGA
wmc312	1A	(GA)10	TGTGCCCGCTGGTGCGAAG	CCGACGCAGGTGAGCGAAG
wmc658	2A	----	CTCATCGTCCTCCTCCACTTTG	GCCATCCGTTGACTTGAGGTTA
wmc313	4A	(CA)18	GCAGTCTAATTATCTGCTGGCG	GGGTCCTTGTCTACTCATGTCT
wmc110	5A	(GT)11	GCAGATGAGTTGAGTTGGATTG	GTACTTGGAAACTGTGTTTGGG
cfa2190	5A	(TC)31	CAGTCTGCAATCCACTTTGC	AAAAGGAAACTAAAGCGATGGA
wmc626	1B	----	AGCCCATAAACATCCAACACGG	AGGTGGGCTTGGTTACGCTCTC
gwm498	1B	----	GGTGGTATGGACTATGGACACT	GGTGGTATGGACTATGGACACT
wmc128	1B	(GA)10	CGGACAGCTACTGCTCTCCTTA	CTGTTGCTTGCTCTGCACCCTT
wmc149	2B	(CT)24	ACAGACTTGGTTGGTGCCGAGC	ATGGGCGGGGGTGTAGAGTTTG
wmc332	2B	(CT)12	CATTTACAAAGCGCATGAAGCC	GAAAACTTTGGGAACAAGAGCA
gwm335	5B	---	CGTACTCCACTCCACACGG	CGGTCCAAGTGCTACCTTTC
gwm630	6B	(GT)16	GTGCCTGTGCCATCGTC	CGAAAGTAACAGCGCAGTGA
gwm146	7B	---	CCAAAAAAACTGCCTGCATG	CTCTGGCATTGCTCCTTGG
wmc76	7B	(GT)19	CTTCAGAGCCTCTTTCTCTACA	CTGCTTCACTTGCTGATCTTTG
gwm333	7B	(GA)19	GCCCGGTCATGTAAAACG	TTTCAGTTTGCGTTAAGCTTTG

3. Results

3.1. Agro-Morphological Variation in Wild Emmer Populations

A total of 169 accessions, collected as 38 populations from the East and West Karacadağ region of Southeast Anatolia (Figure 1), were evaluated for agro-morphological and genetic diversity-related traits. According to the variance analysis (ANOVA), there were significant ($p<0.05$) differences between the evaluated panel of accessions for plant height, peduncle length, flag leaf length and area, length of the uppermost awn in the spikelet, length of the lowermost awn in the spikelet, width and height of the glume, length and width of the anther and 1000 grain weight (Data not shown). Out of 23 agro-morphological parameters evaluated, populations from Karacadag/EAST had the highest values in spike length (9.28 cm), spikelet number (20.69), glume hull thickness (0.26 mm), anther width (0.59 mm). On the other hand, Karadag-1/WEST populations had the highest agro-morphological trait values in heading date (170.38 days), flag leaf area (17.76 cm^2), auriculas width (5.68 mm), length of the uppermost awn in the spikelet (68.41 mm), spikelet length (16.13 mm), lemma length (13.95 mm), palea length (12.88 mm). Finally, populations from Karadag-2/WEST had the highest values in plant height (128.31 cm), heading date (167.49 days), peduncle length (41.48 cm), auriculas length (4.65 mm), lemma width (2.62 mm), palea width (2.03 mm), glume length (12.61 mm), glume height (2.55 mm), anther length (4.10 mm) and maturation day (199.86 days). According to overall data Karadag-2/WEST was the region with the maximum agro-morphological diversity and the highest values in phenotypical traits (Table 3).

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Table 3. Mean values of agro-morphological traits of wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) genotypes sampled from three different sub-regions of Southeast Anatolia.

