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

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Received: 13 April 2021
Accepted: 17 May 2021
Published: 19 May 2021

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 distinctly related lineages, TauL1, TauL2 and TauL3. TauL1 and TauL2 included the only ssp. tauschii, whereas TauL3 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

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 taxonomic 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.

| Origin | TauL1 | TauL2 | TauL3 |
|--------|-------|-------|-------|
| Syria  | AE 1069 IG 47259 | IG 46623 |
| Turkey | KU-2131 KU-2132 KU-2133 KU-2136 KU-2137 PI 486267 | PI 486274 |
|        | KU-2138 KU-2140 KU-2141 PI 486270 | PI 486277 |
|        | 554319 |
| Georgia| AE 254 AE 461 GE12-28- O-2 KU-20-2 KU-2826 AE 1037 GE12-14- O-1 KU-2827 KU-2835B | AE 929 AE 454 |
|        | KU-2829 KU-2832 AE 929a |
| Armenia| AE 245 AE 253 AE 476 AE 721 CGN 10734 AE 229 AE 231 AE 940 AE 941 IG 126991 | |
| Country       | Code | Sequence IDs                      | Code | Sequence IDs                      | Code | Sequence IDs                      |
|--------------|------|-----------------------------------|------|-----------------------------------|------|-----------------------------------|
| Azerbaijan   | AE143| AE 143 AE 220 AE 251 AE 723 AE 724 | AE191| AE 200 AE 201 AE 202 AE 203     | AE197| AE 198 AE 199 AE 204 AE 205 AE 206 AE 207 AE 210 AE 211 AE 216 AE 217 AE 218 AE 219 AE 221 AE 222 AE 223 AE 224 AE 226 AE 230 AE 235 AE 260 AE 261 AE 262 AE 263 AE 264 AE 267 AE 270 AE 272 AE 273 AK 228 IG 47182 IG 47186 IG 47188 IG 47193 IG 47199 IG 47202 IG 47203 IG 2801 KU-2806 |
| Dagestan     | AE234| AE 498 IG 120863 IG 48274 KU-20-1 |
| Iran         | AE183| AE 183 AE 184 AE 541 IG 49095 KU-2082 AE 525 AE 526 AE 20-9 KU-20-10 KU-2109 KU-2113 KU-2115 KU-2120 KU-2069 KU-2075 KU-2079 KU-2080 KU-2083 KU-2090 KU-2092 KU-2093 KU-2152 KU-2153 KU-2154 KU-2157 KU-2158 KU-2096 KU-2097 KU-2098 KU-2100 KU-2101 KU-2102 KU-2103 KU-2104 KU-2105 KU-2106 KU-2110 KU-2111 KU-2112 KU-2118 KU-2124 KU-2126 KU-2155 KU-2156 KU-2159 KU-2160 |
| Turkmenistan | AE141| AE 141 AE 146 AE 242 AE 248 AE 249 AE 192 AE 213 AE 250 IG 120735 |
| Afghanistan  | AE193| 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  | CGN CGN CGN IG 10767 10768 10769 10771 108561 IG 46663 IG 46666 KU-2003 KU-2006 KU-2008 |
| Tajikistan   | AE189| 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   | AE3  | AE 3 AE 239 AE 469 AE 560 IG 120736 AE 692 IG 123910 IG 48539 IG 48565 IG 48567 |
| Kyrgyzstan   | AE256| AE 256 AE 257 AE 1180 IG 131606 |
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, Landbouwhogeschool, 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).

| Country        | Accession |
|----------------|-----------|
| 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 * |

**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.

2.2. Genomic Analysis and Statistical 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., PstI/HpaII and PstI/HhaI were used to select a subset of the corresponding 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.

| 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 5,880 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.

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.

![Figure 2](image-url)
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.

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 |
| NDMI  | 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 | 12.96 | 2.84 | 0.040 |
| FLW   | 4.80   | 11.00  | 8.10  | 1.20 | 4.20 | 10.90 | 7.80  | 1.10 | 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 |
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 *ssp. strangulata* (15 accessions) of *Aegilops tauschii*.

| Trait | *ssp. tauschii* | *ssp. strangulata* | P-Value |
|-------|-----------------|-------------------|---------|
|       | Min | Max | Mean | STD | Min | Max | Mean | STD | (tauschii versus strangulata) |
| 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 | 45.37 | 43.50 | 0.73 | 42.31 | 45.46 | 43.67 | 0.69 | 0.278 |

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 www.mdpi.com/1424-2818/13/5/217/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.

Author Contributions: H.T. conceived the project. H.T., T.-S.C., H.I., Y.Y. and M.M.M.M. designed the research. Y.M. and H.T. provided plant materials. M.M.M.M. conducted the experiments and analyzed the data. Y.S.A.G., N.M.K. and M.A. guided the data analyses. M.M.M.M. prepared the first draft of the manuscript. Y.S.A.G., N.M.K., I.S.A.T. and H.T. critically reviewed and improved the final manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partly supported by the SATREPS Project (JPMJSA1805) funded by Japan Science and Technology Agency (JST), KAKENHI (16H04858) from Japan Society for the Promotion of Science, and by the MRA Project of Tottori University.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Non applicable.

Data Availability Statement: This study did not report any data.

Acknowledgments: We appreciate Michael O. Itam for critical reading of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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