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Traits to Differentiate Lineages and Subspecies of *Aegilops tauschii*, the D Genome Progenitor Species of Bread Wheat

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Abstract: *Aegilops tauschii* Coss., the D genome donor of hexaploid wheat (*Triticum aestivum* L.), is the most promising resource used to broaden the genetic diversity of wheat. Taxonomical studies have classified *Ae. tauschii* into two subspecies, ssp. *tauschii* and ssp. *strangulata*. However, molecular analysis revealed three distantly related lineages, TauL1, TauL2 and TauL3. TauL1 and TauL3 includes the only ssp. *tauschii*, whereas TauL2 includes both subspecies. This study aimed to clarify the phylogeny of *Ae. tauschii* and to find the traits that can differentiate between TauL1, TauL2 and TauL3, or between ssp. *tauschii* and ssp. *strangulata*. We studied the genetic and morpho-physiological diversity in 293 accessions of *Ae. tauschii*, covering the entire range of the species. A total of 5880 high-quality SNPs derived from DArTseq were used for phylogenetic cluster analyses. As a result, we observed wide morpho-physiological variation in each lineage and subspecies. Despite this variation, no key traits can discriminate lineages or subspecies though some traits were significantly different. Of 124 accessions previously lacking the passport data, 66 were allocated to TauL1, 57 to TauL2, and one to TauL3.

Keywords: morpho-physiological diversity; genetic diversity; DArTseq marker; dryland; *Triticum aestivum*

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1. Introduction

Wild relatives attract increasing attention because they can provide characters related to adaptation [1]. The genus *Aegilops* L. (Poaceae) has been intensively studied because of its close relationship with cultivated wheats. The phylogenetic relationship between genera *Aegilops* and *Triticum* L. is widely reported [2–5], and on a world scale, the genus *Aegilops* includes 23 wild annual species, of which 11 are diploids and 12 are allopolyploids [6,7]. The revision of the genus *Aegilops* with regards to its genome and taxonomy results in a total of 27 specific and intraspecific taxa [8,9]. *Aegilops tauschii* Coss. (syn. *Ae. squarrosa* auct. non L.), a wild diploid self-pollinating species (2n = 2x = 14, DD), is the D genome donor of the hexaploid bread wheat (*Triticum aestivum* L.; 2n = 6x = 42, AABBDD). This wild species is found mainly at the edges of wheat fields in eastern Turkey, Iraq, Iran, Pakistan, India, China, Afghanistan, Central Asia, Transcaucasia (South Caucasus) and the Caucasus region [10]. About 8000 to 10,000 years ago, the ancestor of the current bread wheat appeared as a result of natural hybridization between cultivated wheat (*Triticum*

turgidum L., 2n = 4x = 28, AABB) and *Ae. tauschii* [10–12]. Inside this last species, two subspecies were first described by Eig (1929) [13] as *Ae. squarrosa* ssp. *eusquarrosa* and ssp. *strangulata*, and their nomenclature was revised by Hammer (1980) [6] as *Ae. tauschii* ssp. *tauschii* and ssp. *strangulata*. *Ae. tauschii* is genetically and morphologically diverse [13], and the ssp. *tauschii* has elongated cylindrical spikelets, whereas ssp. *strangulata* has quadrate spikelets and empty glumes [6,13]. The ssp. *tauschii* has a wide distribution throughout the species range, whereas ssp. *strangulata* is limited to the south-eastern Caspian coastal region and the Caucasus [14]. Some of the molecular studies supported the subspecies division [15–17], whereas others did not [18,19].

The genetic diversity in *Ae. tauschii* has been studied at the molecular level by using isozymes [20], random amplified polymorphic DNA (RAPD) [21], chloroplast DNA [14,22] amplified fragment length polymorphisms (AFLPs) [23], simple sequence repeats (SSRs) [24] and DArT-array markers [25]. Most of these studies classified *Ae. tauschii* into three lineages: TauL1 including only ssp. *tauschii*, TauL2 including both ssp. *tauschii* and ssp. *strangulata* and TauL3 with intermediate forms. However, Arora et al. [26,27] reported that TauL1 is mainly associated with ssp. *tauschii* and TauL2 with ssp. *strangulata.* Therefore, this study aims to clarify the phylogeny of *Ae. tauschii* and to identify morpho-physiological traits that discriminate between the two main lineages (TauL1 and TauL2), ssp. *tauschii* belonging to TauL1 or TauL2, and the two subspecies (ssp. *tauschii* and ssp. *strangulata*).

