



Taxonomic revision and anatomical studies of the genus *Aegilops* L. (*Poaceae*) with sectional confirmation

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ABSTRACT:

Aegilops L. is a large genus of the tribe *Triticeae*, allied to the family *Poaceae*. It spreads in almost all temperate regions, mainly in the Mediterranean basin, Southwest and Central Asia. The goal of this study was to use morphometric and anatomical methodologies to reappraise the taxonomy of the genus *Aegilops* in Egypt. Clustering was applied to determine the relationship between seven studied species based on sectional delimitation. Results generated from principal component analysis (PCA) and correlation revealed the significance of morphometric and anatomical descriptors traits in the characterization and identification of *Aegilops* species. Heatmap analyses of combining morphological and anatomical data confirmed the sectional classification of the seven species.

KEYWORDS: *Aegilops* species, morphology, Anatomy, Wild wheat, R-software.

1. INTRODUCTION:

Aegilops L. is a large genus of the tribe *Triticeae*, allied to the family *Poaceae*. It spreads in almost all temperate regions, mainly in the Mediterranean basin, Southwest and Central Asia (Van Slageren, 1994). The genus *Aegilops* represents the most significant part of wheat's secondary gene pool, increasing the potential of new variations (Kole, 2011). *Aegilops* is

discriminated from other grass genera by culm usually kneeled at the base, glumes unawned with six or more veins of unequal width, which is at least uppermost fertile spikelet with one or more teeth or awns, and it is also oblong, broadly oblong, obovate, obovate-oblong, obovate-elliptic or subventricose (Davis, 1985; El-Gadi, 1988).

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Conferring to the latest revision of *Aegilops* L. taxonomy, (van Slageren system), *Aegilops* comprises of 23 species (Van Slageren, 1994) . According to the phenotype and genomic analysis, it was allocated into five sections: (*Aegilops* L., *Comopyrum* (Jaub. & Spach) Zhuk., *Cylindropyrum* (Jaub. & Spach) Zhuk., *Sitopsis* (Jaub. & Spach) Zhuk., and *Vertebrata* Zhuk. emend Kihara) encloses various Ploidy Levels. The last treatment of *Aegilops* in Egypt was done by Ibrahim et al. (2016) , who recognized six species of *Aegilops* in Egypt. Whereas Boulos (2009) revealed the presence of seven taxa and Täckholm (1974) reported the existence of seven species and three varieties. Modern geometric morphometric methods (GMMs) increase scientific description accuracy for the essential traits of the phenotypic measurement at biodiversity and proper for differentiating complex taxa. In contrast, classical taxonomy requires handling and admission to specimen's important characteristics of the phenotypic

measurement at biodiversity and appropriate for determining problematic taxa (Ellmouni, 2019; Viscosi & Cardini 2011). Anatomical evidence has been used in plant systematics and is considered to be consistent and reliable for solving taxonomic difficulties among plants (EL-Banhawy et al., 2021). Therefore, the goal of this study was to use morphometric and anatomical methodologies to reappraise the taxonomy of the genus *Aegilops* in Egypt with sectional confirmation. **2. Materials and Methods**

2.1. Plant Materials

Seven species from *Aegilops* distributed in Egypt are *Ae. bicornis* (Forssk.) Jaub. & Spach, *Ae. crassa* Boiss., *Ae. geniculata* Roth, *Ae. kotschy* Boiss., *Ae. longissima* Schweinf. & Muschl., *Ae. peregrina* (Hack. in Fraser) Maire & Weiller, and *Ae. ventricosa* Tausch. Representative specimens were collected from their natural habitats and herbaria subjected to morphological description and anatomical studies (Table 1).

Table 1. Voucher information for taxa used to the morphological, anatomical, Specimens represent seven taxa of genus *Aegilops*.

Latin name	Voucher	Locality	Date
<i>Ae. geniculata</i>	K. Ali, s.n. (FAY)	Alex-Matruh road, Garawlah next to Railroad.	9/4/2021
<i>Ae. kotschy</i>	K. Ali, s.n. (FAY)	Marsa Matrouh, Wadi El- Garawlah and Ras El-hekma.	9/4/2021
	K. Ali, s.n. (FAY)	Mma., Alexandria, Mariut, ' Amria at Mariut Lake, Dab'a, Burg el ' Arab.	27/3/2019
<i>Ae. peregrina</i>	El-khanagry, No. 143 (CAIM)	Experiment Station and the Agricultural Research, Faculty of Agriculture, Cairo	23/5/2002
	Täckholm & El-Hadidi et al., s.n. (CAI)	Marsa Matrouh, wadi Umm El-rakhm between Matrouh and Agiba.	21/3/1975
	Täckholm & El-Hadidi et al., s.n. (CAI)	Wadi Habis, (300 km west of Alexandria).	21/3/1975
<i>Ae. bicornis</i>	K. Ali, s.n (FAY)	Mma. Rosseta next to abo mandour.	28/3/2019
<i>Ae. longissima</i>	N. D. Simpson, NO. 36 (CAI)	Mma. Mariut e.g Abd el Qadir, Burg el' Arab.	22/4/1927
<i>Ae. crassa</i>	I. Abd Elrafee, s.n. (FAY).	South Sinai, Saint Catherine, wadi gebal.	14/6/2020
<i>Ae. ventricosa</i>	V. Täckholm, s.n. (CAI).	El-Amria, Mariut	11/3/1965
	V. Täckholm, s.n. (CAI).	along the inner road, Amria	23/3/1981

The identification of the collected specimen's comparisons and confirmed with the specimens kept in the Egyptian herbaria of Cairo University (CAI), Flora of phytotaxonomy research of Agriculture Research Center (CAIM); the abbreviations of "Index herbarium" ed. 8 (Holmgren et al., 1990).