	Whole Collections	Karacadag/EAST	Karadag-1/WEST	Karadag-2/WEST
Plant height (cm)	126.95 ± 18.45	126.35 ± 19.68	127.64 ± 16.27	128.31 ± 16.28
Heading date (day)	166.06 ± 8.36	164.37 ± 9.12	170.38 ± 7.08	167.49 ± 4.58
Peduncle length (cm)	38.95 ± 6.85	39.17 ± 6.83	35.09 ± 7.09	41.48 ± 5.26
Flag leaf area (cm2)	16.38 ± 6.18	15.66 ± 6.33	17.76 ± 5.76	17.55 ± 4.65
Auriculas length (mm)	4.52 ± 0.64	4.50 ± 0.68	4.45 ± 0.39	4.65 ± 0.65
Auriculas width (mm)	5.42 ± 0.90	5.43 ± 0.88	5.68 ± 0.87	5.19 ± 0.91
Spike length (cm)	9.18 ± 1.16	9.28 ± 1.23	9.11 ± 0.91	8.91 ± 1.08
Spikelet number	20.02 ± 2.49	20.69 ± 2.62	18.89 ± 1.75	18.80 ± 1.67
Length of the uppermost awn in the spikelet (mm)	76.37 ± 18.84	74.91 ± 19.56	80.76 ± 15.78	77.40 ± 18.71
Awn length in the fourth flower (mm)	95.97 ± 19.73	93.68 ± 20.45	100.23 ± 17.338	98.85 ± 18.51
Length of the lowermost awn in the spikelet (mm)	59.49 ± 22.21	56.35 ± 22.72	68.41 ± 18.75	62.11 ± 21.37
Spikelet length (mm)	15.72 ± 1.44	15.50 ± 1.47	16.13 ± 1.49	16.07 ± 1.17
Spikelet width (mm)	4.67 ± 0.82	4.74 ± 0.86	4.38 ± 0.68	4.71 ± 0.72
Lemma length (mm)	13.46 ± 1.66	13.26 ± 1.87	13.95 ± 1.30	13.71 ± 0.91
Lemma width (mm)	2.53 ± 0.35	2.50 ± 0.35	2.51 ± 0.35	2.62 ± 0.37
Palea length (mm)	12.49 ± 1.12	12.32 ± 1.19	12.88 ± 1.02	12.70 ± 0.86
Palea width (mm)	1.81 ± 0.51	1.77 ± 0.26	1.73 ± 0.26	2.03 ± 1.01
Glume length (mm)	12.27 ± 1.12	12.09 ± 1.14	12.59 ± 1.04	12.61 ± 1.04
Glume hull thickness (mm)	0.24 ± 0.09	0.26 ± 0.09	0.22 ± 0.09	0.20 ± 0.06
Glume height (mm)	2.45 ± 0.35	2.43 ± 0.33	2.45 ± 0.35	2.55 ± 0.40
Anther length (mm)	3.73 ± 0.50	3.62 ± 0.50	3.80 ± 0.43	4.10 ± 0.42
Anther width (mm)	0.58 ± 0.10	0.59 ± 0.10	0.53 ± 0.09	0.58 ± 0.11
Maturation (day)	198.12 ± 4.56	97.18 ± 4.97	199.48 ± 4.03	199.86 ± 2.48

3.2. Genetic Diversity Within and Between the Populations and Sub-Regions

Genetic diversity of the evaluated accessions was assessed with analysis of molecular variance (AMOVA), and several diversity parameters including Nei genetic distance and identity values (Tables 4 and 5). AMOVA was calculated to assess the variance within and between 38 populations of wild emmer wheat collected from three sub-regions of Karacadağ mountains (Table 4). There was a significant level of variation within the populations (84%), while variation among the populations was 16%. Nei genetic distance and identity values for the three sub-regions are given in Table 5. According to the results, the regions with the most distance were Karacadag/EAST and Karadag-2/WEST (0.539), while the ones with the closest identity values were Karadag-1/WEST and Karadag-2/WEST (0.788). The lowest distance was seen between two Karacadağ west sub-regions (0.214) and the lowest identity was between Karacadag/EAST and Karadag-2/WEST (0.463).