2. Materials and Methods

2.1. Plant Materials

We used 293 *Ae. tauschii* accessions collected from the entire range of the natural distribution of this species (Table 1, Figure 1). Of these accessions, 201 have full passport data, including geographical coordinates, lineages and subspecies classification [14] (Figure 1). Five of the 201 accessions (AT 55, AT 60, AT 76, PI 499262 and PI 508262) represent adventive populations in the Shaanxi and Henan provinces of China. Among the 201 accessions, 132 belong to TauL1, 64 to TauL2 and 5 to TauL3 [14]. Based on *sensu stricto* criteria for subspecies classification, only accessions with distinctly moniliform spikes were classified to *Ae. tauschii* ssp. *strangulata*. In contrast, accessions having mildly moniliform and cylindrical spikes were classified to *Ae. tauschii* ssp. *tauschii* [14]. Of 293 accessions used in this study, 169 were previously studied by Matsuoka et al. (2009) [14] who classified 110, 55 and 4 to TauL1, TauL2 and TauL3, respectively.

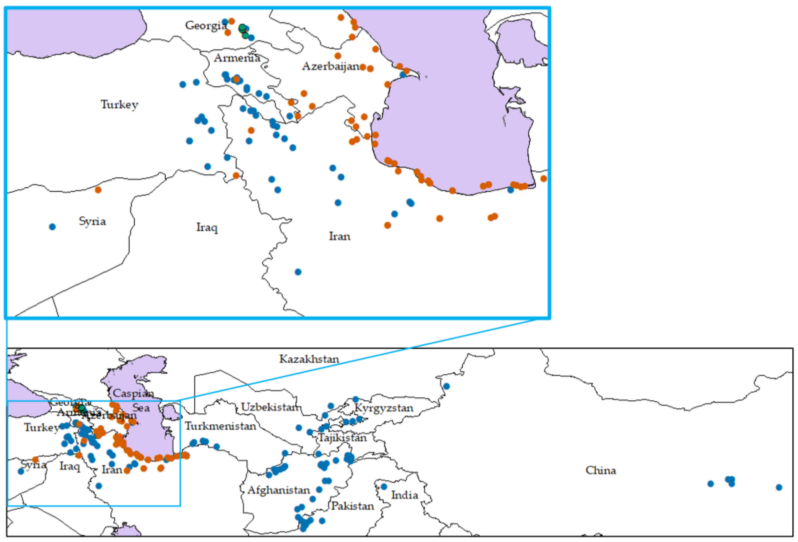

Figure 1. Geographical distribution of 293 *Aegilops tauschii* accessions. Blue circles, lineage 1 accessions (TauL1); red circles, lineage 2 accessions (TauL2); and green circles, lineage 3 accessions (TauL3). Western range is enlarged.

Table 1. *Aegilops tauschii* accessions used in this study.

Origin	TauL1					TauL2					TauL3	
Syria	*AE 1069*	IG 47259				IG 46623						
Turkey	KU-2131 KU-2138 PI 554319	KU-2132 KU-2140	KU-2133 KU-2141	KU-2136 PI 486270	KU-2137 PI 486277	PI 486267	**PI 486274**					
Georgia	*AE 254* KU-2828	*AE 461* KU-2834	*GE12-28-O-2*	*KU-20-2*	KU-2826	AE 1037	*GE12-14-O-1*	KU-2827	KU-2835B		AE 929 KU-2829A *AE 929a*	AE 454 KU-2832
Armenia	*AE 245*	*AE 253*	*AE 476*	*AE 721*	CGN 10734	*AE 229*	*AE 231*	*AE 940*	*AE 941*	IG 126991		
	IG 126273 IG 48758 KU-2821	IG 126280 KU-2809 KU-2822A	IG 126293 KU-2810 KU-2823	IG 126353 KU-2814 KU-2824	IG 48748 KU-2816	**IG 127015**	KU-2811					
Azerbaijan	*AE 143* *AE 725*	*AE 220* *AE 1055*	*AE 251* IG 47196	*AE 723*	*AE 724*	*AE 144* *AE 198* *AE 204* *AE 211* *AE 221* *AE 230* *AE 263* *AE 273* IG 47193 KU-2806	*AE 191* *AE 199* *AE 205* *AE 216* *AE 222* *AE 255* *AE 264* *AK 228* IG 47199	*AE 194* *AE 200* *AE 206* *AE 217* *AE 223* *AE 260* *AE 267* IG 47182 IG 47202	*AE 195* *AE 202* *AE 207* *AE 218* *AE 224* *AE 261* *AE 270* IG 47186 IG 47203	*AE 197* *AE 203* *AE 210* *AE 219* *AE 226* *AE 262* *AE 272* IG 47188 KU-2801		
Dagestan	*AE 234*					*AE 498*	IG 120863	IG 120866	IG 48274	KU-20-1		
Iran	*AE 183* **KU-2109** KU-2121 KU-2152	*AE 184* KU-2113 KU-2142 KU-2153	*AE 541* KU-2115 KU-2143 KU-2154	IG 49095 KU-2116 KU-2144 KU-2157	KU-2082 KU-2120 KU-2148 **KU-2158**	*AE 525* * KU-2069 KU-2086 KU-2096 KU-2102 KU-2110 KU-2126	*AE 526* KU-2075 * KU-2088 * KU-2097 KU-2103 KU-2111 KU-2155	KU-20-8 KU-2079 * KU-2090 * KU-2098 KU-2104 KU-2112 KU-2156	KU-20-9 * KU-2080 * KU-2092 * KU-2100 KU-2105 KU-2118 KU-2159	KU-20-10 KU-2083 KU-2093 * KU-2101 KU-2106 KU-2124 KU-2160		
Turkmenistan	*AE 141* *AE 291* *AE 637* IG 48518	*AE 146* *AE 398* *AE 964*	*AE 242* *AE 472* IG 126387	*AE 248* *AE 473* IG 126489	*AE 249* *AE 499* IG 48508	*AE 192*	*AE 213*	*AE 250*	*CGN 10733*	**IG 120735**		