2.2. Morphological and Anatomical Analyses

For morphological description, the specimens were examined by a binocular head zoom stereo microscope, model number MS003A unit. Growth parameters of all studied *Aegilops* taxa were recorded based on ten plants for two replicates per taxon. For anatomical analyses, Cross-sections (20-25 μ thick) were obtained by cutting μ in culms at the second internodes near the roots. Sectioning for anatomical investigations was performed according to Johansen (1940). The observation was based on LM at magnification ranging from x40 to x100. Anatomical parameters of all tested *Aegilops* taxa were recorded in three replicates per taxon. All the quantitative characteristics were measured using image analysis software (Schneider et al., 2012).

2.3. Statistical Analysis

All quantitative data were analyzed using R-software with the necessary packages installed (R_Core_Team 2018). Cluster analysis processed with the package "pvclust" (Suzuki and Shimodaira 2013). The principal component analysis (PCA), were designed by "factoextra" and

"FactoMineR" packages (Kassambara and Mundt, 2017). The "corrplot" package was used to depict the correlation between descriptors (Soetewey, 2020). The "pheatmap" and "ggplot2" packages were used to visualize the dissimilarity and similarity among species (Kassambara 2020). Boxplots were produced via the "ggplot2" library (Wickham 2016). Analysis of variance (ANOVA) was accomplished, and it is followed by a Post Hoc Tukey Honestly Significant Difference (HSD) test.

3. RESULTS AND DISCUSSION:

3.1. Morphological Studies

All species are characterized by spike length less than 10 cm except *Ae. longissima* more than 10 cm tall, length of leaf sheath less than 10 cm tall except *Ae. bicornis* more than 10 cm, leaf blade length less than 10 cm except *Ae. ventricosa* more than 10 cm, spikelets number less or equal 10 but more than 10 in *Ae. longissima*, caryopsis length very small in *Ae. pergerina* & *Ae. ventricosa*, leaf blade width less than 5 mm in all species except *Ae. crassa* & *Ae. ventricosa*. number of glume's awns zero in *Ae. bicornis*, *Ae. longissima*, *Ae. crassa* & *Ae. ventricosa* and number of lemma's awns zero in *Ae. pergerina*, *Ae. longissima* & *Ae. crassa*. Morphological studies of investigated species are based on the most discriminative quantitative morphological characters summarized in Table 2.

Table 2. Quantitative traits used for Morphological analysis for seven *Aegilops* species under investigation with descriptive data, Min (Mean \pm S.D.) Max.

Morphological descriptors	Abbreviation	Min (Mean \pm S.D.) Max.
Length of culm / cm	Culum_L	9.6(19.54 \pm 6.4)29
Length of leaf sheath /cm	Leaf sheath_L.	1(5.76 \pm 4.22)12.6
Length of leaf blade /cm	Leaf blade_L	2(4.63 \pm 2.84)10.8
Width of Leaf blade/cm	Leaf blade_W.	2(3.14 \pm 1.35)5
length of the ligules/mm	Ligules_L.	1(1.43 \pm 0.79)3
length of auricles	Auricles_L.	1(1.43 \pm 0.53)2
Length of the spike/cm	Spike_L.	2.9(7.91 \pm 5.27)18.7
Length of spike including axns (cm)	Spike&Awn_L.	0(7.39 \pm 6.69)18.7
Number of nodes in spike central axis	Spike_Node_No.	3(7.29 \pm 3.73)13
Number of the spikelets on each node	Spikelets_No.	1(1 \pm 0)1
Number of spikelets	Spikelets/spike_No.	5(7.86 \pm 2.41)11
Length of spikelet /mm	Spikelets&Awn_L.	0(7.39 \pm 6.69)18.7
Width of the spikelets/mm	Spikelets_W.	2(2.71 \pm 0.49)3
Length of spikelet (excluding/ awn) / mm	Spikelets_L	0.8(6.43 \pm 3.18)9
Number of ribs in glume	Glume ribs_No.	5(7 \pm 1)8
Length of glume/mm	Glume_L.	5(6.71 \pm 1.25)9
Width of the glume / mm	Glume_W.	1(2.79 \pm 0.99)4
Number of florets/spikelets	Florets_No.	2(2.43 \pm 0.53)3
Number of lemmas ribs	lemma ribs_No.	3(4.43 \pm 1.81)8
Length of lemma /mm	lemma_L.	6(7.57 \pm 1.27)9
Width of lemma/mm	lemma_W.	1(2.14 \pm 1.03)3.5
Number of lemma awns	Awn_No.	0(0.86 \pm 0.9)2
Length of lemma awn / cm	Awn_L.	0.7(2.21 \pm 1.94)5.6
Length of palea/mm	Palea_L.	4(6.71 \pm 1.38)8
Width of Palea/mm	Palea_W.	1(1.57 \pm 0.79)3
Length of glume awn/mm	Anther_L.	1(2.71 \pm 0.76)3
Length of caryopsis/ mm	Caryopsis_L.	1(3.14 \pm 2.41)6

All examined species are characterized by glabrous leaf sheath and curved Rachillae except *Ae. longissima* showed hairy at margin and zigzag (flexuous) rachillae; ligule margin lacerated in all species except for *Ae. pergerina* is ciliate and *Ae. crassa* entire. awnless Glume and lemma in *Ae. Crassa* & *Ae. longissima* where the uppermost spikelet with 2 long awns. Whereas, awned glume and lemma in *Ae. kotschyi*, and *Ae. geniculata*. awned glume

and Awnless lemma in *Ae. pergerina*. awnless glume and awned lemma in *Ae. bicornis* & *Ae. ventricosa*. Finally, caryopsis shape appears elliptic-oblong at *Ae. kotschyi*, *Ae. bicornis*, *Ae. longissima*, *Ae. crassa*, obovate at *Ae. pergerina*, *Ae. ventricosa* while ovate *Ae. geniculata*. Morphological studies of investigated species are based on the most discriminative qualitative morphological characters summarized in **Table 3**.

Table 3. Qualitative traits used for morphological characters for seven *Aegilops* species under investigation according to section

Species	Section: <i>Aegilops</i>			Section: <i>Sitopsis</i>		Section: <i>Vertebrata</i>	
	<i>Ae. geniculata</i>	<i>Ae. kotschyi</i>	<i>Ae. pergerina</i>	<i>Ae. bicornis</i>	<i>Ae. longissima</i>	<i>Ae. crassa</i>	<i>Ae. ventricosa</i>
1-Surface of the leaf sheath	Glabrous with Hairy margin	Glabrous with ciliated margin	glabrous	glabrous	Glabrous with Hairy margin	glabrous	glabrous
2-Surface of the leaf blade	glabrous	Glabrous & sparsely hairy ciliate margin	Pubescent with hairy margin	sparsely hairy ciliate margin	Pubescent on both sides	Glabrous	Glabrous with Hairy margin
3-Ligules Shape	truncate	truncate	truncate	truncate	truncate	truncate	truncate
4-Ligule apex	lacerate	lacerate	ciliate	lacerate	lacerate	entire	lacerate
5-Rachillae	Slightly Curved or bent	Slightly Curved or bent	Slightly Curved or bent	Slightly Curved or bent	flexuous	Slightly Curved or bent	Slightly Curved or bent
6-Rachis Shape	ovate	wedged	Oblong-ovate	wedged	Oblong-ovate	ovate	Oblong-ovate
7-Spike shape	Wide erect, ovate, bilateral	lanceolate, bilateral	lanceolate or ovate	linear, bilateral	Linear	± Cylindrical	± Cylindrical
8-Spikelet Shape	Ovate laterally compressed	Elliptic-Oblong laterally compressed	Oblong – ovate	Elliptic laterally compressed	Oblong laterally compressed	Oblong laterally compressed	Ovate swollen at base
9-Glume tip	2-3awned	3-awned	3-awned	2-toothed	2-toothed	3-toothed	1-toothed
10-lemma Tip	2-awned	3-awned	3-toothed	1-awned	awnless	2-toothed	1or2awned
11-Caryopsis Shape	ovate	Elliptic-oblong	obovate	Elliptic-oblong	Elliptic-oblong	Elliptic-oblong	obovate

3.2. Anatomical Studies

Culms internode of the seven studied *Aegilops* species in T.S. has a sub-epidermal layer of the supporting tissue, interrupted by the outermost vascular bundle's circle. Fiber strands were observed between the smaller bundles and the epidermis, with alternating strands of chlorenchyma. Vascular bundles are enclosed by bundle sheath. Ground tissue is broken at the middle of the culm forming a central cavity. The transverse sections from the basal internodes appear rounded with a central cavity. Fine ribs are facing sclerenchyma associated with

the outermost small vascular bundles (Figure 1).

The shape of the section outline of stem appears as rounded or rounded with slight ribs in all species whereas undulating with deep ribs at *Ae. longissima*. Chlorenchyma tissue is present in scattered shape only at *Ae. longissima*, *Ae. kotschyi* & *Ae. geniculata*. The supporting tissue ring seems to be completed at *Ae. bicornis* and Undulated with shallow ribs at most species. Vascular bundle arranged in two circles at *Ae. longissima* & *Ae. geniculata*, while three-circles at the other species **Table 4**.

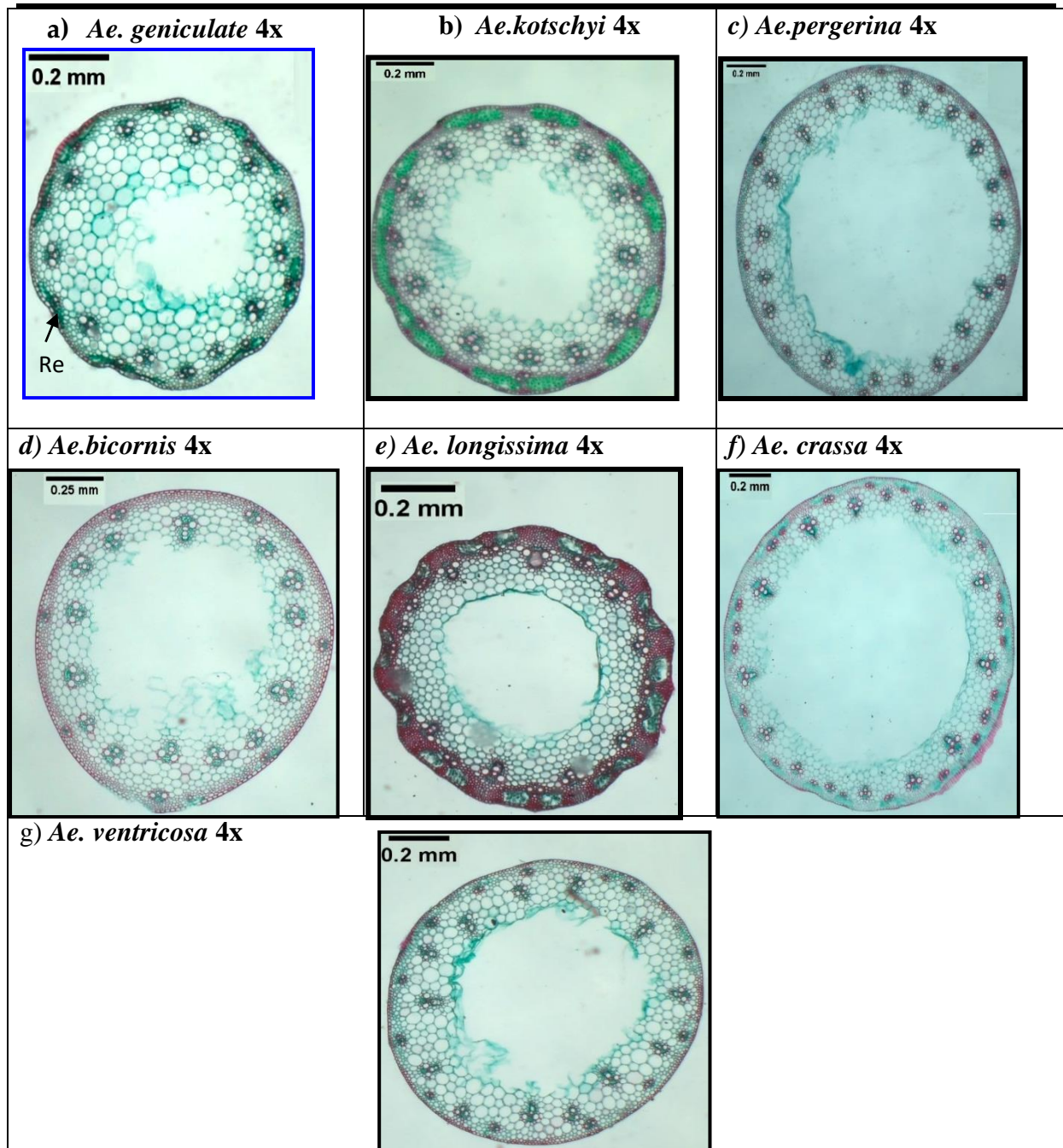


Fig. 1. Light microscopy of internodes cross sections of *Aegilops Sp.*

Table 4. Qualitative traits used for anatomical characters for seven *Aegilops* species under investigation according to section

Characters	Section: <i>Aegilops</i>		Section: <i>Sitopsis</i>			Section: <i>Vertebrata</i>	
	<i>Ae. geniculata</i>	<i>Ae. kotschy</i>	<i>Ae. pergerina</i>	<i>Ae. bicornis</i>	<i>Ae. longissima</i>	<i>Ae. crassa</i>	<i>Ae. ventricosa</i>
1. Shape of the section outline	Rounded with slight ribs	Rounded with slight ribs	rounded	rounded	Undulating with deep ribs	rounded	rounded
2. Shape of the supporting tissue ring	Undulated with shallow ribs	Patches of S alternating with Chlorenchyma	Undulated with shallow ribs	Completed ring	Undulated with shallow ribs	Undulated with shallow ribs	Undulated with shallow ribs
3. Type of the supporting tissue	sclerenchyma	Sclerenchyma	Sclerenchyma	Sclerenchyma	Sclerenchyma	Sclerenchyma	Sclerenchyma
4. Arrangement of Vascular bundle	In 2- circles	In 3-circles	In 3-circles	In 3-circles	In 2-circles	In 3-circles	In 3-circles
5. Ground tissue	parenchymatous	parenchymatous	parenchymatous	parenchymatous	parenchymatous	parenchymatous	parenchymatous
6. Assimilatory parenchyma	Scattered	scattered	absent	absent	scattered	absent	absent
7. Type of bundle sheath	sclerenchyma-tous	Sclerenchyma-tous	Sclerenchyma-tous	Sclerenchyma-tous	Sclerenchyma-tous	Sclerenchyma-tous	Sclerenchyma-tous

The maximum diameter for stem diameter was recorded in *Ae. pergerina*, while the minimum was recorded in *Ae. geniculata*; the maximum distance from pith to the vascular bundle is recorded in *Ae. longissima*, but the minimum in *Ae. ventricosa*. The vascular bundle with maximum width was recorded in *Ae. bicornis*, however, the Minimum

in *Ae. ventricosa*; only two circles of V.B. in *Ae. geniculata* & *Ae. longissima*. Chlorenchyma thickness absence in *Ae. bicornis*, *Ae. pergerina*, *Ae. crassa* & *Ae. ventricosa*. Sclerenchyma Thickness against V.B. and also under epidermis absence in *Ae. geniculata*. **Table 5.**

Table 5. Quantitative traits used for anatomical analysis for seven *Aegilops* species under investigation with descriptive data, Min (Mean ± S.D.) Max.

Anatomical descriptors	Abbreviation	Min (Mean ± S.D.)	Max.
Thickness of stem except the pith	Stem_Di.1	0.9(1.26±0.31)	1.7
Number of parenchyma rows between the two vascular bundles	Stem_Di.2	0.44(0.5±0.07)	0.63
Number of parenchyma cells from the pith until the nearest vascular bundle	Parenchyma_Row_No.1	2(2.71±0.49)	3
Distance between the pith and the nearest vascular bundle	Parenchyma_Row_No.2	1(1.43±0.53)	2
Distance between the two vascular bundles	Distance pith_VB	0.02(0.04±0.02)	0.07
Thickness of phloem tissue of large vascular bundle	Distance_VB	0.17(0.23±0.04)	0.3
Diameter of xylem vessels of large vascular bundle	Phloem_Th.	0.02(0.02±0)	0.03
Thickness of xylem tissue of large vascular bundle	Xylem vessels_Di.	0.06(0.07±0.01)	0.08
Number of xylem vessels in large vascular bundle	Xylem tissue_Th.	0.04(0.06±0.01)	0.07
Maximum diameter of stem	Xylem vessels_No.	3(3±0)	3
Width of large vascular bundle	Vascular bundle_W.	0.05(0.08±0.01)	0.1
Thickness of large vascular bundle	Vascular bundle_Th.	0.06(0.09±0.02)	0.13
Number of vascular bundle rings	Vascular bundle_R_No.	2(2.71±0.49)	3
Number of parenchyma rows from sclerenchyma or collenchyma until pith	Parenchyma_Row_No.3	5(5.71±0.95)	7
Thickness of chlorenchymatous tissue	Chlorenchyma_Th.	0(0.02±0.03)	0.06
Width of chlorenchymatous tissue	Chlorenchyma_W.	0(0.06±0.12)	0.32
Thickness of sclerenchyma against the vascular bundle	Sclerenchyma_Th.1	0(0.04±0.02)	0.06
Thickness of sclerenchyma under epidermis	Sclerenchyma_Th.2	0(0.03±0.02)	0.06
Thickness of Collenchyma.	Collenchyma_th	0(0.011±0.01415)	0.032
Number of parenchyma rows	Parenchyma.Row	0(0.286±0.756)	2.000

3.3. Traits analysis

In the cluster analysis for morphological and anatomical traits for the seven *Aegilops* sp. has been separated into two clusters at an approximately unbiased (AU) P value of 99 (Figure 2). The morphological and

anatomical clusters assembled *Ae. bicornis* and *Ae. longissima* in the same cluster and *Ae. pergerina*, *Ae. geniculata* and *Ae. crassa* in the other clusters, only *Ae. ventricosa* and *Ae. kotschy* swapped between clusters.