We have also calculated several genetic diversity parameters to estimate the variation within and between the populations. These parameters were the number of alleles per locus (Na), the number of effective alleles per locus (Ne), Shannon's information index (I), expected heterozygosity (He), and unbiased heterozygosity (uHe). The highest number of alleles per locus was in Karacadag/EAST populations, while the lowest was in Karadag-1/WEST. Similarly, Karacadag/EAST populations had the highest values in Ne, I, He, and uHe compared to Karadag-1/WEST and Karadag-2/WEST populations (Table 6). In contrast, the lowest values in all parameters were obtained in Karadag-1/WEST populations. According to the above results, maximum and minimum genetic diversity was obtained in the populations from Karacadag/EAST and Karadag-1/WEST populations, respectively.

Table 4. Analysis of molecular variance (AMOVA) summary for the variation within and between populations of *Triticum turgidum* ssp. *dicoccoides*.

Source	df	SS	MS	Est. Var.	%
Among Pops	2	439.551	219.776	4.429	16
Within Pops	166	3803.206	22.911	22.911	84
Total	168	4242.757		27.340	100

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Table 5. Nei genetic distance (above diagonal) and Nei identity (below diagonal) values among 38 *Triticum turgidum* ssp. *dicoccoides* populations.

Population	Karacadag/EAST	Karacadag-1/WEST	Karacadag-2/WEST
Karacadag/EAST	---	0.518	0.539
Karadag-1/WEST	0.484	---	0.214
Karadag-2/WEST	0.463	0.788	---

Table 6. Summary statistics of genetic variation among three different regions, Karacadag/EAST, Karadag-1/WEST, and Karadag-2/WEST. The number of alleles per locus (Na), number of effective alleles per locus (Ne), Shannon's information index (I), expected heterozygosity (He), and unbiased heterozygosity (uHe) of 38 *Triticum turgidum* ssp. *dicoccoides* populations.

Pops	N	Na	Ne	I	He	uHe
Karacadag/EAST	108	9.938 ± 1.871	5.470 ± 0.869	1.692 ± 0.177	0.725 ± 0.046	0.729± 0.046
Karadag-1/WEST	28	3.938 ± 0.470	2.747 ± 0.308	1.030 ± 0.118	0.561 ± 0.051	0.572 ± 0.052
Karadag-2/WEST	33	6.125 ± 0.861	4.135 ± 0.644	1.348 ± 0.181	0.621 ± 0.070	0.632 ± 0.071

3.3. PCoA and Neighbor-Joining Grouping Patterns in Wild Emmer Populations

The PCoA analysis was applied to define the population interactions, and 169 accessions from 38 populations were separated into 2 major clusters, without any mixture between Karacadag/EAST and both Karadag-1/WEST and Karadag-2/WEST. The distinction between East and West was quite sharp and there was no mixture (Figure 1) between these regions. Two sub-sets of Karadağ/WEST (1 and 2) were almost entirely mixed. So, it was not possible to separate them further into smaller sub-sets. Of the sub-cluster in Karacadag/EAST, there were several accessions located far away from the rest of the accessions.

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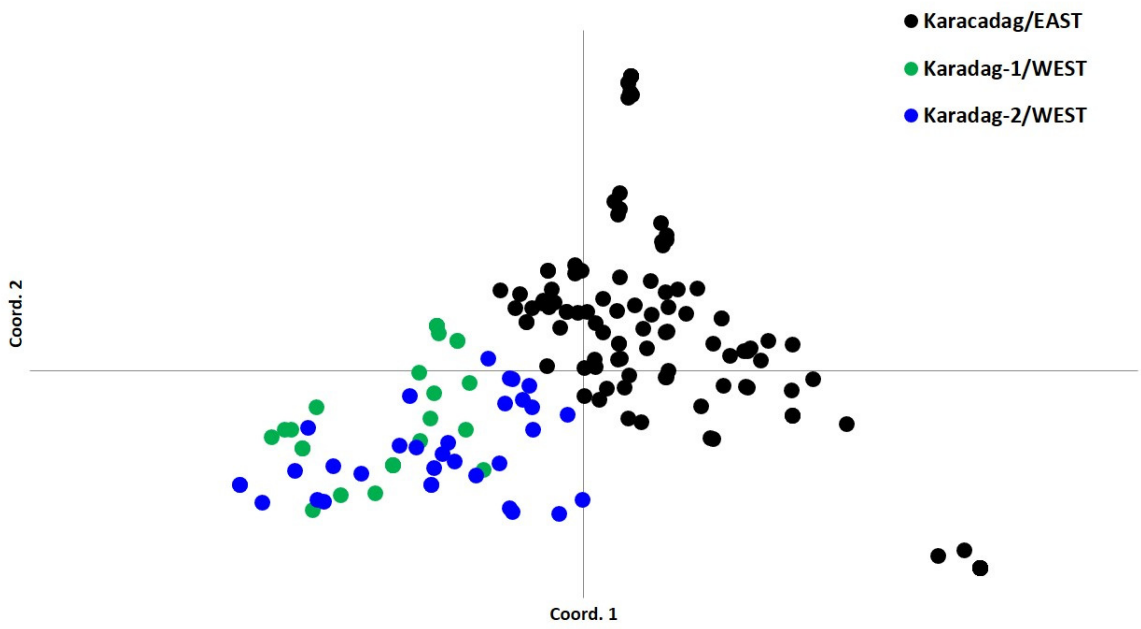

Figure 1. PCoA clustering of 38 Triticum turgidum ssp. dicoccoides populations were collected from the Karacadağ region of Southeast Anatolia, central Fertile Crescent. Populations were; Karacadag/EAST, Karadag-1/WEST and Karadag-2/WEST.

The relationships within and between the three regional groups were also evaluated using Neighbor-Joining analysis (Figure 2). Neighbor-Joining tree dendrograms produced a similar distribution to PCoA clustering. Two main branches were formed with accessions from WEST and EAST. These two main clusters did not have any admixture. There were several sub-clusters on the main clusters EAST and WEST. There were several sub-clusters in the main cluster WEST, which are almost entirely constituted from the mixed accessions of WEST-1 and WEST-2. There were only a few sub-clusters (WEST-uppermost branch) with a clear divergence from the rest of the group. Even though there were mixtures over the entire main cluster WEST, each small sub-set was formed with at least several accession from the same sub-region.

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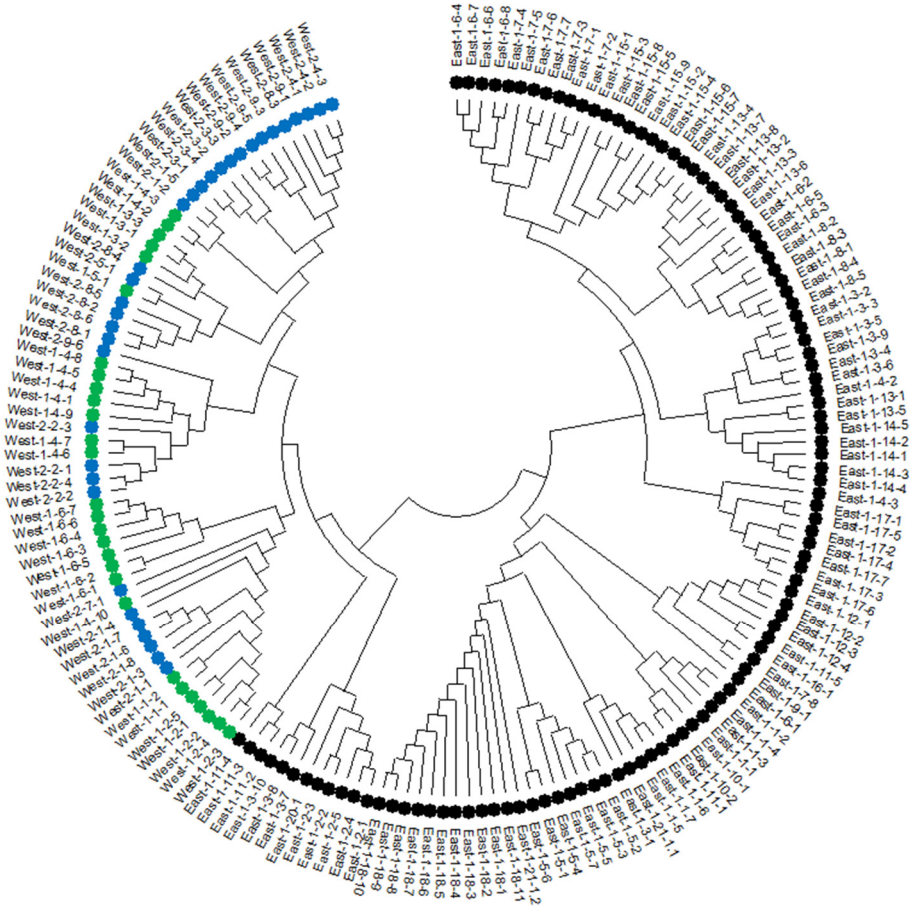

