



## EPIDERMAL FEATURES OF GLUMES AND FLORETS IN *AEGILOPS GENICULATA* ROTH AND *AEGILOPS PEREGRINA* (HACK.) MAIRE ET WEILLER × *SECALE CEREALE* L. HYBRIDS, AMPHIPLOIDS AND PARENTAL FORMS

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**ABSTRACT.** Micromorphological features of glumes, lemmas and paleas were examined under light and scanning electron microscopes in amphiploids of *Aegilops geniculata*, *Ae. peregrina* × *Secale cereale* and their parental species. The principal features include the size and shape of silica bodies, crown cells, prickles, macrohairs, stomata and the morphology of long cells. The SEM observations of glumes separate two parental forms of *Aegilops* by the type of prickles and types of wax on palea surface. The abaxial surfaces of lemmas in the parental species and amphiploids show the most taxonomical features. Amphiploids in many features exhibit an extended range of variation in comparison to their parental species, particularly in stomatal length. Epidermal features in amphiploids are inherited after *Ae. geniculata* and *Ae. peregrina*. Results of this study provided data on new quantitative and qualitative traits of the glumes, lemmas and paleas in the studied taxa.

**KEY WORDS:** *Aegilops geniculata*, *Aegilops peregrina*, amphiploids, glumes, lemma, palea, micromorphology, *Secale cereale*, SEM

### INTRODUCTION

*Aegilops* presents many difficulties to taxonomists because its numerous species hybridize both with each other and with different species of *Triticum* (KILIAN et al. 2011). This type of behaviour has led to wide variations within the various taxa and consequently to difficulties in developing an adequate key to meet all the variable divergent characters of this genus. The spikelet and its parts are considered as diagnostic characters, which show a wide variation between *Aegilops* species, especially in relation to the lemma, palea, keel, and awn of the lemma and glume (MHADAT et al. 1998). PERRINO et al. (1993), ZAHARIEVA et al. (1999), BANDOUI et al. (2009) using morphological, phenological and biochemical characters found great variation between *Ae. geniculata* populations in Italy, Bulgaria and Algeria, respectively. This variation was mainly explained by the adaptation of this species to different pedoclimatic conditions. Intraspecific variation with *Ae. geniculata* Roth could also be due

to the occurrence of natural hybridization with other tetraploid *Aegilops* (PAZY & ZOHARY 1965, KIMBER & FELDMAN 1987). FELDMAN (1965) described many intermediate and introgressed types found in mixed populations of *Ae. peregrina* (Hack.) Maire et Weiller, *Ae. biuncialis* Vis and *Ae. geniculata*. In view of this considerable variability some taxonomists proposed different classifications (MAIRE 1955, QUEZEL & SANTA 1962). The utility and importance of micromorphological characters for systematic studies of the family Poaceae have been confirmed by several authors, e.g. METCALFE (1960), ELLIS (1979), THOMASSON (1986) and KLIMKO et al. (2009). The principal features of taxonomic significance include the pattern and type of silica bodies (METCALFE 1960, ELLIS 1979, PALMER & TUCKER 1981, 1983), papillae and prickles especially in the lemma epidermis (SHAW & SMEINS 1981, THOMASSON 1986). The micromorphological characters of the abaxial lemma surface have phylogenetic value (PETERSON 1989, SNOW 1996, MEJIA-SAULES & BISBY 2003, ORTÚÑEZ & DE LA FUENTE 2010) and utility in

the identification of fossil grasses (THOMASSON 1978, THOMASSON et al. 1986). This work is a continuation of the micromorphological analysis in *Aegilops kotschyi*, *Ae. biuncialis*, *Secale cereale* hybrids, amphiploids and parental forms (KLIMKO et al. 2009). The aim of the present report was to present detailed observations of microepidermal characters of glumes, lemmas and paleas in *Ae. geniculata*, as well as *Ae. peregrina* × *S. cereale* amphiploids and their parents.

## MATERIAL AND METHODS

Plant material consisted of parental species *Ae. geniculata*, *Ae. peregrina*, *S. cereale*, and *Ae. geniculata* × *Secale cereale*, *Ae. peregrina* × *S. cereale* F<sub>1</sub> hybrid and amphiploid. Material was collected from greenhouse-grown plants from the Institute of Plant Genetics, Polish Academy of Sciences in Poznań. *Aegilops geniculata* × *Secale cereale* (2480 K, KT 205) F<sub>1</sub> hybrids (2n=3x=21) were obtained via embryo culture and the amphiploids (2n=42) through in vitro propagation of the F<sub>1</sub> hybrid (WOJCIECHOWSKA & PUDELSKA 2002a, KALINOWSKI & WOJCIECHOWSKA 2003). *Aegilops peregrina* × *S. cereale* amphiploids were produced by chromosome doubling of embryo-calluses derived from the F<sub>1</sub> hybrids by colchicine treatment and tissue culture (WOJCIECHOWSKA & PUDELSKA 1999). The amphiploids numbered 408 B and 408 F were obtained from the F<sub>1</sub> hybrids 408 B and 408 F from the F<sub>1</sub> 408/0/8 and 408/0/2, respectively. Flowers were collected from the colchicine-derived plants of the C<sub>4</sub> generations (408 B) and callus-regenerated plants of the R<sub>2</sub> (408 F) generations. Amphiploids 408 F were regenerated from the inflorescence culture (KALINOWSKI et al. 2001). The glumes and florets (lem-

mas and paleas) were analysed in the lowest floret of the spikelet. Data were gathered from the abaxial surface in the middle part of the glumes and lemmas, while due to the considerable variation of characters in the paleae near the apex observations were conducted on the middle part and along veins, following the earlier study on the subject (KLIMKO et al. 2009). Observations were conducted under a ZEISS EVO 40 scanning electron microscope (SEM). Measurements of the main characteristics, i.e. five quantitative and six qualitative features (Tables 1–4), were performed under a light microscope (Olympus BX-43). Stains such as Sudan III were used to aid the identification of cork cells using LM. Samples were sonicated in xylene for at least thirty minutes to remove epicuticular wax that may obscure surface features (ACEDO & LLAMAS 2001). In this paper we followed ELLIS (1979) for the description of lemma micromorphology, since lemmas are homologous to leaves (SNOW 1996), and extended the terminology to glumes and paleas, because they show similar epidermal characteristics.

## RESULTS AND DISCUSSION

A description of micromorphology of glumes, lemmas and paleas in the abaxial surface of the studied taxa is given below and illustrated with selected SEM photographs (Figs 1–28). The microepidermal features are summarized in Tables 1–4.

SEM micrographs of the outer abaxial surface show important features which could be used to identify *Aegilops* species. Seven types of epidermal features have been shown: long cells, cork cells, crown cells (siliceous papillae), prickles, macrohairs, stomata and cuticle ornamentation.

Table 1. Micromorphological characters of lower glume. Length in  $\mu\text{m}$

Lower glume	Parental species			Amphiploid		
	<i>Ae. geniculata</i>	<i>Ae. peregrina</i>	<i>Secale cereale</i>	KT 205	408 B	408 F
Long cells						
length	33.5–83.5	46.8–76.6	90.5–183.5	68.5–139.6	48.7–117.9	55.2–113.9
anticlinal wall	sinuous	sinuous	sinuous	sinuous	sinuous	sinuous
periclinal wall	convex	convex	convex	convex	convex	convex
Cork cells						
shape	reniform elliptic	reniform elliptic	reniform elliptic	reniform polygonal	reniform elliptic	reniform elliptic
length	16.3–23.1	17.6–24.6	15.5–24.5	17.7–22.7	14.7–21.3	14.7–19.9
Crown cells						
cells shape	rounded	rounded	rounded	rounded	rounded	rounded
protrusion shape	conical	conical	conical	conical	conical	conical
length	36.3–51.7	31.4–45.2	25.5–36.8	34.3–48.4	29.2–54.2	30.5–50.7
Prickles						
length	104.5–153.5	105.9–333.7	81.1–137.8	141.2–204.8	114.7–297.5	162.5–324.4
Macrohairs	not observed	not observed	not observed	not observed	not observed	not observed
Stomata						
length	26.9–42.5	31.5–38.9	39.1–49.7	45.5–56.5	41.9–51.3	39.6–53.9
Cuticle	smooth, slightly striate	smooth	smooth	smooth	smooth	smooth

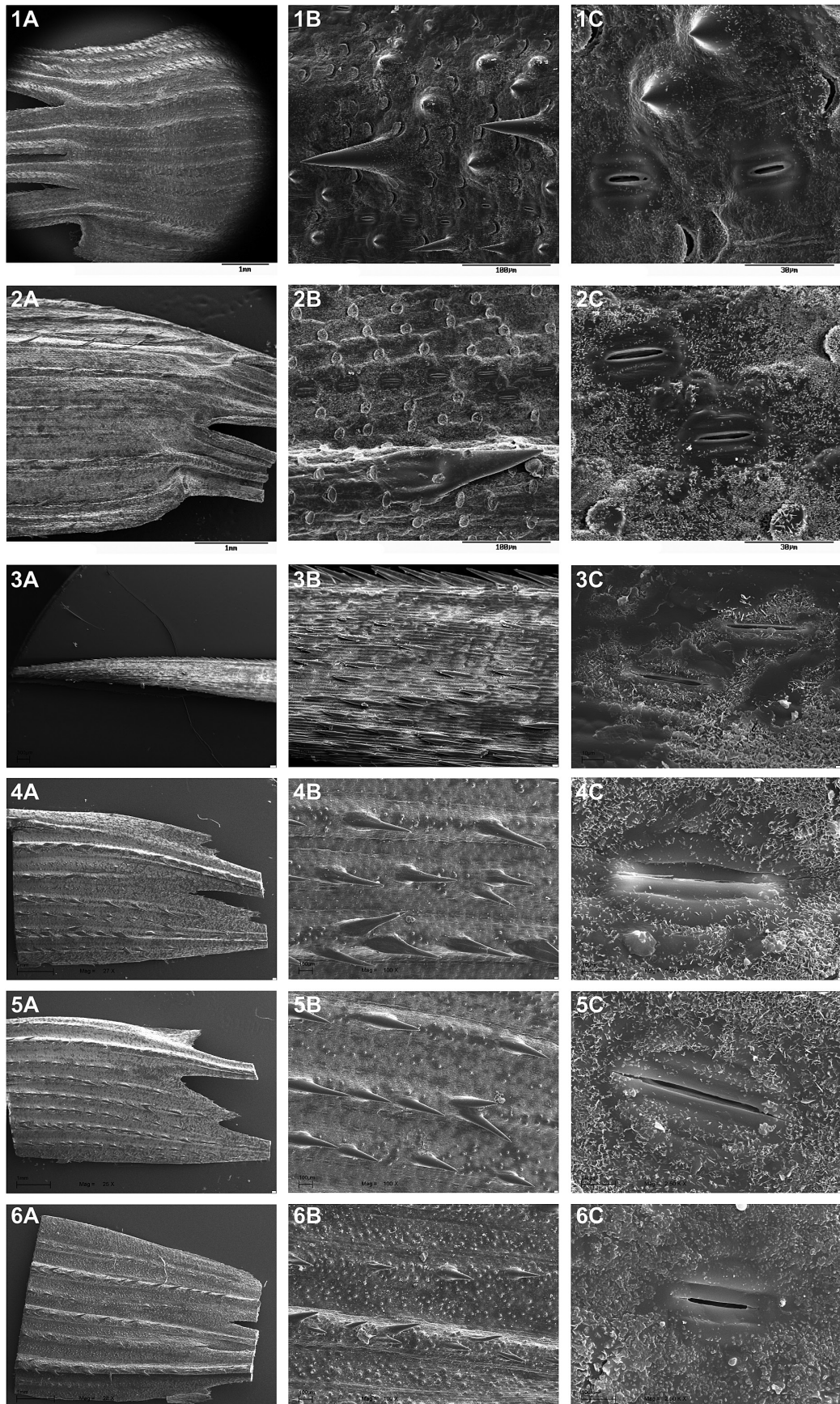


Fig. 1–6. SEM. Lower glume surface: 1. *Aegilops geniculata*; 2. *Aegilops peregrina*; 3. *Secale cereale*; 4. *Aegilops peregrina* × *Secale cereale* (408B); 5. *Aegilops peregrina* × *Secale cereale* (408 F); 6. *Aegilops geniculata* × *Secale cereale* (KT 205)

## LOWER GLUME (TABLE 1, FIGS 1–6)

The lower glume is rigid, boat-shaped with a projecting keel extending a short distance below the apical awns to the base of the bracts as prominent ribs.

In *Ae. geniculata* nine veins were observed, in *Ae. peregrina* eight and in both amphiploids *Ae. peregrina* × *S. cereale* (408B, 408F) seven and eight, respectively. In *Ae. geniculata* × *S. cereale* (KT 205) six veins were found. In *S. cereale* the lower glume is linear, coriaceous, 1-keeled, keeled along its entire length, 1-veined. Primary vein scabrous, lateral veins absent.

- Long cells are rectangular and their length ranges from 33.5  $\mu\text{m}$  (*Ae. geniculata*) to 183.5  $\mu\text{m}$  (*S. cereale*). Maximum length of elongated cells in amphiploids of *Ae. geniculata* × *S. cereale* (KT 205) is 139.6  $\mu\text{m}$  and in *Ae. peregrina* × *S. cereale* (408 B, 408 F) it is 117.9  $\mu\text{m}$  and 113.9  $\mu\text{m}$ , respectively. In amphiploids the length of elongated cells is identical to the range of lengths in *S. cereale*. The length of long cells in the parental forms *Ae. geniculata* and *Ae. peregrina* is 33.5–83.5  $\mu\text{m}$  and 46.8–76.6  $\mu\text{m}$ , respectively. The anticlinal walls are parallel and highly sinuous, with  $\Omega$ -shaped waves. In contrast, the periclinal walls are smooth and convex.
- Cork/silica cells. The size of cork cells ranges from 14.7  $\mu\text{m}$  (amphiploids 408 B and 408 F) to 24.6  $\mu\text{m}$  (*Ae. peregrina*). The anticlinal walls are straight and their periclinal walls are slightly convex and collapse upon dehydration. The shape of these cells is similar and the cork/silica pair cells are reniform/elliptic except in KT 205, where the pair cells are reniform/polygonal.

- Crown cells (papillae) appear as rounded, raised with convex surface and the protrusion shape is conical. The protrusion in the examined taxa is formed by most of the external periclinal wall of cells, similarly as in *Ae. kotschyi* and *Ae. biuncialis* (KLIMKO et al. 2009), although in some species from the genus *Bromus* the protrusion is formed only by the central portion of the outer periclinal cell wall (ACEDO & LLAMAS 2001). They vary in size from 25.5  $\mu\text{m}$  (*S. cereale*) to 54.2  $\mu\text{m}$  (amphiploid 408 B). The maximal length of the crown cells in both amphiploids 408 B and 408 F is greater than in the parental forms, similarly as the range of crown cell lengths observed in amphiploid KT 205 is wider.
- Prickles: they occur as rows on the outer glumes lower surface, appearing over the veins as single or double prickles. The SEM observation shows the presence of two types of prickles: erect in *Ae. geniculata* and couched prickles in *Ae. peregrina* and amphiploids. The couched prickles in *Ae. peregrina* are similar to those in *Ae. biuncialis* (MHAI DAT et al. 1998). However, semi-erect prickles are found e.g. in *Ae. kotschyi* Boiss. In *Aegilops* sp. the prickles can be scattered over the entire surface as in *Ae. longissima* Schweinf et Muschl., or clustered in groups of prickle hairs as in *Ae. crassa* Boiss (MHAI DAT et al. 1998). The terminology used in this study followed GOMEZ-CAMPO (1981). Prickles of glumes in the *Aegilops* parental species and amphiploids were found to be rigid, sharp pointed with thick walls and with an oval base, which is consistent with the description given by METCALFE (1960). The surface of prickles is striate in

Table 2. Micromorphological characters of upper glume; length in  $\mu\text{m}$

Upper glume	Parental species			Amphiploid		
	<i>Ae. geniculata</i>	<i>Ae. peregrina</i>	<i>Secale cereale</i>	KT 205	408 B	408 F
Long cells						
length	40.4–75.4	42.9–76.1	77.5–213.7	63.7–165.4	50.7–103.7	41.1–113.8
anticlinal wall	sinuous	sinuous	sinuous	sinuous	sinuous	sinuous
periclinal wall	convex	convex	convex	convex	convex	convex
Cork cells						
shape	polygonal elliptic	polygonal elliptic	reniform elliptic	polygonal elliptic	reniform elliptic	reniform elliptic
length	13.4–21.5	15.1–25.7	13.5–23.7	17.6–24.1	15.4–19.8	15.5–18.9
Crown cells						
cells shape	rounded	rounded	rounded	rounded	rounded	rounded
protrusion shape	conical	conical	conical	conical	conical	conical
length	37.3–55.1	20.2–48.1	29.5–42.2	29.5–44.2	33.4–62.1	25.6–43.6
Prickles						
length	76.2–204.1	120.9–179.3	84.6–142.2	not observed	146.6–213.1	162.5–324.3
Macrohairs						
length	not observed	not observed	not observed	99.4–232.7	not observed	not observed
Stomata						
length	38.4–45.9	28.8–40.9	34.5–53.3	40.4–54.3	41.9–56.7	35.5–48.5
Cuticle	striate	smooth, striate	smooth	smooth	striate	smooth, slightly striate

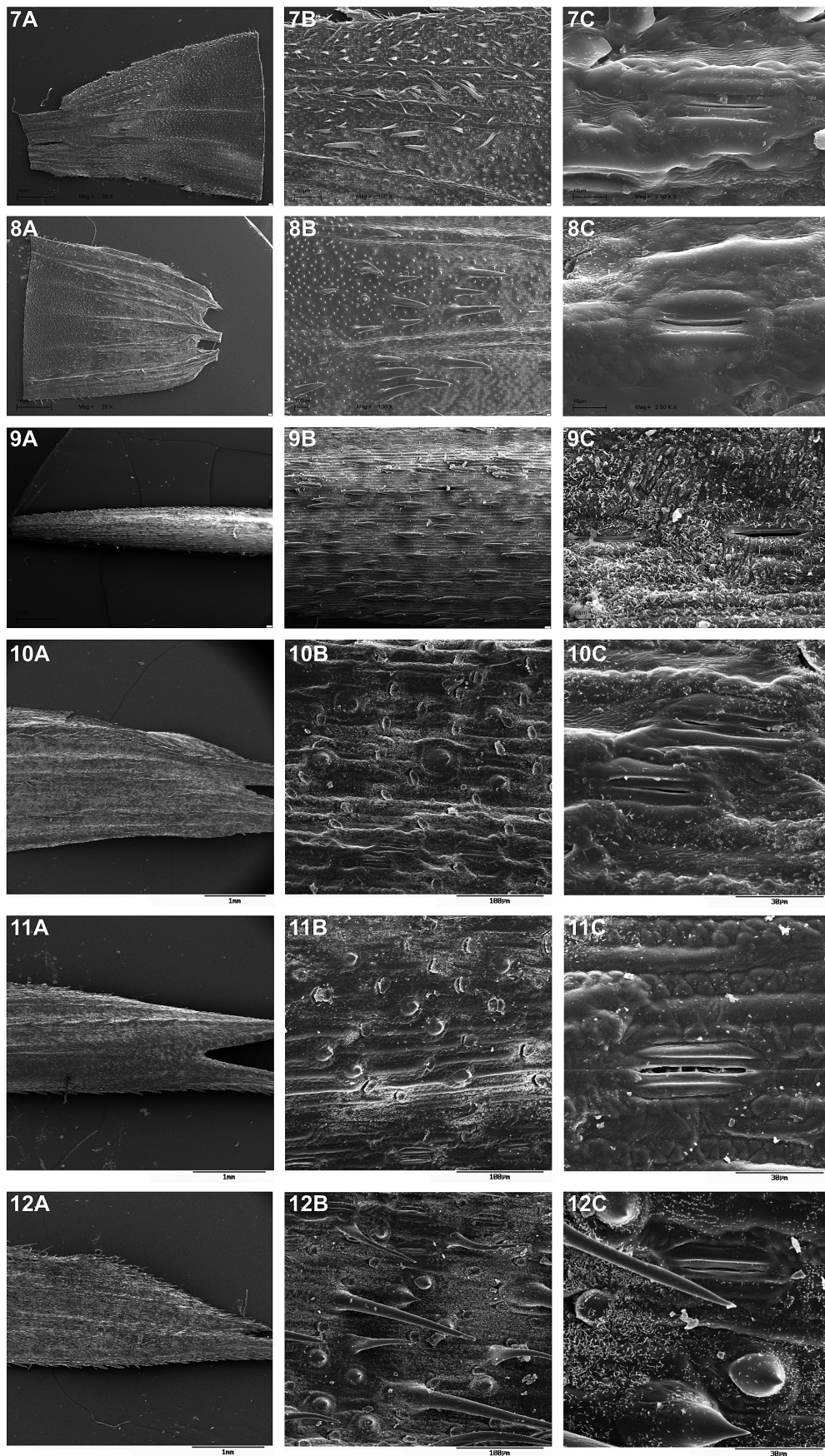


Fig. 7–12. SEM. Upper glume surface: 7. *Aegilops geniculata*; 8. *Aegilops peregrina*; 9. *Secale cereale*; 10. *Aegilops peregrina* × *Secale cereale* (408B); 11. *Aegilops peregrina* × *Secale cereale* (408 F); 12. *Aegilops geniculata* × *Secale cereale* (KT 205)

*Ae. peregrina* and smooth-striate in *Ae. geniculata*. Prickles vary in size from 81.1  $\mu\text{m}$  (*S. cereale*) to 333.7  $\mu\text{m}$  (*Ae. peregrina*). In amphiploids (KT 205) prickles have a greater range of lengths than in the parental forms: from 141.2 to 204.8  $\mu\text{m}$ , while in amphiploids 408 B and 408 F the length is comparable to that in *Ae. peregrina*. MHAIDAT et al. (1998) examined prickles in a wild Jordan population of *Ae. geniculata* and *Ae. peregrina*. In reference to their results in *Ae. geniculata* the length of prickles varies from 69 to 276  $\mu\text{m}$ , while in *Ae. peregrina* it is from 100 to 567  $\mu\text{m}$ . The SEM observation of the lower glumes also separated the parental species of *Aegilops* by the type of prickles. Macro-hairs in *Aegilops* are absent on the lower glume. In *S. cereale* macrohairs are found only at the margin of the lower glume.

- Stomata are arranged linearly, along veins. The length of stomata varies from 26.9  $\mu\text{m}$  (*Ae. geniculata*) to 56.5  $\mu\text{m}$  (amphiploid KT 205). The mean length from 30 measurements of stomata in the abaxial epidermis of lower glumes in *Ae. geniculata* and *Ae. peregrina* is similar, amounting to 36.2  $\mu\text{m}$  (*Ae. geniculata*), 34.6  $\mu\text{m}$  (*Ae. peregrina*), and 41.3  $\mu\text{m}$  in *S. cereale*. In amphiploids stomata are much larger. In amphiploid KT 205 their mean length is 49.75  $\mu\text{m}$ . Both amphiploids, 408 B and 408 F, have larger stomata than the parental species, but their mean length differs only slightly, amounting to 47.34  $\mu\text{m}$  (408 B) and 48.78  $\mu\text{m}$  (408 F), respectively.
- Cuticle: The surfaces of lower glumes are covered by the cuticular layer, which is smooth/slightly striate in *Ae. geniculata* and smooth in *S. cereale*. Waxes dense, covering almost all the surface (Figs. 25, 26). More differences are found on

short cells than on long cells. On the basis of epidermal micromorphology of lower glumes it may be inferred that the examined taxa have similar epidermal cell types.

#### UPPER GLUME (TABLE 2, FIGS 7–12)

The upper glume is rather oblong, less obovate, coriaceous without keels. The surface is scabrous and rough on veins. In *Ae. peregrina* and *Ae. geniculata* five veins were observed. In both amphiploids 408 B and 408 F six veins and in (KT 205) five veins were found. In *Secale cereale* the upper glume linear, coriaceous, 1-keeled, keeled throughout its length, 1-veined. Primary vein scabrous, lateral veins absent.

- The long-cells are rectangular and their length ranges from 40.4 (*Ae. geniculata*) to 165.4  $\mu\text{m}$  (KT 205). Two parental species of *Aegilops* have similar long cells of 40.4–75.4  $\mu\text{m}$  (*Ae. geniculata*) and 42.9–76.1  $\mu\text{m}$  (*Ae. peregrina*). Three amphiploids 408 B and 408 F and KT 205 have longer elongate cells, with lengths comparable to that in *S. cereale*, similarly as on the lower glume. The anticlinal walls are parallel and highly sinuous, with  $\Omega$ -shaped waves. Periclinal walls are smooth and convex.
- Cork/silica cells. The size of cork cells ranges from 13.4  $\mu\text{m}$  (*Ae. geniculata*) to 25.7  $\mu\text{m}$  (*Ae. peregrina*). The anticlinal walls are straight and their periclinal walls are slightly convex and collapse upon dehydration. The shape of these cells is similar, with cork/silica pair cells being reniform/elliptic in amphiploids 408 B and 408 F and polygonal/elliptic in amphiploids KT 205 and in the parental species.
- Crown cells (papillae) appear as rounded, raised with a convex surface and the protrusion shape is

Table 3. Micromorphological characters of lemma; length in  $\mu\text{m}$

Lemma	Parental species			Amphiploid		
	<i>Ae. geniculata</i>	<i>Ae. peregrina</i>	<i>Secale cereale</i>	KT 205	408 B	408 F
Long cells						
length	64.4–135.2	31.7–95.1	34.3–133.5	25.9–101.2	51.2–100.5	47.9–149.9
anticlinal wall	sinuous	sinuous	sinuous	sinuous	sinuous	sinuous
periclinal wall	convex	convex	convex	convex	convex	convex
Cork cells						
shape	polygonal elliptic	polygonal elliptic	polygonal elliptic	reniform polygonal	reniform elliptic	reniform elliptic
length	13.1–20.1	11.5–18.9	16.3–18.3	12.5–17.4	13.8–17.9	16.1–21.4
Crown cells						
cells shape	rounded	rounded	rounded	rounded	rounded	rounded
protrusion shape	conical	conical	conical	conical	conical	conical
length	17.1–29.9	23.1–57.3	20.3–36.8	28.5–38.1	36.5–66.5	24.6–63.7
Prickles	not observed	not observed	1–3(4)	not observed	1–2	1–2
Macrohairs						
length	not observed	not observed	not observed	112.7–222.8	not observed	not observed
Stomata						
length	34.1–46.1	35.2–46.8	33.3–50.9	45.4–53.3	36.3–51.2	35.6–46.7
Cuticle	smooth	smooth, striate	smooth	smooth	smooth	smooth

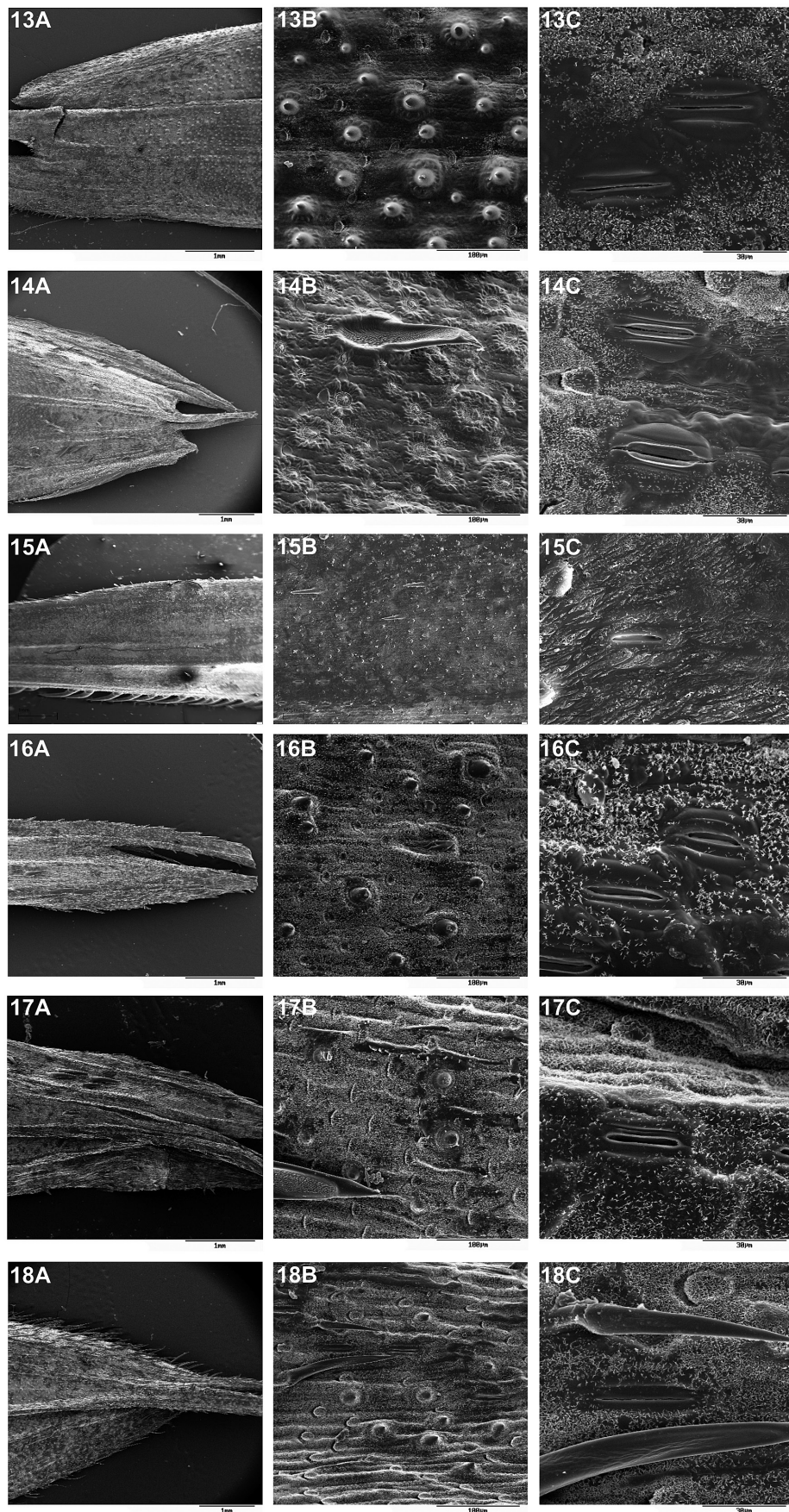


Fig. 13–18. SEM. Lemma surface: 13. *Aegilops geniculata*; 14. *Aegilops peregrina*; 15. *Secale cereale*; 16. *Aegilops peregrina* × *Secale cereale* (408B); 17. *Aegilops peregrina* × *Secale cereale* (408 F); 18. *Aegilops geniculata* × *Secale cereale* (KT 205)

- conical. The protrusion is formed by most of the external periclinal wall of cells. Crown cells have crenulate walls, with pronounced waxes similar to those of the long cells. They vary in size from 20.2  $\mu\text{m}$  (*Ae. peregrina*) to 62.1  $\mu\text{m}$  (408 B). In amphiploid KT 205 the minimum length is smaller than in the parental species, amounting to 29.5  $\mu\text{m}$ , while in 408 B and 408 F it is 33.4  $\mu\text{m}$  and 25.6  $\mu\text{m}$ , respectively.
- Prickles absent in the middle part of the upper glume in *Ae. geniculata* and *Ae. peregrina*. They occur below the tip of the apex and in the basal part in *Ae. peregrina*, and occasionally in *Ae. geniculata*. In all amphiploids they occur over the veins as single or double. In *S. cereale* single, prickles present. The SEM observation shows the presence of two types of prickles as in the lower glumes. Prickles on the upper glumes are rigid and sharp pointed. In all taxa the surface of prickles is smooth, less often smooth-striate. The size of prickles varies from 76.2  $\mu\text{m}$  (*Ae. geniculata*) to 324.3  $\mu\text{m}$  (408 F). Relatively short prickles are found in *S. cereale*. Their length ranges from 84.6 to 142.2  $\mu\text{m}$ . In amphiploid KT 205 prickles not observed. Macro-hairs on the upper glume absent, except for amphiploid KT 205, where macrohairs are present.
  - Stomata, similarly as on the lower glume, are arranged along veins. The length of stomata varies from 28.8  $\mu\text{m}$  (*Ae. peregrina*) to 56.7  $\mu\text{m}$  (amphiploids 408 B). Significant differences are observed in the mean length of stomata in the parental species at 41.68  $\mu\text{m}$  (*Ae. geniculata*), 35.32  $\mu\text{m}$  (*Ae. peregrina*) and 43.2  $\mu\text{m}$  (*S. cereale*). In amphiploid (KT 205), the mean stomatal length is 49.55  $\mu\text{m}$  and in amphiploids 408 B and 408 F it is identical, i.e. 47.5  $\mu\text{m}$ . Again larger stomata were found in polyploids (WINKELMANN & GRUNEWALDT 1995).
- Cuticle: The surface of upper glumes is covered by the cuticle, longitudinally striated in *Ae. geniculata* and amphiploid 408B, smooth in KT 205 and smooth, slightly striate in the other taxa; wax dense, covering almost all the surface.

#### THE LEMMA (TABLE 3, FIGS 13–18)

The lemma is oblong, leathery and coriaceous. In *Ae. geniculata*, *Ae. peregrina* and amphiploids: 408 F as well as in KT 205, five veins were observed, while in 408 B only four veins were found. In *Secale cereale* the lemma is lanceolate, coriaceous, keeled, 5-veined. Midvein pectinately ciliate, margins ciliate.

- The long-cells are rectangular and their length ranges from 31.7  $\mu\text{m}$  (*Ae. peregrina*) to 135.2  $\mu\text{m}$  (*Ae. geniculata*). Maximum length of elongated cells in amphiploids of KT 205 is 101.2  $\mu\text{m}$  and in 408 B and 408 F it is 100.5  $\mu\text{m}$  and 149.9  $\mu\text{m}$ , respectively. In amphiploids the length of elongate cells falls within the range observed in *S. cereale*. The length of long cells in the parental forms *Ae. geniculata* and *Ae. peregrina* is 64.4–135.2  $\mu\text{m}$  and 31.7–95.1  $\mu\text{m}$ , respectively. The anticlinal walls are parallel and highly sinuous, with  $\Omega$ -shaped waves. The periclinal walls are smooth and convex.
- Cork/silica cells. The size of cork cells varies from 11.5  $\mu\text{m}$  (*Ae. peregrina*) to 21.4  $\mu\text{m}$  (amphiploid 408 F). The anticlinal walls are straight and their periclinal walls are slightly convex and collapse upon dehydration. The shape of this cells is similar and the cork/silica pair cells are reniform/

Table 4. Micromorphological characters of palea; length in  $\mu\text{m}$

Palea	Parental species			Amphiploids		
	<i>Ae. geniculata</i>	<i>Ae. peregrina</i>	<i>Secale cereale</i>	KT 205	408 B	408 F
Long cells						
length	37.1–75.9	51.2–114.5	58.6–182.4	44.8–112.7	37.5–124.7	59.6–140.6
anticlinal wall	sinuous	sinuous	sinuous	sinuous	sinuous	sinuous
periclinal wall	convex	convex	convex	convex	convex	convex
Cork cells						
shape	reniform	reniform	reniform	reniform	reniform	reniform
length	12.5–21.8	14.04–18.6	15.4–23.4	13.4–19.5	14.7–20.8	10.8–17.6
Crown cells						
cells shape	rounded	rounded	rounded	rounded	rounded	rounded
protrusion shape	conical	conical	conical	conical	conical	conical
length	14.8–21.7	30.7–46.1	20.2–32.0	17.9–27.4	25.5–46.9	24.4–40.3
Prickles	not observed	not observed	not observed	not observed	not observed	not observed
Macrohairs						
length	55.3–100.5	not observed	1–2	32.7–76.4	59.4–89.7	60.1–116.6
Stomata						
length	30.5–44.1	29.9–41.4	30.1–43.8	36.5–43.3	37.2–54.4	39.4–55.1
Cuticle	smooth	striate	smooth	smooth	smooth	smooth, striate



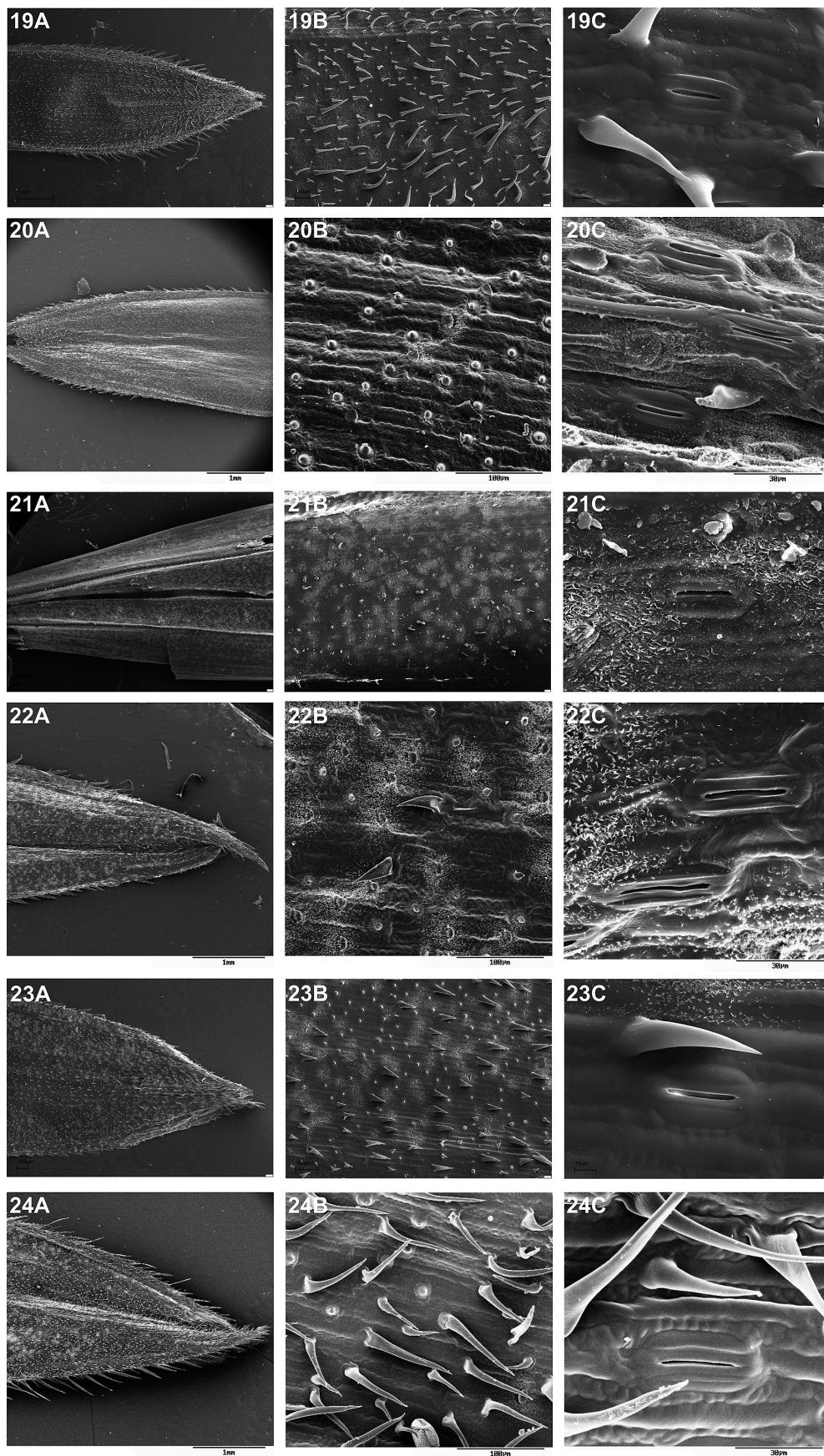


Fig. 19–24. SEM. Palea surface: 19. *Aegilops geniculata*; 20. *Aegilops peregrina*; 21. *Secale cereale*; 22. *Aegilops peregrina* × *Secale cereale* (408B); 23. *Aegilops peregrina* × *Secale cereale* (408 F); 24. *Aegilops geniculata* × *Secale cereale* (KT 205)

- elliptic or reniform/polygonal, except for *Ae. peregrina*, where the pair cells are polygonal/elliptic.
- Crown cells (papillae) appear as rounded, raised with a convex surface and the protrusion shape is conical. The protrusion is formed by most of the external periclinal wall, similarly as on glumes. They vary in size from 17.1  $\mu\text{m}$  (*Ae. geniculata*) to 66.5  $\mu\text{m}$  (amphiploid 408 B). The maximal length of the crown cells in both amphiploids 408 B and 408 F and in KT 205 is greater than in the parental species.
  - Prickles: In the middle part of lemma prickles absent or observed singly in amphiploids 408 B and 408 F.
  - Macrohairs: unicellular, rigid, straight or hooked, observed on the surface in *S. cereale* and amphiploid KT 205, of 112.7–222.8  $\mu\text{m}$  in length.
  - Stomata – similarly as on glumes arranged on rows on both sides of veins. They range in length from 33.3  $\mu\text{m}$  (*S. cereale*) to 53.3  $\mu\text{m}$  in KT 205. The mean length of stomata in the abaxial epidermis of the lemmas in *Ae. geniculata* and *Ae. peregrina*

- grina* is similar, at 37.34  $\mu\text{m}$  and 36.76  $\mu\text{m}$ , and in *S. cereale* it is 37.8  $\mu\text{m}$ . In the amphiploids mean stomatal length is greater than in the parental species and amounts to 39.45  $\mu\text{m}$  (KT 205), 46.95  $\mu\text{m}$  (408 B) and 44.88  $\mu\text{m}$  (408 F).
- Cuticle ornamentation is smooth, except for *Ae. peregrina*, where it is smooth, longitudinal, striated. Wax abundant throughout the surface.

#### THE PALEAS (TABLE 4, FIGS 19–24)

In all taxa of *Aegilops* the palea is elliptic and membranous, 2-veined, on the margins hairy, equaling or subequaling the lemma, ciliate along keels. In *S. cereale* the palea is elongate, membranous, 2-veined. Keels scaberulous.

- Long cells are rectangular and their length ranges from 37.1  $\mu\text{m}$  (*Ae. geniculata*) to 182.4  $\mu\text{m}$  (*S. cereale*). Maximal length of elongated cells in amphiploids of KT 205 is 112.7  $\mu\text{m}$  and in 408 B, 408 F it is 124.7  $\mu\text{m}$  and 140.6  $\mu\text{m}$ , respectively. In amphiploids the length of elongate cells falls

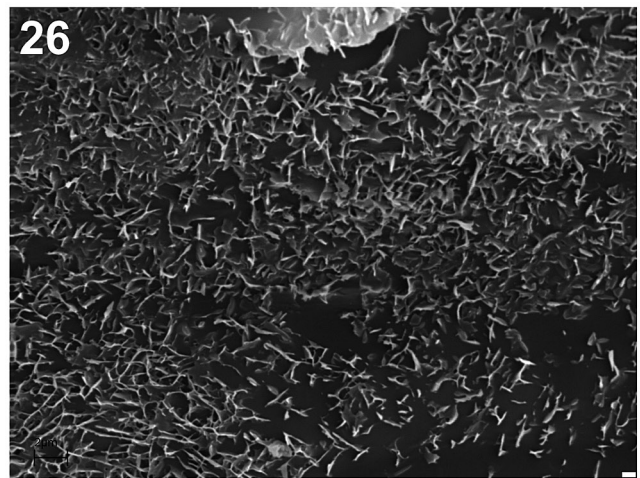
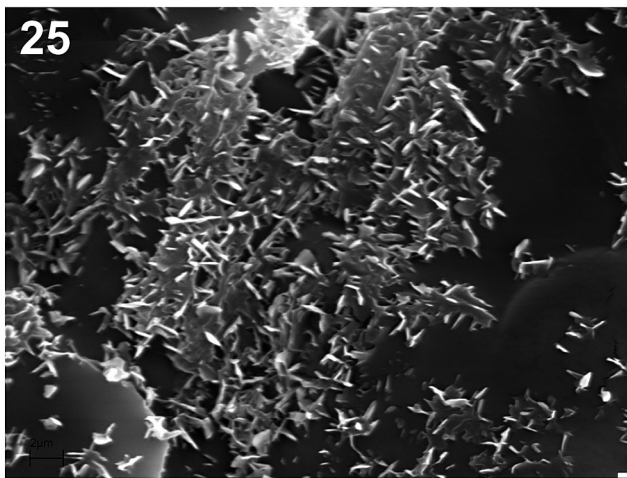


Fig. 25–26. SEM. Ornamentation of wax on upper glume: 25. *Aegilops geniculata*; 26. *Aegilops geniculata*  $\times$  *Secale cereale* (KT 205)

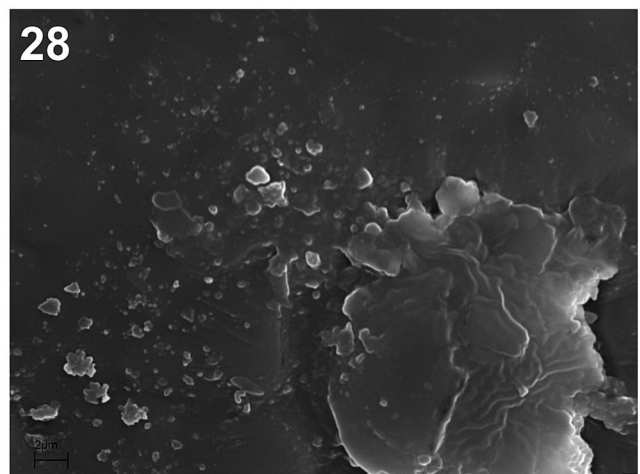
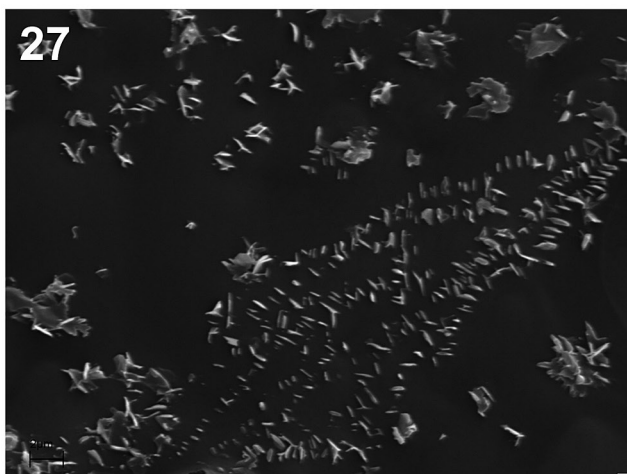


Fig. 27–28. SEM. Ornamentation of wax on palea surface: 27. *Aegilops geniculata*; 28. *Aegilops peregrina*

within the range found in *S. cereale*. Certain differences are found in the length of long cells in the parental species of *Ae. geniculata* at 37.1–75.9  $\mu\text{m}$  and *Ae. peregrina* it is 51.2–114.5  $\mu\text{m}$ , respectively. The anticlinal walls are parallel and highly sinuous. The periclinal walls are smooth and convex.

- Cork/silica cells. The size of cork cells ranges from 10.8  $\mu\text{m}$  (amphiploid 408 F) to 23.4  $\mu\text{m}$  (*S. cereale*). The anticlinal walls are straight and their periclinal walls are slightly convex and collapse upon dehydration. The shape of these cells is similar and the cork/silica pair cells are reniform/elliptic.
- Crown cells (papillae) appear as rounded, raised with a convex surface and the protrusion shape is conical. The protrusion is formed by most of the external periclinal wall of cells. They vary in size from 14.8  $\mu\text{m}$  (*Ae. geniculata*) to 46.9  $\mu\text{m}$  (amphiploid 408 B). The maximum length of the crown cells in both amphiploids 408 B and 408 F is comparable to that in *Ae. peregrina*. In *S. cereale* the crown cells range in size from 20.2 to 32.0  $\mu\text{m}$ .
- Prickles: not observed in all taxa. As it was reported by METCALFE (1960), ELLIS (1979) and SNOW (1996), sometimes prickles are longer and then it is difficult to differentiate between prickles and macrohairs. It results from our studies that significant differences between prickles and macrohairs are found in the shape of the base, oval or bulbous, respectively, and in their width. Prickles are markedly wider than macrohairs.
- Macrohairs: the upper glume densely covered with hairs was observed in *Ae. geniculata* and in amphiploid KT 205. In amphiploids 408 B and 408 F single macrohairs are found, similarly as in *S. cereale*. Macrohairs are straight or bent on the apex with a bulbous base. In terms of pubescence the surface of paleas in *Ae. geniculata* is similar to that in *Ae. biuncialis* (Ab 7), (KLIMKO et al. 2009).
- Stomata arranged along veins. The length of stomata ranges from 29.9  $\mu\text{m}$  (*Ae. peregrina*) to 55.1  $\mu\text{m}$  (amphiploid 408 F). The mean length of stomata in the parental species is similar: 37.34  $\mu\text{m}$  in *Ae. geniculata*, 36.76  $\mu\text{m}$  in *Ae. peregrina* and 37.8  $\mu\text{m}$  in *S. cereale*. Non-significant differences in stomatal length are observed in amphiploid KT 205 in comparison to the parental species. The mean length of stomata is 39.45  $\mu\text{m}$ . In turn, in both amphiploids 408 B and 408 F stomata are markedly larger, of 46.95  $\mu\text{m}$  and 44.88  $\mu\text{m}$ , respectively.
- Cuticle: The surface of paleas are covered by the cuticle – longitudinally striated in *Ae. peregrina*, and smooth in *Ae. geniculata*, *S. cereale* and amphiploids KT 205. Some differences in the cuticle occur between amphiploids 408 B, where the cuticle is smooth-striate and 408 F, where the cuticle is smooth. Differences in wax were found between *Ae. geniculata* and *Ae. peregrina* (BARTHLOTT et al. 1998).

The results of this study confirm the taxonomic significance of the micromorphological characters in the genus *Aegilops*, *Secale cereale*, and their amphiploids. The principal features of taxonomic value in glume and floret surfaces include the shape and size and distributions of prickles, macrohairs, cork silica, crown cells and the shape and diameter of long cells and stomata are presented in Tables 1–4. Our study shows that the micromorphology of the abaxial surface is the most important.

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#### REFERENCES

- ACEDO C., LLAMAS F. (2001): Variation of micromorphological characters of lemma and palea in the genus *Bromus* (Poaceae). *Annales Botanici Fennici* 38: 1–14.
- BANDOU H., RODRIGUEZ-QUIJANO M., CARRILLO J.M., BRANLARD G., ZAHARIEVA M., MONNEVEUX P. (2009): Morphological and genetic variation in *Aegilops geniculata* from Algeria. *Plant Systematics and Evolution* 277: 85–97. DOI 10.1007/s00606-008-0106-z.
- BARTHLOTT W., NEINHUIS C., CUTLER D., DITSCH F., MEUSEL I., THEISEN I., WILHELM H. (1998): Classification and terminology of plant epicuticular waxes. *Botanical Journal of Linnean Society* 126: 237–260.
- ELLIS R.P. (1979): A procedure for standardizing comparative leaf anatomy in the Poaceae II. The epidermis as seen in surface view. *Bothalia* 12: 641–671.
- FELDMAN M. (1965): Further evidence for natural hybridization between tetraploid species of *Aegilops* section *Pleionathera*. *Evolution* 19: 162–174.
- GOMEZ-CAMPO G. (1981): Taxonomic and evolutionary relationships in the genus *Vella*. L. (Cruciferae). *Botanical Journal of Linnean Society* 82: 165–179.
- KALINOWSKI A., WINIARCZYK K., WOJCIECHOWSKA B. (2001): Pollen proteins after two-dimensional gel electrophoresis and pollen morphology of the amphiploids *Aegilops kotschyi* and *Ae. variabilis* with *Secale cereale*. *Sexual Plant Reproduction* 14: 153–161.
- KALINOWSKI A., WOJCIECHOWSKA B. (2003): Pollen and leaf proteins 2-D electrophoresis of the *Aegilops geniculata*  $\times$  *Secale cereale* hybrids, amphiploids and parental forms. *Euphytica* 133: 201–207.
- KILIAN B., MAMMEN K., MILLET E., SHARMA R., GRANNER A., SALAMINI F., HAMMER K., OZKAN H. (2011): *Aegilops*. In: *Wild crop relatives: Genomic and breeding resources, cereals*. Springer Verlag, Ber-

- lin–Heidelberg. DOI 10-1007/978-3-642-142 28-4-1.
- KIMBER G., FELDMAN M. (1987): Wild wheat. An introduction. Special Report 535. College of Agriculture, University of Missouri, Columbia.
- KLIMKO M., PUDELSKA H., WOJCIECHOWSKA B., KLIMKO W. (2009): