*Indian J. Plant Genet. Resour. 33(3): 360–366 (2020)*
DOI 10.5958/0976-1926.2020.00050.9

RESEARCH ARTICLE

# Collection, Conservation and Morphological Characterization of *Aegilops tauschii* Coss. Accessions from Kashmir, India

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(Received: 18 July, 2020; Revised: 27 November, 2020; Accepted: 05 December, 2020)

*Aegilops tauschii* is an important crop wild relative of bread wheat (*Triticum aestivum*) and its gene pool is currently considered the most important resource for wheat improvement as potential gene donor for drought, disease and pest resistance. *Aegilops tauschii* subsp. *tauschii* grows abundantly at several places in Kashmir but is somewhat uncommon. Fourteen germplasm accessions were collected for the first time from diverse locations mainly in Budgam and Srinagar districts of Kashmir and conserved in the National Genebank. Analysis of data recorded on morphological traits of plant height, days to 50% flowering, days to 50% maturity, spike length, spikelets/spike, no. of seeds/spikelet, seed yield/plant and 100-grain weight revealed considerable variability. Significant positive association of days to flowering was observed with spike length and number of spikelets/spike. Interestingly, spike length revealed a significant positive association with altitude of place from where accessions were originally collected. Principal component analysis revealed that first two components having Eigen values greater than one contributed nearly 60% variance. Cluster analysis grouped the accessions into three distinct groups mainly on the basis of spike length. The study provides basic information about this valuable crop wild relative and the information may help in its proper utilization.

**Key Words: *Aegilops tauschii* subsp. *tauschii*, Cluster analysis, Conservation, CWR, PCA**

## Introduction

The genus *Aegilops* L. (Family: Poaceae) with 22 species comprises both diploids and polyploids that originated in the Middle East (van Slageren, 1994). *Aegilops tauschii* Coss. is commonly called as Tausch's goatgrass or rough-spike hard grass and is found in Temperate Asia, Eastern Europe and the Caucasus region. This species is native to Eastern Europe (Ukraine-Crimea), western and middle Asia, the Caucasus, the Indian Subcontinent and China (USDA, ARS, National Genetic Resources Program, 2017). *Aegilops tauschii* is uncommon throughout most of its range but is locally abundant, found growing in dry grasslands, fallow, steppes and moderately disturbed sites such as wastelands, roadsides and edges or within cultivation of, for example, barley, bread wheat and fruit trees besides in woodlands, irrigated fields as well as stony slopes (van Slageren, 1994). It is believed to be the diploid (2n = 2x = 14) progenitor of hexaploid wheat (*Triticum aestivum*) contributing D-genome. A spontaneous natural hybridization event is believed to have occurred some 8,000 years ago between *Triticum turgidum* ssp. *dicoccoids* (AABB) and *Aegilops tauschii* (DD) somewhere in the Fertile Crescent resulting in hexaploid (2n = 42) wheat (Feldman, 2001). Gene pool of this secondary wild relative of wheat is currently considered the most important gene resource for wheat improvement as potential gene donor to confer traits for disease and pest resistance (Kaur *et al*., 2010). Despite the difficulties involved in handling of wild species, such as crossability and incompatibility, *Aegilops* species have significantly contributed to wheat breeding. Infact, this wild relative has gathered more attention as a new resistance source against Ug99 stem rust and wheat blast diseases (Kishii, 2019). Some germplasm lines of *Aegilops tauschii* have been found to exhibit hyper-resistance to leaf rust fungi (*Puccinia triticina*) at both seedling and adult stages (Lee *et al*., 2020).

According to Genesys Report (2017) there are 1,726 germplasm accessions of wild origin of which 561 are backed up at a second gene bank. Furthermore, 415 accessions are duplicated and conserved *ex situ* in the Svalbard Seed Vault. Out of the 1,726 accessions, 1,432 have been collected within the species' native range including two from India. In India, *Aegilops tauschii* subsp. *tauschii* is found growing in Kashmir valley of Jammu & Kashmir and we have for the first

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time collected 14 diverse germplasm accessions from the region. These accessions have been conserved in the National Genebank at ICAR-NBPGR New Delhi.

*Aegilops tauschii* is an annual herbaceous plant found growing especially on dry *karewa* lands (elevated table-land of lacustrine deposits) mainly in the areas of Budgam and Pulwama where we have observed its fragmented populations together with other grass species. Some decades back *Aegilops tauschii* was found very commonly growing in stony places at Shankaracharya hill near Srinagar (Mehra and Sharma, 1977). Although one accession of this plant was collected by us from this area, we have not found it so common here nowadays. *Aegilops tauschii* has been assessed as endangered species in Europe (Smekalova *et al.*, 2011) and as of least concern in China (Sung and Yan, 2004). As of now *Aegilops tauschii* subsp. *tauschii* occurs not so commonly in Kashmir. However, habitat loss, uncontrolled grazing and agricultural expansion remain major threats. The plant often forms clusters or tufts of erect or ascending clums on an average 50 cms tall. Its leaf (flag leaf) blades are 8-15 cm long and 0.4-0.8 cms broad. Each clum terminates in a compact spike, 5-12 cms long excluding the terminal awn. The rachis of the spike contains nodes and internodes and being fragile, breaks at nodes when mature and internode segments fall to the ground along with attached spikelets. Each internode segment on an average is 0.8 cms long roughly same as that of flag leaf width. The internodes are composed of rachis segment and one sessile spikelet which remains closely packed to its broadside. Each spike may contain 8-12 internode segments with as much spikelets, sometimes with one or two vestigial spikelets at the base. Glumes of spikelets are leathery, 4-6 mm long with truncate or slightly toothed apex. Each mature spikelet contains 2-3 (commonly 2) small wheat like grains. Under natural conditions in Kashmir, the spikes start maturing around first week of June as is the case with wheat crop and by the end of the month there is almost complete shattering.

Better understanding of *Aegilops tauschii*, its occurrence, distribution and morphological characteristics will help in proper utilization as well as for future collection and genetic resource management of this most important crop wild relative (CWR) of wheat in the country especially in the absence of earlier reports from the region. Apart from collection and conservation, the objective of the present study was to assess the morphological diversity in the collected accessions.

## Materials and Methods

### *Collection of Germplasm*

An exploration and germplasm collection programme was conducted during the year 2018 in the months of June and July and 14 accessions of *Aegilops tauschii* subsp. *tauschii* were collected mainly from Budgam and Srinagar districts of Kashmir from oats fields on dry *karewa* lands, roadsides, graveyards, almond orchards, apple orchards, foot hill slopes and banks of irrigation channels at an altitudinal range of 1591-1842 masl (Table 1). The collected germplasm has been conserved in the National Genebank at ICAR-NBPGR New Delhi.

**Table 1. Collection sites and geographical coordinates of 14 germplasm accessions of *Aegilops tauschii* subsp. *tauschii* used in the present study**

| Accession No. | Collection site | Latitude | Longitude | Altitude (masl) |
|---|---|---|---|---|
| IC0628095 | Apple orchard | 33° 59′ 19″ | 74° 47′ 89″ | 1661 |
| IC0628096 | Apple orchard | 33° 59′ 19″ | 74° 47′ 89″ | 1661 |
| IC0628097 | Oat field | 33° 54′ 42″ | 74° 46′ 26″ | 1830 |
| IC0628098 | Oat field | 33° 54′ 42″ | 74° 46′ 26″ | 1830 |
| IC0628099 | Almond orchard | 33° 53′ 15″ | 74° 47′ 11″ | 1842 |
| IC0628100 | Roadside | 33° 54′ 57″ | 74° 48′ 19″ | 1708 |
| IC0628101 | Graveyard | 34° 01′ 30″ | 74° 49′ 35″ | 1592 |
| IC0628102 | Shady stream bank | 33° 59′ 10″ | 74° 49′ 22″ | 1598 |
| IC0628103 | Roadside | 33° 58′ 03″ | 74° 47′ 53″ | 1606 |
| IC0628104 | Roadside | 33° 58′ 06″ | 74° 48′ 01″ | 1611 |
| IC0628105 | Wasteland | 34° 01′ 56″ | 74° 44′ 32″ | 1591 |
| IC0628106 | Hill slope | 34° 04′ 52″ | 74° 50′ 47″ | 1750 |
| IC0628107 | Roadside | 34° 02′ 15″ | 74° 48′ 15″ | 1592 |
| IC0628108 | Protected National Park | 34° 09′ 06″ | 74° 55′ 17″ | 1731 |

### *Morphological Characterization*

Morphological characterization of 14 germplasm accessions of *Aegilops tauschii* subsp. *tauschii* was carried out under rainfed conditions at Experimental Farm of ICAR-NBPGR Regional Station Srinagar, Jammu and Kashmir (33°59′ N latitude, 74°47′ E longitude, 1639 m above sea level) during *rabi* season of 2018-19. The accessions were sown in the second week of November. Experiment was laid out in randomized block design with three replications per accession. Bed width of 2 m and row to row and plant to plant distance of 40 cm and 15 cm respectively were maintained. Weeding was done by hand before the emergence of spikes. Data were recorded following descriptors established for *Aegilops* (IBPGR 1981). Data were taken on plant height (cm), days to 50% spike emergence, days to 50% maturity, spike length (cm), spikelets/spike, no. of seeds/spikelet and seed yield/plant (g) in seven randomly selected plants per accession per replicate ignoring those on peripheries. 100-seed weight (g) was recorded in triplicate in each genotype. Days to 50% spike emergence were counted as days from sowing to spike emergence in 50% of the plants per row while days to 50% maturity were counted as days from sowing to appearance of first sign of maturity in 50% of the plants. Spike length was measured from bottom to the top of the spike using a scale ignoring the terminal awn. Number of spikelets/ spike and number of seeds/spikelet were counted in each spike and averaged. Seed yield was measured by weighing the total seeds produced by a plant. All of the above mentioned parameters were recorded from minimum of seven plants in each replicate randomly chosen in each row and mean of quantitative data sets were used for analysis.

### *Statistical Analysis*

The data were subjected to analysis of variance and Least Significant Difference (LSD) computed at p = 0.05. Correlations between different traits as well as altitude of original collection sites were determined by Pearson's correlation. The quantitative traits were then analyzed by cluster and principal component analysis (PCA). PCA was carried out on the correlation matrix calculating the mean data of the accessions to investigate the importance of different characters in explaining multivariate polymorphism (Mallikarjuna Swamy *et al*., 2003) and then the accessions were plotted according to their first two principal component scores.

## Results and Discussion

Fourteen germplasm accessions of *Aegilops tauschii* subsp. *tauschii* collected from various areas of Kashmir were evaluated for various morphological traits. Variability was observed in plant height, spike length and spikelet size (Fig. 1). Mean values of data on various quantitative traits recorded in these 14 germplasm accessions of *Aegilops tauschii* has been presented in Table 2. Plant height among the accessions varied from 45.9-61.9 cm with an average value of 56.2 cm. Accession IC-0628095 was significantly shorter ($P< 0.05$) than all other accessions except for IC-0628101. Days to 50% flowering among the accessions varied from 175.7-182.7 days with accession IC-0628105 and IC-0628108 being significantly early ($P<0.05$) flowering compared to the late flowering accession IC-0628098. Days to 50% maturity ranged from 212.7-2 15.3 days with an average value of 213.7 days. The spike length ranged from 7.5-10.1 cm while the No. of spikelets/ spike ranged from 8.9-11 with a mean value of 10.2. Accession IC-0628098, a collection from place with higher altitude exhibited maximum spike length as well as highest number of spikelets/spike. Number of seeds per spikelet ranged from 2.1-2.3 with an average of 2.0 seeds/spikelet. Seed yield/plant among the accessions ranged from 2.095-3.818 g with a mean yield of 2.823 g. Maximum seed yield/plant was recorded in IC-0628097 followed by IC-0628096. 100-seed weight among the accessions ranged from 1.164-1.697 g with IC-0628103 recording maximum100-seed weight. Basic statistics of these traits revealed considerable variability. Hence they present the potential genetic resource for generating synthetic hexaploid wheat- a prerequisite for breeding common wheat with desired traits and conducting advanced genetic studies (Lee *et al*., 2020). High genetic variance was recorded for plant height. On the other hand medium genetic variance was recorded for days to 50% flowering while all other traits viz., spike length, spikelets/spike, days to 50% maturity, number of seeds/spikelet, seed yield/plant and 100-seed weight showed small genetic variance. LSD computed at 0.05 level of probability has revealed more significant differences in agro-morphological trait of plant height than other traits among various genotypes. In order to assess relationship between different traits simple correlation coefficients between different components has been computed and is presented in Table 3. Significant ($P<0.05$) and positive correlations of days to 50%

**Table 2. Agro-morphological characteristics of 14 germplasm accessions of *Aegilops tauschii* subsp. *tauschii***

| Accession No. | Plant height (cms) | Days to 50% flowering | Days to 50% maturity | Spike length (cms) | No. of spikelets/ spike | No. of seeds/ spikelet | Seed yield/ plant (g) | 100-Seed weight (g) |
|---|---|---|---|---|---|---|---|---|
| IC0628095 | 45.9 | 178.3 | 212.7 | 8.9 | 9.8 | 2.2 | 2.462 | 1.272 |
| IC0628096 | 55.5 | 178 | 212.7 | 8.7 | 10.4 | 2.1 | 3.483 | 1.445 |
| IC0628097 | 57.9 | 181 | 215.3 | 10 | 10.9 | 2.2 | 3.818 | 1.389 |
| IC0628098 | 59.1 | 182.7 | 214 | 10.1 | 11 | 2.1 | 2.706 | 1.231 |
| IC0628099 | 57.9 | 179 | 213.7 | 9.1 | 10.3 | 2.2 | 2.722 | 1.483 |
| IC0628100 | 59.3 | 178 | 213.3 | 9.3 | 10.9 | 2.3 | 3.001 | 1.186 |
| IC0628101 | 49.8 | 178.7 | 213 | 7.5 | 9.3 | 2.1 | 3.119 | 1.164 |
| IC0628102 | 60.5 | 179.3 | 214.7 | 9 | 10.3 | 2.2 | 2.238 | 1.235 |
| IC0628103 | 61.9 | 178.7 | 213 | 8.9 | 10 | 2.1 | 3.017 | 1.697 |
| IC0628104 | 61.5 | 177.7 | 213.7 | 8.9 | 10.1 | 2 | 3.003 | 1.260 |
| IC0628105 | 55.8 | 175.7 | 214.7 | 8.7 | 9.9 | 2.3 | 2.535 | 1.290 |
| IC0628106 | 53.5 | 177.3 | 213.3 | 8.7 | 10.5 | 2.1 | 2.095 | 1.167 |
| IC0628107 | 54.1 | 177.7 | 213.7 | 9.3 | 10.5 | 2.1 | 2.789 | 1.445 |
| IC0628108 | 54.9 | 176.3 | 214 | 8.3 | 8.9 | 2.1 | 2.541 | 1.389 |
| Mean | 56.2 | 178.4 | 213.7 | 8.9 | 10.2 | 2.1 | 2.823 | 1.332 |
| Minimum | 45.9 | 175.7 | 212.7 | 7.5 | 8.9 | 2.0 | 2.095 | 1.164 |
| Maximum | 61.9 | 182.7 | 215.3 | 10.1 | 11.0 | 2.3 | 3.818 | 1.697 |
| Variance | 20.3 | 3.1 | 0.6 | 0.4 | 0.3 | 0 | 0.215 | 0.022 |
| LSD (p=0.05) | 6.3 | 3.3 | 3.5 | 1.7 | 1.2 | 0.4 | 1.849 | 0.436 |


**Fig. 1. *Aegilops tauschii* subsp. *tauschii* a. Mature plants in an Oat field in Budgam district of Kashmir; b. Variability in the spike morphology among the accessions collected, characterized and conserved; c. Figure revealing the color and structure of mature spikes & spikelets and d. Relative size of different components of a mature spike**