Figure 2. Neighbor-Joining analysis of 38 Triticum turgidum ssp. dicoccoides populations were collected from the Karacadağ region of Southeast Anatolia, central Fertile Crescent.

4. Discussion

Crop wild relatives are one of the main sources of allelic diversity, and so the hub for climate-resilient crop breeding. To "feed the billions" [59] and meet the pace of climate change and population increase, there is a continuous need for allele mining and germplasm screening [60–64]. Wild emmer wheat, one of the closest relatives of durum wheat [65], is a possible shortcut to the wide wild allelic gene pool in Triticum sp. [8,66–68]. As highlighted, there is a need for a "walk on the wild side" [61]. With this objective in mind, we characterized a set of *In-situ* germplasm accessions from the Southeast Anatolia, Karacadağ mountains, the home of Göbeklitepe, the oldest known temple, for genetic diversity assessment [69,70].

4.1. Agro-Morphological Diversity

As a result of germplasm collection from the three different sub-regions around Karacadağ mountains in Southeast Anatolia, 169 accessions within 38 populations were characterized. Agro-morphological characterization and genetic variation assessment through SSR markers were utilized to estimate the natural population diversity of this region. Wild emmer is thought to be domesticated near the southern Levant [6,9], and there has been intensity in the number of reports from the possible domestication center. However, it has a much wider species distribution [6,8,9] and the number of reports from other regions (Turkey and Iran) is quite limited [26,27,71]. In addition, none of the

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previous reports from Turkey built an in-depth agro-morphological and/or genetic diversity evaluation among natural (*In-situ*) populations.

Here we obtained significant agro-morphological and genetic diversity among and within the subsets of wild emmer wheat collected (Tables 3 and 4). The main component of the traits we evaluated were spike, plant growth, and phenological traits.

Even though there were a vast number of studies in relation to biotic stress tolerance, such as powdery mildew [20,72], fusarium head blight [13], and stripe rust [15] in wild emmer, we did not find any reports concerning spike and phenology traits within this region. The results highlight the potential of *In-situ* populations and hidden gems in the wild [73] reports that several spike-related traits such as purple coleoptile, purple auricle, purple culm, hairy auricle, hairy rachilla, and fragility of spike were controlled by single dominant genes, making the transfer of such traits much straightforward compared to quantitatively inherited traits. Further studies should try to utilize germplasm resources with novel allelic diversity into the common crops as candidates for abiotic and biotic stress tolerance as well as yield and growth-related traits [13,20,72,74,75].

4.2. Genetic Diversity of In-Situ Populations

To evaluate the genetic diversity within and between the populations, 16 specific SSR markers were applied (Table 2). According to the results of AMOVA and other genetic diversity parameters, there was significant diversity among the evaluated panel of *In-situ* accession. There were 84% within and 16% between populations diversity. The results were in a similar range with previous reports in tetraploid wheat. In similar studies, [76] reported 19 and 81% within and between population diversity, while Teklu, et al. [77] reported wider levels of diversity among 73 wild emmer accessions from 11 different geographic regions. Nei genetic distance values in this study ranged between 0.214 (between Karadag-1/WEST and Karadag-2/WEST) and 0.539 (between Karacadag/EAST and Karadag-2/WEST). The results showed a clear-cut separation between the EAST and WEST populations. Mountainous landscape of the region seems to reduce genetic drift and mixture even within a relatively close distance of about 200 km. The distance between WEST-1 and WEST-2 populations were closer, about 55 km and their genetic distance was quite small, compared to WEST-EAST distance (Table 5). According to Harlan and Zohary [78], Zohary and Hopf [79] and Ozkan, Willcox, Graner, Salamini and Kilian [9] wild emmer has a wide distribution from the Levant to Turkey and south/southwest Iran. The level of observed diversity within the Karacadağ region demonstrates the potential of this unexplored habitat for crop improvement [6,11,80].