Fig. 1. Agglomerative hierarchical cluster for *Aegilops* sp. based on quantitative character (a) morphological (b) anatomical traits, with pictures represent the difference in spike and stem sections.

The first five components with Eigenvalues higher than one described 94 percent of the overall variance in morphological (Table 6) and anatomical (Table 7) quantitative characteristics, according to principal component analysis (PCA). The first dimension (Dim.1) of the quantitative morphological traits PCA described 33.56% of the total variation and was linked to leaf-sheath, spike, spike & awn, lengths, number of nodes of spike central axis, and number of spikelets /spike.

The second dimension (Dim.2) was linked to leaf blade width and lemma length and explained 21.95 % of the total variation. The third component (Dim.3) accounted for 19.97% of the total variance and was mainly related to awn length and caryopsis length; the fourth component (Dim.4) accounted for 12.85% of the total variation and was particularly associated with anther length (Table 6).

Table 6. Principle component loading of 27 quantitative morphological traits

Morphological traits	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
Culum_L	4.43E ⁻⁰¹	-2.26E ⁻⁰¹	-7.90E ⁻⁰¹	3.43E ⁻⁰¹	7.35E⁻⁰²
Leaf sheath_L.	8.03E⁻⁰¹	-3.70E ⁻⁰¹	2.61E ⁻⁰¹	3.66E ⁻⁰¹	-5.65E⁻⁰²
Leaf blade _L	4.02E ⁻⁰¹	4.06E ⁻⁰¹	-2.96E ⁻⁰¹	7.46E⁻⁰¹	1.32E ⁻⁰¹
Leaf blade_W.	-3.15E ⁻⁰²	8.15E⁻⁰¹	5.36E ⁻⁰²	4.80E ⁻⁰¹	3.20E ⁻⁰¹
Ligules_L.	6.21E ⁻⁰¹	4.61E ⁻⁰²	-2.50E ⁻⁰¹	-6.62E ⁻⁰¹	2.86E ⁻⁰¹
Auricles_L.	1.55E ⁻⁰¹	3.41E ⁻⁰¹	-3.90E ⁻⁰²	-6.75E ⁻⁰¹	5.65E ⁻⁰¹
Spike_L.	8.03E⁻⁰¹	4.35E ⁻⁰¹	-9.50E ⁻⁰²	-3.13E ⁻⁰¹	1.94E ⁻⁰¹
Spike&Awn_L.	8.68E⁻⁰¹	4.36E ⁻⁰¹	2.14E ⁻⁰¹	7.54E⁻⁰³	-3.41E ⁻⁰²
Spike_Node_No.	9.07E⁻⁰¹	1.72E ⁻⁰¹	2.42E ⁻⁰¹	1.84E ⁻⁰¹	1.48E ⁻⁰¹
Spikelets_No.	-3.69E ⁻¹⁶	2.65E ⁻¹⁶	-5.06E ⁻¹⁶	4.06E ⁻⁰¹⁶	-3.20E ⁻¹⁶
Spikelets/spike_No.	9.83E⁻⁰¹	3.50E ⁻⁰²	3.45E ⁻⁰²	1.52E ⁻⁰¹	-4.65E ⁻⁰²
Spikelets&Awn _L.	3.36E ⁻⁰¹	-5.82E ⁻⁰¹	6.24E⁻⁰¹	2.27E ⁻⁰¹	-2.23E ⁻⁰¹
Spikelets_W.	-7.92E ⁻⁰¹	2.72E ⁻⁰¹	-4.96E ⁻⁰¹	1.97E ⁻⁰¹	3.20E ⁻⁰²
Spikelets_L	3.53E ⁻⁰¹	6.73E⁻⁰¹	4.75E ⁻⁰¹	7.54E⁻⁰²	-4.34E ⁻⁰¹
Glume nerves_No.	-3.29E ⁻⁰¹	4.68E ⁻⁰¹	-6.25E ⁻⁰¹	1.69E ⁻⁰¹	2.04E ⁻⁰¹
Glume_L.	-9.59E ⁻⁰²	5.96E ⁻⁰¹	-6.50E ⁻⁰¹	3.95E ⁻⁰¹	-8.26E ⁻⁰²
Glume_W.	-8.73E ⁻⁰¹	3.05E ⁻⁰³	-3.00E ⁻⁰¹	2.26E ⁻⁰¹	-3.03E ⁻⁰¹
Florets_No.	-2.02E ⁻⁰²	6.43E⁻⁰¹	2.79E ⁻⁰¹	-6.26E ⁻⁰¹	-3.29E ⁻⁰¹
lemma nerves_No.	-3.70E ⁻⁰¹	-6.27E ⁻⁰¹	-5.21E ⁻⁰¹	-3.37E ⁻⁰¹	1.22E ⁻⁰¹
lemma_L.	-2.12E ⁻⁰¹	8.66E⁻⁰¹	8.36E⁻⁰²	-1.21E ⁻⁰¹	-3.98E ⁻⁰¹
lemma_W.	-9.47E ⁻⁰¹	-3.57E ⁻⁰¹	1.83E ⁻⁰¹	-1.61E ⁻⁰¹	-2.22E ⁻⁰²
Awn_No.	-4.73E ⁻⁰¹	1.72E ⁻⁰¹	4.98E ⁻⁰¹	5.28E ⁻⁰¹	4.33E ⁻⁰¹
Awn_L.	-5.05E ⁻⁰²	-2.02E ⁻⁰¹	8.94E⁻⁰¹	1.67E ⁻⁰¹	1.26E ⁻⁰¹
Palea_L.	2.78E ⁻⁰¹	6.84E⁻⁰¹	-1.67E ⁻⁰¹	-5.81E ⁻⁰²	-1.52E ⁻⁰¹
Palea_W.	-7.62E ⁻⁰¹	4.75E ⁻⁰¹	4.20E ⁻⁰¹	-1.22E ⁻⁰¹	2.58E ⁻⁰²
Anther_L.	3.81E ⁻⁰¹	-6.24E ⁻⁰¹	-4.94E ⁻⁰¹	9.15E⁻⁰²	-3.13E ⁻⁰¹
Caryopsis_L.	-5.25E ⁻⁰¹	-1.13E ⁻⁰¹	7.63E⁻⁰¹	2.11E ⁻⁰¹	2.59E ⁻⁰¹
Standard deviation	8.727124	5.708647	5.193378	3.343579	1.645011
Proportion of Variance	33.56586	21.95633	19.97453	12.85992	6.326963
Cumulative Proportion	33.56586	55.52219	75.49672	88.35664	94.68361

component (Dim.2) which described 24.49% of the total variation. The third component (Dim.3) explained 16.38% of the total variation being mainly linked with “Thickness of sclerenchyma against the vascular bundle”; and the fourth component (Dim.4) accounted for 11.53% of the total variation and was correlated “Thickness of sclerenchyma under epidermis” (Table 7).

In the anatomical quantitative traits PCA, the first principal component (Dim.1) represented 30.17% of the total variation, and covered the most of such as “Number of parenchyma cells from the pith until the nearest vascular bundle” and “Distance between the pith and the nearest vascular bundle” while “Thickness of phloem tissue of large vascular bundle” and “Maximum diameter of stem” were related to the second

Table 7. Principle component loading of 18 quantitative anatomical traits

Anatomical traits	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
Stem_Di.1	3.02E ⁻⁰³	8.34E⁻⁰¹	3.45E ⁻⁰¹	4.20E ⁻⁰¹	-7.65E ⁻⁰²
Stem_Di.2	5.34E ⁻⁰¹	3.18E ⁻⁰¹	-3.57E ⁻⁰¹	4.14E ⁻⁰¹	-2.48E ⁻⁰¹
Parenchyma_Row_No.1	6.56E⁻⁰¹	1.19E ⁻⁰¹	2.20E ⁻⁰¹	-5.51E ⁻⁰¹	4.47E ⁻⁰¹
Parenchyma_Row_No.2	9.16E⁻⁰¹	-3.38E ⁻⁰¹	-1.16E ⁻⁰¹	5.90E ⁻⁰²	1.17E ⁻⁰¹
Distance_pith_VB	8.48E⁻⁰¹	-2.10E ⁻⁰¹	-1.28E ⁻⁰¹	5.64E ⁻⁰²	-4.65E ⁻⁰¹
Distance_VB	5.58E ⁻⁰¹	-4.87E ⁻⁰¹	6.41E⁻⁰¹	-1.54E ⁻⁰³	-8.55E ⁻⁰²
Phloem_Th.	4.99E ⁻⁰²	9.23E⁻⁰¹	7.36E⁻⁰²	-2.96E ⁻⁰¹	1.88E ⁻⁰¹
Xylem vessels_Di.	5.12E ⁻⁰¹	6.91E⁻⁰²	-5.75E ⁻⁰¹	1.16E ⁻⁰¹	7.64E⁻⁰²
Xylem tissue_Th.	4.80E ⁻⁰¹	4.55E ⁻⁰¹	-2.12E ⁻⁰¹	-7.00E ⁻⁰¹	1.49E ⁻⁰¹
Xylem vessels_No.	-2.20E ⁻¹⁶	1.25E ⁻¹⁵	6.25E⁻¹⁶	-1.22E ⁻¹⁵	1.27E ⁻¹⁵
Vascular bundle_W.	5.39E ⁻⁰¹	2.21E ⁻⁰¹	-6.23E ⁻⁰¹	4.58E ⁻⁰¹	5.54E ⁻⁰²
Vascular bundle_Th.	7.12E⁻⁰¹	6.13E ⁻⁰¹	-1.58E ⁻⁰¹	1.84E ⁻⁰¹	1.36E ⁻⁰¹
Vascular bundle_R_No.	7.09E⁻⁰²	7.10E⁻⁰¹	2.70E ⁻⁰¹	1.82E ⁻⁰¹	5.14E ⁻⁰¹
Parenchyma_Row_No.3	7.02E⁻⁰¹	-3.45E ⁻⁰²	-1.68E ⁻⁰²	-4.95E ⁻⁰¹	-4.40E ⁻⁰¹
Chlorenchyma_Th.	5.27E ⁻⁰¹	-7.18E ⁻⁰¹	2.61E ⁻⁰¹	1.33E ⁻⁰¹	3.31E ⁻⁰¹
Chlorenchyma_W.	3.58E ⁻⁰¹	-5.46E ⁻⁰¹	1.11E ⁻⁰¹	3.05E ⁻⁰¹	6.84E⁻⁰¹
Sclerenchyma_Th.1	2.59E ⁻⁰¹	3.11E ⁻⁰¹	8.20E⁻⁰¹	2.72E ⁻⁰¹	-1.42E ⁻⁰¹
Sclerenchyma_Th.2	5.28E ⁻⁰¹	2.28E ⁻⁰¹	6.55E⁻⁰¹	6.84E⁻⁰²	-3.45E ⁻⁰¹
Standard deviation	5.129087	4.163905	2.786161	1.960959	1.763249
Proportion of Variance	30.1711	24.49356	16.38918	11.53505	10.37205
Cumulative Proportion	30.1711	54.66466	71.05384	82.58889	92.96094

Furthermore, the phenotypic and anatomical variety among the seven species under investigation, as well as how widely distinct they are along the two axes, was shown by the distribution of the examined

species based on the first two components (Figure 3). The correlogram correlation analysis exposed significant relationships among numerous traits (Figure 4).

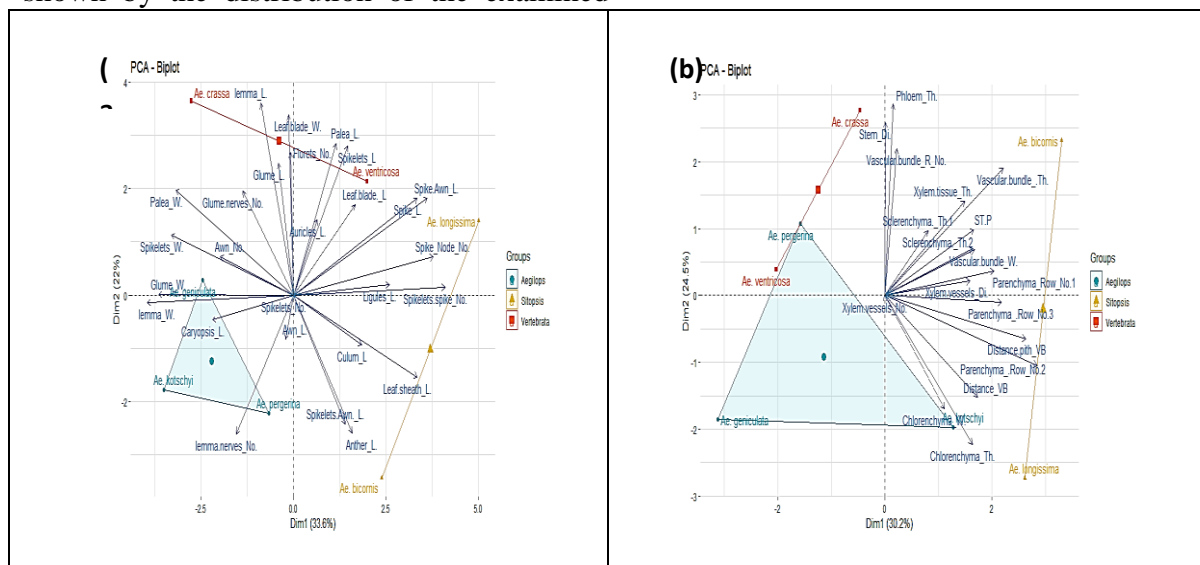


Fig. 3. Visualized Principal Component Analysis (PCA) of (a) morphological (b) anatomical traits.

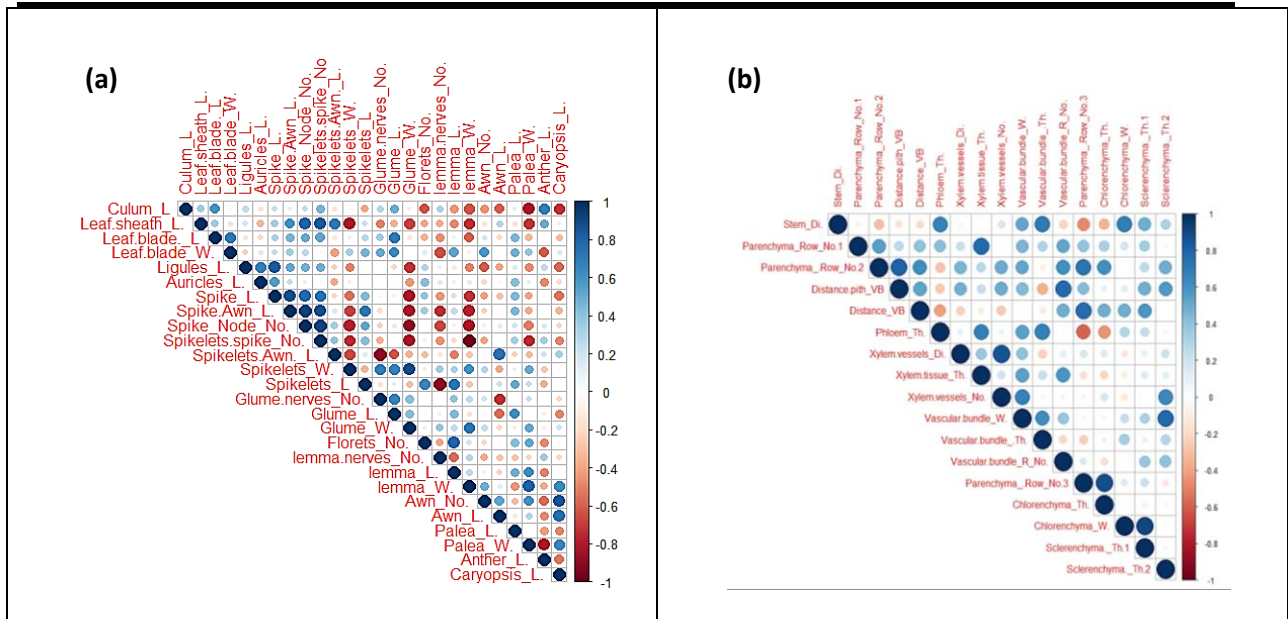


Fig. 4. Correlation of the quantitative traits, positive correlations in blue color and negative correlations in red color. (a) morphological (b) anatomical traits.

Finally, the heatmap based on combination of morphological and anatomical data succeeded in clustering the section of seven species under investigation into two main clusters. The first cluster includes, "*Ae. bicornis* and *Ae. longissima*" belong to section Sitopsis. The other five species go together in the second cluster, divided into two subclusters; the first contains *Ae. ventricosa*, and *Ae. crassa* linked to section

Vertebrata. The other had three species, "*Ae. kotschyi*, *Ae. peregrina*, and *Ae. geniculata*", related to section Aegilops. Boxplot for of morphological and anatomical data was displayed the variation between three section. Geom-boxplot and ANOVA show a variation and significant between three section Aegilops, Sitopsis and *Vertebrata* with p-value ($p = 5.424 \times 10^{-5}$, R-squared = 0.9946) (Figure 5)

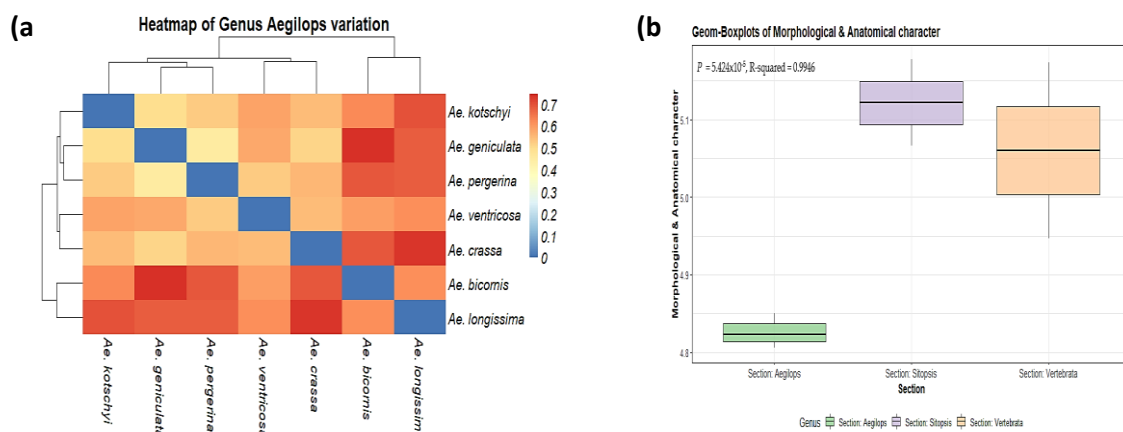


Fig. 5. Based on combined quantitative data of morphological and anatomical data, (a) Heatmap to visualize the similarity and dissimilarity between seven species. (b) Boxplots to show the variation between three sections.

The current study represented the first taxonomic revision for seven species of the Egyptian wild wheat (*Aegilops* L.) based on morphological and anatomical studies. The morphological data were examined by scoring 27 discriminative characters at *Aegilops* sp., including habit, culms, leaves, spikelets, florets and caryopses shape.

Anatomical characteristics have been utilized to differentiate closely related *Aegilops* species based on leaf anatomical character variation across ploidy levels, to present the best anatomical characters for differentiating *Aegilops* species, and to determine the taxonomic relationships among these species (Kharazian, 2007; Mavi et al., 2011).

The current research confirms the effectiveness of 18 anatomical attributes for discrimination among seven *Aegilops* growing in Egypt. The anatomy of the internodes cross-sections provides a critical significance on the taxonomy of the studies taxa. The variations among taxa extend to include the arrangements of these tissues, lignification and number of layers. However, *Ae. longissima* was discriminated by its undulating outline with deep ridges and furrows than the remaining taxa.

According to Van Slageren (1994), Seven *Aegilops* species understudied are classified inside three sections, sect. *Aegilops* include *Ae. kotschyi*, *Ae. pergerina*, and *Ae. geniculata*, which its 2-3 awned glumes can distinguish. Sect. *Vertebrata*, include *Ae. crassa* and *Ae. ventricosa*, which its awnless glumes can determine. Sect. *Sitopsis*. Include *Ae. bicornis* and *Ae. longissima*, which can be distinguished by its laterally compressed spikeletes and awnless glumes. Pheatmap which allows contemporary visualization of clusters of

taxa and traits. The heatmap clustering generated based on the combination between anatomical and morphological characters goes in line with this sectional classification of investigated species. In parallel to Boxplot revealed to the variation between sections.

Conclusion:

Plant architecture, especially inflorescence, is the best criterion for the systematic classification of *Aegilops* species. Culm anatomy (assimilating parenchyma and supporting tissues) indicates significant variations among *Aegilops* species. Combination between the morphological and anatomical studies establishes the variation between *Aegilops* species and it was in consistent with sections according to van Slageren. *Aegilops* contains many important genes that might be transferred to cultivated wheat and utilized in their improvement evolution and played a major role in wheat domestication. Thus, the genus *Aegilops* represents the largest part of wheat's secondary gene pool, increasing the potential of new variations, so We recommend stopping urban sprawl along the northern coast to preserve wild plants from extinction.

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مراجعة تصنيفية ودراسة تشريحية لجنس الأيجلوبس (العائلة النجيلية) مع التأكيد على أقسام الأنواع

الملخص العربي

يعتبر جنس الأيجلوبس أحد أكبر الأجناس التي تنتمي إلى العائلة النجيلية تحت عائلة "بوويدي" ومجموعة "تريبتيسي" ويضم حوالي ٢٢ نوعاً حول العالم. فهو يحتوي على العديد من الجينات المهمة التي يمكن نقلها إلى القمح المزروع، وبذلك يلعب دوراً رئيسياً في استئناس القمح مما يعزز إمكانية وجود أصناف جديدة. اعتمد تصنيف أنواع جنس الأيجلوبس على الوصف الشكلي الذي تم تأكيده بالفحص التشريحي لها. تم جمع العينات من منطقة البحر الأبيض المتوسط، رشيد، مرسى مطروح وسانت كاترين. يتواجد في مصر ٧ أنواع لجنس الأيجلوبس وهما *Ae. bicornis*, *Ae. crassa*, *Ae. geniculate*, *Ae. longissimi*, *Ae. pergerina*, *Ae. kotschy* and *Ae. ventricosa*. أظهرت النتائج المتحصلة عليها عن تحليل المكون الرئيسي (PCA) والارتباط أهمية الصفات المورفولوجية والتشريحية في وصف وتحديد أنواع الأيجلوبس. أكدت العلاقة المورفولوجية والتشريحية للأنواع السبعة. بالتوازي مع Boxplot و ANOVA كشفوا الاختلاف الكبير بين الأقسام. حيث *Ae. bicornis* و *Ae. longissima* ينتميان إلى قسم *Sitopsis*. بينما تتواجد الأنواع الخمسة الأخرى معاً في المجموعه الثانيه ، التي تنقسم إلى مجموعتين فرعيتين ؛ الأولى تحتوي على *Ae. crassa* و *Ae. ventricosa* حيث ينتموا لقسم *Vertebrata* والأخرى تضم أنواع ، " *Ae. geniculata* ، *Ae. peregrina* ، *Ae. kotschy* ، ويتبعوا قسم *Aegilops* .