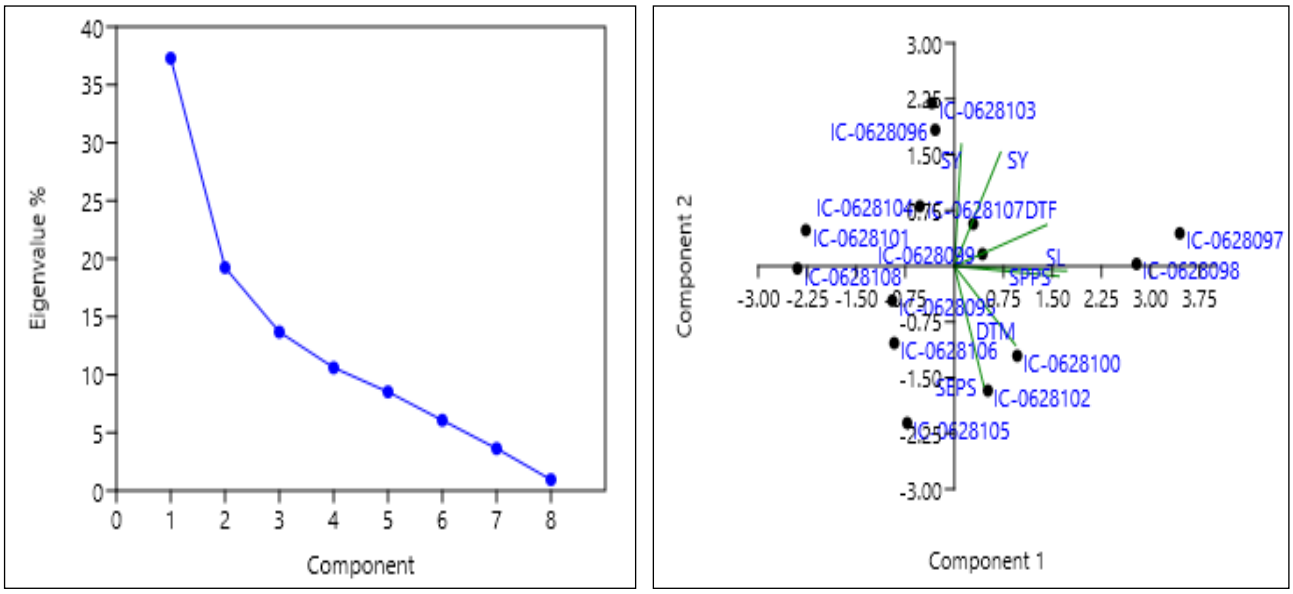


**Fig. 2. Scree plot and biplot of 14 *Aegilops tauschii* subsp. *tauschii* accessions. The spread of accessions in the plot show agro-morphological diversity**

**Table 3. Correlations between different agro-morphological traits and altitude of original collection site in 14 germplasm accessions of *Aegilops tauschii* subsp. *tauschii***

| Trait | PH | DTF | DTM | SL | NSPPS | NSEPS | SY | SW | ALT |
|---|---|---|---|---|---|---|---|---|---|
| PH | 1.000 | 0.252 | 0.400 | 0.449 | 0.419 | -0.058 | 0.189 | 0.301 | 0.114 |
| DTF | 0.252 | 1.000 | 0.204 | 0.634* | 0.568* | -0.096 | 0.319 | -0.017 | 0.528 |
| DTM | 0.400 | 0.204 | 1.000 | 0.446 | 0.220 | 0.320 | 0.037 | -0.083 | 0.246 |
| SL | 0.449 | 0.634* | 0.446 | 1.000 | 0.828** | 0.195 | 0.195 | 0.133 | 0.546* |
| NSPPS | 0.419 | 0.568* | 0.219 | 0.828** | 1.000 | 0.210 | 0.231 | -0.071 | 0.417 |
| NSEPS | -0.058 | -0.096 | 0.320 | 0.195 | 0.210 | 1.000 | -0.088 | -0.153 | 0.109 |
| SY | 0.189 | 0.319 | 0.037 | 0.195 | 0.231 | -0.088 | 1.000 | 0.331 | 0.126 |
| SW | 0.301 | -0.017 | -0.083 | 0.133 | -0.071 | -0.153 | 0.331 | 1.000 | 0.008 |
| ALT | 0.114 | 0.528 | 0.246 | 0.546* | 0.417 | 0.109 | 0.126 | 0.008 | 1.000 |

PH=Plant height, DTF=Days to 50% flowering, DTM=Days to 50% maturity, SL=Spike length, NSPPS=No. of spikelets/spike, NSEPS=No. of seeds/spike, SY=Seed yield/plant, SW=100-Seed weight, ALT=Altitude of original collection site

*Correlation significant at the 0.05 level (2-tailed), **Correlation significant at the 0.01 level (2-tailed)

**Table 4. Eigenvectors and percent explained variation by the four major principal components in 14 germplasm accessions of *Aegilops tauschii* subsp. *tauschii***

| Agro-morphological characters | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|
| Plant height | 0.371 | 0.179 | 0.413 | –0.449 |
| Days to 50% flowering | 0.419 | 0.089 | –0.471 | –0.049 |
| Days to 50% maturity | 0.308 | –0.326 | 0.458 | –0.186 |
| Spike length | 0.530 | –0.082 | –0.071 | –0.007 |
| No. of spikelets/spike | 0.488 | –0.116 | –0.262 | 0.071 |
| No. of seeds/spikelet | 0.106 | –0.542 | 0.321 | 0.620 |
| Seed yield/plant | 0.228 | 0.455 | –0.030 | 0.582 |
| 100-Seed weight | 0.088 | 0.575 | 0.468 | 0.179 |
| Eigen value | 2.676 | 1.508 | 1.006 | 0.700 |
| Percent variance | 38.29 | 21.54 | 14.37 | 10.01 |
| Cumulative percent variance | 38.29 | 59.83 | 74.20 | 84.21 |

flowering were observed with spike length and No. of spikelets/spike. Moreover, spike length revealed a highly significant (P< 0.01) positive association with no. of spikelets/ spike. Interestingly, a significant (P< 0.05) positive association was observed between spike length and altitude of original collection sites of the accessions.

Multivariate statistical analysis of the agro-morphological data obtained on 14 *Aegilops tauschii* subsp. *tauschii* germplasm accessions during the course of present investigation has been done for deeper understanding of relationship between the traits. Evaluation of phenotypic variability by multivariate analysis helps in identifying the most suitable resources for special traits (Goel *et al*., 2015). Therefore, extent of genetic diversity in *Aegilops tauschii* germplasm has been measured by principal component analysis (PCA) and cluster analysis for their effective evaluation. Results from the principal component analysis showed that four components of PC1, PC2, PC3 and PC4 accounted for more than 80% of the total variation in agro-morphological traits studied contributing 38.29%, 21.54%, 14.37% and 10.01% of the total variation respectively (Table 4). Eigen values given in the table show that relative discriminating power of the principal components was high for PC1 (2.676) followed by PC2 (1.508), PC3 (1.006) and PC4 (0.700). The most effective traits in the first component were spike length and no. of spikelets/spike (Table 4). Major effective traits in the second component were no. of seeds/spikelet, seed yield/ plant and 100-seed weight. In the third component days to 50% flowering, days to 50% maturity and 100-seed weight were the main effective traits while in fourth component main effective traits were no. of seeds/spikelet and seed yield/plant. The first PC thus, was more related to vegetative growth while second, third and fourth PCs were related to both yield and yield contributing traits. Traits loading more on first two principal components and having Eigen values greater than 1 and contributing nearly 60% variance were used to cluster 14 genotypes into closely related groups. The number of components was also determined with the help of the highest slope in Scree plot (Fig. 2). Scree plot explains the percentage of variance associated with each principal component. Actually, according to Chahal and Gosal (2002), characters with largest absolute values closer to unity within the first component influence the clustering more than those with lower absolute values closer to zero. Clustering was done through Ward's method. The critical examination of the dendrogram generated after the cluster analysis and PCA, revealed three clusters (Fig. 3). Cluster I included two accessions with spike length of 7.5 and 8.3 cms, cluster II included five accessions with spike length of 9.1-10.1 cms while cluster III included remaining 7 genotypes with spike length of 8.7-9.0 cms. Clearly spike length appears to be the most effective trait in differentiating clusters. Interestingly, we have also found positive association between spike length and altitude of the original collection site. Knaggs *et al*. (2000) studied the plant morphology of 54 accessions of *Aegilops tauschii* and identified subspecies of *strangulata* and *tauschii* from each other; however, the first two principal components could not distinguish accessions according to their country of origin. In similar study on 55 accessions of *Aegilops tauschii*, the first two principal components suggested that evaluated characters were useful for distinguishing the two subspecies of *Aegilops tauschii*, the accessions of subsp. *strangulata* showed the larger dispersion than the accessions of subsp. *tauschii* (Naghavi and Amirian, 2005). In yet another study on different *Aegilop*s species, principal component analysis identified three components that explained 79.95% of

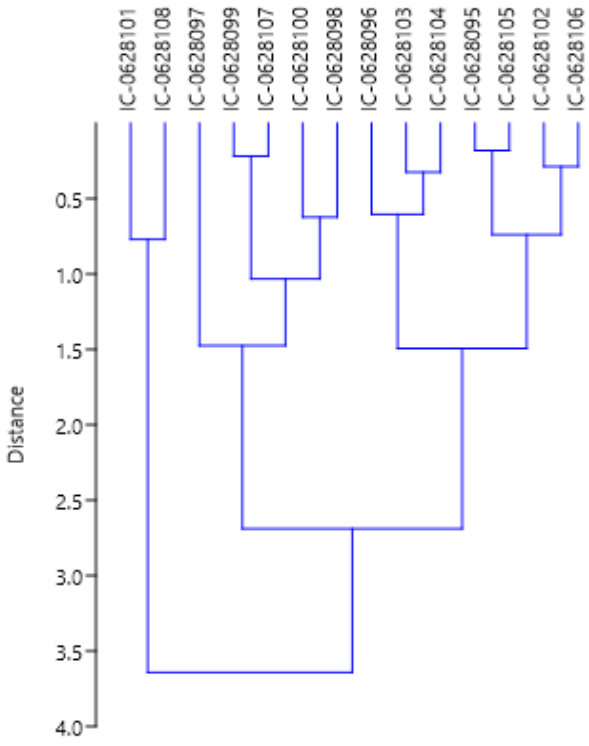


**Fig. 3. Dendrogram of 14 *Aegilops tauschii* subsp. *tauschii* accessions based on agro-morphological characters using hierarchical cluster analysis (Ward's method)**

the total variation of measured traits and dendrogram obtained from cluster analysis revealed that phenological and agro-morphological traits could distinguish different species from each other (Pour-Aboughadareh *et al*., 2018). Interestingly, in our study with 14 accessions of *Aegilops tauschii*, the 7 accessions in cluster III possessed features typical of *Aegilops tauschii* subsp. *tauschii* while accessions in Cluster I and II look closer to *Aegilops tauschii* subsp. *strangulata*, although the accessions were originally collected randomly and bulked. Based on these observations, we argue that spike length and related morphological traits could be very important in discriminating different forms of *Aegilops tauschii*. We believe that the basic information generated and provided here may be helpful in proper utilization as well as for future collection and genetic resource management of this very important CWR genetic resource of wheat for enhancing climate resilience in later case by conferring traits of drought tolerance and of disease and pest resistance.

## Conclusion

*Aegilops tauschii* is an important crop wild relative of bread wheat (*Triticum aestivum*) believed to be its progenitor. Its gene pool is currently considered most important for wheat improvement as potential gene donor for drought, disease and pest resistance. 14 germplasm accessions were collected from diverse locations and conserved in National Gene Bank at ICAR-NBPGR New Delhi. Significant positive association of days to flowering was observed with spike length and number of spikelets/spike. Interestingly, spike length revealed a significant positive association with altitude of place from where accessions were originally collected. Cluster analysis grouped the accessions into three distinct groups mainly on the basis of spike length. The study provides basic information about this valuable CWR and may help in its further evaluation and proper utilization.

## Acknowledgement

The authors are highly thankful to the Director, ICAR–INBPGR, New Delhi for guidance and help.

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