The number of alleles per locus (Na) is an indicator of the diversity at the gene level [81]. Here, we observed a three-fold difference between three regional groups from 3.93 in Karadag-1/WEST to 9.93 in Karacadag/EAST. Since all accessions were from the same species within the same wider region, this level of difference may be due to unequal population size distribution among sub-sets or some other unknown reasons. Li, Roder, Fahima, Kirzhner, Beiles, Korol and Nevo [43] evaluated the number of microsatellites among 105 individuals from the Yehudiyya region of Israel, the Na values they obtained were lower than the current study. In their follow-up study, the same group obtained 1.88, 3.86, and 5.89 Na values at chromosome, genome, and genome x chromosome levels. In a similar study, on a different region, Li, Fahima, Peng, Roder, Kirzhner, Beiles, Korol and Nevo [39] reported an average of 7.1 Na value using 28 microsatellite markers among 155 individuals from two sub-regions of Tabigha, Israel. In our study, the number of effective alleles (Ne) followed the same trend with the Na values, which ranged between 2.75 and 5.50 per locus. The Ne values were higher than Arystanbekkyzy, Nadeem, Aktas, Yeken, Zencirci, Nawaz, Ali, Haider, Tunc, Chung and Baloch [26], who reported an average 1.962 Ne value among 29 wild and 4 cultivated emmer wheat collected from different regions of Turkey.

We obtained a Shannon index (I) between 1.030 (Karadag-1/WEST) and 1.692 (Karacadag/EAST). Expected heterozygosity (He) was between 0.561 and 0.725. The I and He values we obtained was significantly higher compared to Dong, Wei, Chen, Li, Wang, Nevo and Zheng [7] and Fahima, Sun, Beharav, Krugman, Beiles and Nevo [37], while it was in similar ranges with Fahima, Roder,

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Wendehake, Kirzhner and Nevo [42]. A similar study [28], compared accessions from Israel and Turkey (Diyarbakır region) for genetic diversity using AFLP markers. The He values they reported were much lower compared to the current study. Ozbek, et al. [82] evaluated a set of accessions (120) from Israel and also reported lower He values compared to our results. Here, Na, Ne, I, He, and uHe values we obtained, shows the genetic diversity of the natural wild emmer populations in the Karacadağ region.

4.3. PCoA and Neighbor-Joining Analysis

PCoA and Neighbor-Joining trees followed the same trend (Figures 1 and 2). Both methods separated WEST and EAST populations and did not create any mixture zones. On the other hand, two sub-sets of WEST (1 &2) were almost entirely mixed and it was not possible to make a distinction between these two. Neighbor-Joining Dendrograms and PCoA clustering were similar to our previous report with AFLP markers [9], which distributed EAST and WEST populations in two separate clusters. When we closely looked at the Neighbor-Joining dendrogram for branching and genotypes positions (Figure 3), it was seen that most of the individuals from the specific populations were located closely on the same branch, with some exceptions that did not follow any trend.

5. Conclusions

Wild and domesticated emmer wheat are well-characterized and excessively utilized species for crop improvement, especially in biotic stress tolerance. Here, a panel of wild emmer wheat was characterized for agro-morphological traits and genetic diversity. According to genetic diversity values obtained, PCoA and Neighbor-Joining dendrograms, two regional groups with approximately 200 km distance had significantly different characteristics in terms of allelic distribution and some phenotypic traits. The screening and utilization of *In-situ* germplasm sources from this region would help widen the genetic diversity in durum as well as common wheat breeding.

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