Table 1. *Cont.*

Afghanistan	*AE 193*	*AE 275*	*AE 276*	*AE 277*	*AE 279*					
	AE 280	*AE 281*	*AE 1087*	KU-2010	KU-2012					
	KU-2016	KU-2018	KU-2022	KU-2025	KU-2027					
	KU-2035	KU-2039	KU-2042	KU-2043	KU-2044					
	KU-2050	KU-2051	KU-2056	KU-2059	KU-2061					
	KU-2063	KU-2066	*KU-2616*	KU-2617	KU-2619					
	KU-2621	KU-2624	KU-2630	KU-2632	KU-2633					
	KU-2635	KU-2636	KU-2638	KU-2639	PI 476874					
Pakistan	CGN 10767	CGN 10768	CGN 10769	CGN 10771	IG 108561					
	IG 46663	IG 46666	KU-2003	KU-2006	KU-2008					
Tajikistan	*AE 189*	*AE 233*	*AE 647*	*AE 817*	*AE 858*					
	AE 955	*AE 956*	AE 1038	*AE 1039*	*AE 1040*					
	IG 48554	IG 48559	IG 48564							
Uzbekistan	*AE 3*	*AE 239*	*AE 469*	*AE 560*	IG 120736	*AE 692* *				
	IG 123910	IG 48539	IG 48565	IG 48567						
Kyrgyzstan	*AE 256*	*AE 257*	*AE 1180*	IG 131606						
Kazakhstan	AE 1090									
China	AT 55	AT 60	AT 76	PI 499262	PI 508262					
Unknown location	*AE 32*	*AE 67*	*AE 147*	*AE 150*	*AE 422*	*AE 426* *	*AE 428* *	*AE 429* *	*AE 430* *	*AE 431*
	AE 427	*AE 433*				*AE 432*	*AE 434* *			

Roman accessions are known from Matsuoka et al. (2009) [14]. Italic accessions are classified in this study into TauL1, TauL2 or TauL3. Bold accessions have different taxonomy based on chloroplast DNA. AE accessions were received from the Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Germany; AT accessions from the Faculty of Agriculture, Okayama University, Japan; CGN accessions from the Instituut Voor Planten Veredeling, Landbouwhoge School, Wageningen, the Netherlands; IG accessions from the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria; KU accessions from the Germplasm Institute, Faculty of Agriculture, Kyoto University, Japan; and PI accessions from the US Department of Agriculture. * Ssp. *strangulata*. The subspecies classified morphologically following Matsouka et al. (2009) [14] and confirmed by cluster analysis in this study (Figure S1).

2.2. Genomic Analysis and Statistaical Analysis of Molecular Data

Genomic DNA was extracted using the CTAB method [28]. The DNA samples (30 µL; 50–100 ng μL^{-1}) were sent to Diversity Arrays Technology Pty. Ltd, Canberra, Australia (http://www.diversityarrays.com, accessed on 29 January 2018) for a whole-genome scan using the DArTseq platform. Sequencing-based DArT genotyping applies two complexity-reduction methods optimized for several plant species i.e., *Pst*I/*Hpa*II and *Pst*I/*Hha*I were used to select a subset of the corresponding fragments [29]. At the DArT facility, the DArT soft marker extraction pipeline was used to filter and identify the informative markers. We performed the hierarchical clustering analysis in the statistical software R with the pvclust package [30]. The DArTseq SNPs data of 5880 markers without any missing data for 293 accessions of *Ae. tauschii* from 16 countries (some accessions are from unknown origin) were used for the analysis. Pvclust package computes the AU (approximately unbiased) *p*-value and BP (bootstrap probability) value via multiscale bootstrap resampling. These values can show how strong the clustering result is supported by the data. The dendrogram was generated by using the Euclidean distance matrix and complete method. The summary of SNP data sequences used for constructing phylogenetic tree was provided in Table S1.

2.3. Morpho-Physiological Evaluation

The morphological and physiological traits of all the accessions were measured at the research field of the Arid Land Research Center, Tottori University (Tottori, Japan; 35°32′N 134°13′E) during the winter and spring seasons of 2016/17 and 2017/18 by using an augmented complete block design with three randomly selected accessions as checks (GE12-14-O-1, GE12-28-O-2 and KU-20-2), and five plants were grown per accession. To estimate the phenotypic variation, we measured two leaf parameters (flag leaf length, FLL; flag leaf width, FLW), four spike parameters (spike length, SPL; spike width, SPW; seed number per spike, SN/SP; spike weight, SPWg), days to heading (DH), biomass weight (Bio) and three physiological traits (Normalized Difference Vegetative Index, NDVI; canopy temperature, CT; and chlorophyll content, SPAD). To measure SPWg, we covered the spikes with a transparent envelope before physiological maturity to avoid shattering. The measurement methods are summarized in Table 2.

Table 2. Phenotypic traits analyzed.

Trait	Abbreviation (Unit)	Measurement/Definition
Flag leaf length	FLL (cm)	Measured from three tillers per accession.
Flag leaf width	FLW (mm)	Measured from three tillers per accession.
Spike length	SPL (cm)	Measured from the middle five spikes after maturity stage.
Spike width	SPW (cm)	Measured from the middle of five spikes after maturity stage.
Seed number/spike	SN/SP	Counted from five spikes at harvesting.
Spike weight	SPWg (g)	Weighed from five spikes (one per tiller) using a sensitive scale.
Days to heading	DH	Recorded when the whole spike above the flag leaf fully emerged on the earliest tiller in each plant of each accession.
Biomass weight	Bio (g)	Weighed after harvesting and drying of five plants in a glasshouse.
Normalized Difference Vegetation Index	NDVI	A vegetative index that compares reflectance in the red and near-infrared regions. Measured during flowering using a handheld optical sensor unit (Green Seeker), NTech Industries, Inc., Ukiah, CA, USA.
Canopy temperature	CT (°C)	Measured during flowering using an infrared thermometer AD-5611A.
Chlorophyll content	SPAD	Measured at the flowering stage from the middle of the flag leaf of three tillers using a Minolta brand chlorophyll meter (Model SPAD-502; Spectrum Technologies Inc., Plainfield, IL, USA).

2.4. Statistical Analysis of Morpho-Physiological Data

Analyses of the phenotypic data, including mean, standard deviation, range distribution and analysis of variance (*F* and *p*-values in one-way ANOVA) for the morpho-physiological variations were calculated using Plant Breeding Tools (PBTools) version 1.4 (International Rice Research Institute, http://bbi.irri.org/products, 15 February 2020). Due

to the significant genotype × season interaction, best linear unbiased predictions (BLUPs) were estimated for each trait.

3. Results

3.1. Phylogenetical Allocation of Uncertain Accessions by Molecular Markers

Following Matsouka et al. (2009) [14], we carefully observed the key morphological traits of the 124 accessions that lacked taxonomical information and identified 7 accessions as ssp. *strangulata* and the remaining 117 as ssp. *tauschii*. Among the seven accessions identified as ssp. *strangulata*, AE 525 was collected from Iran, AE 692 from Uzbekistan and AE 426, AE 428, AE 429, AE 430 and AE 434 from unknown regions. To know the lineages (TauL1, TauL2 or TauL3) of all 124 accessions, we conducted cluster analysis using 5880 DArTseq markers. As a result, 66, 57 and 1 were clustered in TauL1, TauL2 and TauL3, respectively (Figure 2, Figure S1). All the accessions in TauL1 were ssp. *tauschii*, whereas in TauL2, 50 were ssp. *tauschii* and 7 were ssp. *strangulata*. The accessions in the TauL3 were ssp. *tauschii*. These findings supported previous results that the ssp. *strangulata* is present only in TauL2.

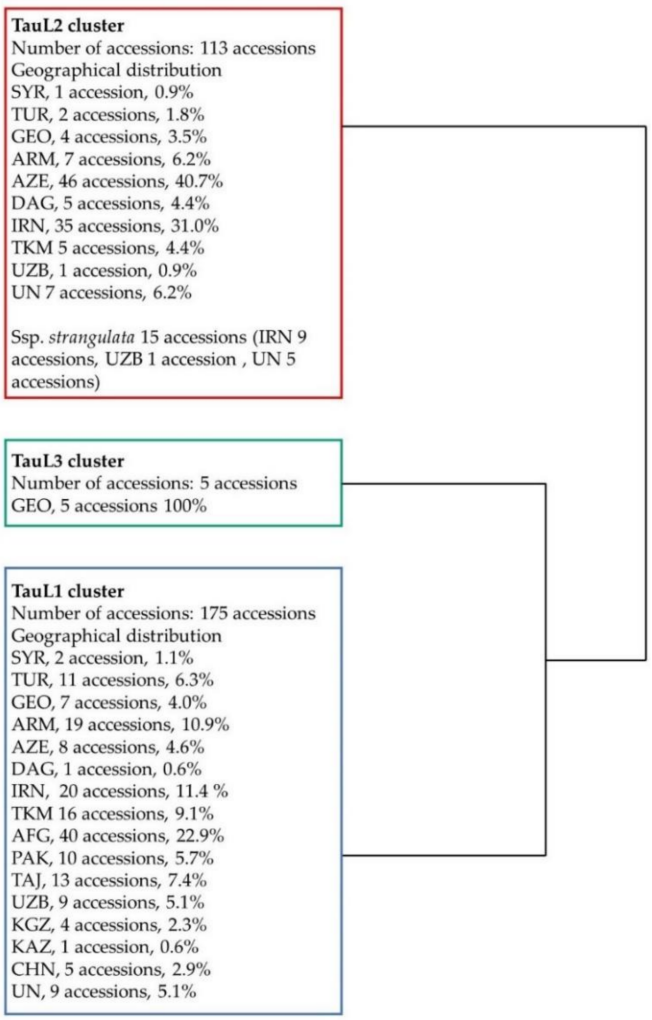

Figure 2. Schematic form of hierarchical clustering of 293 *Ae. tauschii* accessions showing the classification of TauL1, TauL2 and TauL3 based on high-quality SNPs derived from 5880 DArTseq markers. Origin of accessions: SYR, Syria; TUR, Turkey; GEO, Georgia; ARM, Armenia; AZE, Azerbaijan; DAG, Dagestan; IRN, Iran; TKM, Turkmenistan; AFG, Afghanistan; PAK, Pakistan; TAJ, Tajikistan; UZB, Uzbekistan; KGZ, Kyrgyzstan; KAS, Kazakhstan; CHN, China and UN, unknown country.

Previously, Matsuoka et al. (2009) [14] classified *Ae. tauschii* accessions into TauL1, TauL2 and TauL3 based on the chloroplast DNA. To confirm their result, we analyzed the 169 accessions used in Matsuoka et al. (2009) [14] using DArTseq markers. Most of the accessions were clustered as expected with 5 exceptions: KU-2109 and KU-2158 were in TauL1, whereas PI 486274, IG 127015 and IG 120735 were in TauL2.

From these studies, we found that all 293 accessions of *Ae. tauschii* were classified as 175 TauL1, 113 TauL2 and 5 TauL3. In TauL2, 15 accessions were ssp. *strangulata* and others including accessions in TauL1 and TauL3 were ssp. *tauschii*.

The TauL1 cluster contained accessions from Syria, Turkey, Georgia, Armenia, Azerbaijan, Dagestan, Iran, Turkmenistan, Afghanistan, Pakistan, Tajikistan, Uzbekistan, Kyrgyzstan, Kazakhstan, China and unknown countries. The TauL2 cluster contained accessions from Syria, Turkey, Georgia, Armenia, Azerbaijan, Dagestan, Iran, Turkmenistan, Uzbekistan and unknown countries (Table 1, Figure 2, Figure S1). The ssp. *strangulata* accessions were clustered in one clade in TauL2, and most of the accessions were from Iran.

3.2. Morpho-Physiological Differences between TauL1 and TauL2

A large variation was observed for all the morpho-physiological traits in TauL1 and TauL2 (Table 3). Statistical analyses showed a significant difference between these two lineages in SPW, SPWg, DH and Bio. The means in these traits were larger in TauL2 than in TauL1, indicating that the accessions in TauL2 tend to be higher than TauL1. On the other hand, the means of the physiological traits (NDVI, CT and SPAD), and leaf traits (FLL and FLW) were not significantly different between them. The ranges of these traits overlapped between the two lineages, and thus we cannot discriminate the two groups with these traits (Table 3).

Table 3. Morpho-physiological variation in two *Aegilops tauschii* lineages, TauL1 (175 accessions) and TauL2 (113 accessions).

Trait	TauL1				TauL2				*p*-Value (TauL1 versus TauL2)
	Min	Max	Mean	STD	Min	Max	Mean	STD	
FLL	5.35	20.65	13.74	2.48	5.77	20.32	12.96	2.84	0.052
FLW	4.80	11.00	8.10	1.20	4.20	10.90	7.80	1.10	0.145
SPL	9.08	17.55	12.61	1.50	8.80	17.27	12.03	1.55	0.325
SPW	0.40	0.71	0.53	0.06	0.40	0.75	0.58	0.07	0.011
SN/SP	15.82	29.67	22.00	2.32	15.42	29.93	19.51	2.05	0.081
SPWg	0.35	0.67	0.50	0.06	0.34	0.71	0.54	0.07	0.005
DH	150.78	184.03	169.19	5.78	159.77	191.45	174.39	4.04	0.000
Bio	60.53	189.78	99.24	23.61	73.90	227.09	134.50	37.11	0.000
NDVI	0.60	0.63	0.62	0.01	0.60	0.64	0.62	0.01	0.389
CT	15.11	25.14	18.34	1.91	14.49	24.50	17.91	1.84	0.303
SPAD	40.92	45.37	43.50	0.73	42.06	45.46	43.69	0.71	0.413

3.3. Morpho-Physiological Variation between ssp. tauschii Belonging to TauL1 and TauL2

We designated ssp. *tauschii* in TauL1 and TauL2 as 'TauL1T' and 'TauL2T', respectively, and compared accessions in these groups. A large variation was observed for all the morpho-physiological traits in TauL1T and TauL2T (Table 4). Statistical analyses showed significant differences between the two groups in FLL, DH and Bio. The mean of FLL was higher in TauL1T, whereas those of DH and Bio were higher in TauL2T. On the other hand, the means of the physiological traits (NDVI, CT and SPAD), and spike traits (SPL, SPW, SN/SP and SPWg) were not significantly different between them. The ranges of these traits overlapped between TauL1T and TauL2T, and thus we cannot discriminate the two groups with these traits (Table 4).

Table 4. Morpho-physiological variation in ssp. *tauschii* in TauL1 (TauL1T, 175 accessions) and TauL2 (TauL2T, 98 accessions).

Trait	TauL1T				TauL2T				*p*-Value (TauL1T versus TauL2T)
	Min	Max	Mean	STD	Min	Max	Mean	STD	
FLL	5.35	20.65	13.74	2.48	5.77	20.32	12.78	2.89	0.040
FLW	4.80	11.00	8.10	1.20	4.20	1.90	7.80	1.20	0.239
SPL	9.08	17.55	12.61	1.50	8.80	16.75	12.19	1.41	0.271
SPW	0.40	0.71	0.53	0.06	0.40	0.72	0.57	0.07	0.145
SN/SP	15.82	29.67	22.00	2.32	16.13	29.93	19.72	2.07	0.106
SPWg	0.35	0.67	0.50	0.06	0.37	0.67	0.53	0.06	0.091
DH	150.78	184.03	169.19	5.78	159.77	191.45	174.46	4.28	0.001
Bio	60.53	189.78	99.24	23.61	73.90	227.09	135.39	37.28	0.000
NDVI	0.60	0.63	0.62	0.01	0.60	0.64	0.62	0.01	0.327
CT	15.11	25.14	18.34	1.91	14.49	24.50	17.84	1.87	0.377
SPAD	40.92	45.37	43.50	0.73	42.31	45.46	43.67	0.69	0.278

3.4. Morpho-Physiological Variation between ssp. tauschii and ssp. strangulata

A large variation was observed for all the morpho-physiological traits in ssp. *tauschii* and ssp. *strangulata* (Table 5). Statistical analyses showed significant difference between these two subspecies in SPL, SN/SP, SPWg and DH. The means of SPL and SN/SP were higher in ssp. *tauschii* than in ssp. *strangulata*, whereas those of SPWg and DH were higher in ssp. *strangulata* than in ssp. *tauschii*. On the other hand, the means of the leaf traits (FLL and FLW), SPW and physiological traits (NDVI, CT and SPAD) were not significantly different between them. The ranges of these traits overlapped between the two subspecies (Table 5).

Table 5. Morpho-physiological variation in ssp. *tauschii* (273 accessions) and spp. *strangulata* (15 accessions) of *Aegilops tauschii*.

Trait	Ssp. *tauschii*				Ssp. *strangulata*				*p*-Value (*tauschii* versus *strangulata*)
	Min	Max	Mean	STD	Min	Max	Mean	STD	
FLL	5.35	20.65	13.40	2.68	11.04	17.97	14.15	2.17	0.228
FLW	4.20	11.00	8.00	1.20	6.40	9.50	7.90	0.90	0.123
SPL	8.80	17.55	12.46	1.48	8.82	17.27	11.00	1.97	0.027
SPW	0.40	0.72	0.54	0.07	0.58	0.75	0.66	0.06	0.432
SN/SP	15.82	29.93	21.18	2.48	15.42	20.42	18.13	1.30	0.006
SPWg	0.35	0.67	0.51	0.06	0.34	0.71	0.58	0.09	0.004
DH	150.78	191.45	171.08	5.86	170.37	178.51	173.94	1.72	0.000
Bio	60.53	227.09	112.21	34.01	89.70	223.05	128.67	35.40	0.294
NDVI	0.60	0.64	0.62	0.01	0.60	0.63	0.62	0.01	0.088
CT	14.49	25.14	18.16	1.91	16.11	21.55	18.37	1.55	0.280
SPAD	40.92	45.46	43.56	0.72	42.06	44.83	43.82	0.84	0.151

3.5. Morpho-Physiological Variation of Accessions in TauL3

In this study, only five accessions (AE 454, AE 929, AE 929a, KU-2829A and KU-2832) belong to TauL3. Therefore, we did not compare them with TauL1 and TauL2. All the accessions originated from Georgia and showed a similar plant morphology to ssp. *tauschii* with an intermediate spike shape between TauL1 and TauL2. Genomic analysis revealed that these accessions are clearly differentiated from both TauL1 and TauL2.

4. Discussion

4.1. Geographical Clines of Morphological Variation in Subspecies and Lineage Classification

The main putative area of origin of *Ae. tauschii* is the Transcaucasus, from which it has spread to the east and south [10] (Figure 1). While ssp. *tauschii* has cylindrical spike forms and ssp. *strangulata* moniliform spike forms, some *Ae. tauschii* accessions have

mildly moniliform spike forms (TauL3) which suggest a hybrid origin. Overall, spikelet morphology is the main trait not only for discriminating the two subspecies but also for intraspecific diversification in *Ae. tauschii*, even though the genetic basis of spikelet morphology divergence has not yet been studied. Nishijima et al. (2017) [31] divided *Ae. tauschii* into two main lineages TauL1 and TauL2, and a minor lineage (TauL3) by Bayesian population structure analysis with genome-wide marker genotyping. Using DArTseq genotyping of a large number of accessions, we confirmed their results (Figure 2, Figure S1). The TauL1 accessions are spread from the western geographical range (Transcaucasus, northern regions of Iran) to the eastern geographical range (Pakistan and Afghanistan), whereas TauL2 is limited only to the western range, and ssp. *strangulata* is included only in TauL2.

This result is consistent with Mizuno et al. (2010) [23] using AFLPs. Thus, the differentiation of the ssp. *strangulata* is believed to have occurred in TauL2. Furthermore, we found that the most probable origin of ssp. *strangulata* is Iran and that this subspecies clusters in one clade within TauL2 (Figure 2, Figure S1). This finding strongly indicates that speciation had occurred in the ssp. *tauschii* included in TauL2, resulting in appearance of ssp. *strangulata*-type spike morphology. The D genome of ssp. *strangulata* is involved in the D genome of bread wheat. This was revealed by sequencing [32], single nucleotide polymorphisms [33], variation in the *AP2* homoeologs, the genes underlying lodicule development [34], SSR markers [35], NADP-dependent aromatic alcohol dehydrogenase [36] and aspartate aminotransferase and alcohol dehydrogenase isoenzymes [37]. Overall, using the DArTseq genotyping platform, we have allocated 124 accessions with no previous lineage description into TauL1, TauL2 or TauL3. Furthermore, based on this data, we have reclassified 5 accessions: 2 accessions from Iran (KU-2109 and KU-2158) formerly classified in TauL2 by chloroplast DNA [14] were now placed in TauL1, and 3 accessions (PI 486274 from Turkey, IG 127015 from Armenia and IG 120735 from Turkmenistan) formerly classified in TauL1 were now placed in TauL2. The inconsistency of the nucleus and cytoplasmic genomes may be attributable to the cytoplasmic substitution origin by hybrids between the two lineages and the backcrossing in the evolution of these accessions. Furthermore, previous studies reported that accessions in TauL2 were distributed in the regions near the Caspian Sea. However, here we found that five accessions (AE 192, AE 213, AE 250, CGN10733 and IG 120735) which originated from Turkmenistan and AE 692 from Uzbekistan were clustered in TauL2 (Table 1). These accessions may have been transferred to the regions naturally or by human activity.

4.2. Potential for Adaptive Convergence in Ae. tauschii Evolution

Molecular evolutionary studies have explained the origin of crops more clearly than before [38,39], especially for the main crops that were domesticated without ploidy modification. Phylogeographic analyses based on nuclear and chloroplast DNA sequences have shown multiple evolutionary origins of cultivated rice in East Asia [40] and barley in the Fertile Crescent and Central Asia [41,42], whereas phylogenetic analysis based on multilocus microsatellite genotyping has shown a single domestication event for maize ca. 9000 years ago [43]. One of the fundamental problems in understanding the evolution of *Ae. tauschii* is the relationship between the different lineages and subspecies. In the current study, although some traits examined differed significantly between the lineages and subspecies, the range of the diversity was overlapped (Tables 3–5). The phenotypes convergence may have originated through either divergent genetic solutions [44,45] or the same pathways, genes or even nucleotide positions in independent lineages [46,47]. Convergence at the genetic level can in turn result from (i) mutations arising independently in separate populations or organisms (parallel genetic evolution); (ii) evolution of a polymorphic allele in a common ancestral population or species (trans-specific polymorphism); and (iii) evolution of an allele introduced by hybridization (introgression) from one population to another (e.g., TauL1 and TauL2). Another possibility that can explain the phenotypic similarities between the different *Ae. tauschii* lineages is the occurrence

of genetic differentiation after the geographical isolation under similar environmental condition without morphological or physiological differentiation. Local standing genetic diversity combined with spatial population structure restricting dispersal in an ecologically patchy area promotes rapid convergence [48].

4.3. Implications of Ae. tauschii Diversity in Wheat Breeding

Among the species in genus *Aegilops*, only *Ae. tauschii* can be used efficiently for wheat improvement owing to the mostly regular pairing of its chromosomes with the D genome chromosomes of bread wheat [49]. It is believed that *Ae. tauschii* is an excellent source to widen the narrow genetic base of bread wheat. Currently, with the new advances in plant science and the rapid development of sequencing and genome-editing tools, identification and characterization of genes of interest in wheat are in progress and can be expected to become easier and more straightforward in the coming decades. Once the gene in question is identified and characterized, it is easy to transfer and utilize the gene in breeding programs. This will pave the way to utilize the genes from *Ae. tauschii* as it will help to overcome the limitations related to the irregular chromosome pairing.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/d13050217/s1, Figure S1: Hierarchical clustering of 293 *Ae. tauschii* accessions showing the classification of TauL1, TauL2, and TauL3 based on high-quality SNP markers derived from 5880 DArTseq markers. Values at branches are AU values (upper, red), BP values (down, blue), and cluster labels (medium, gray). Ssp. *strangulata* is indicated, and others belongs to ssp. *tauschii*. UN, unknown lineages or country. Origin of accessions: SYR, Syria; TUR, Turkey; GEO, Georgia; ARM, Armenia; AZE, Azerbaijan; DAG, Dagestan; IRN, Iran; TKM, Turkmenistan; AFG, Afghanistan; PAK, Pakistan; TAJ, Tajikistan; UZB, Uzbekistan; KGZ, Kyrgyzstan; KAS, Kazakhstan and CHN, China. The two black circles indicate where these two trees are connected, Table S1: The summary of SNP data sequences used for constructing phylogenetic tree.

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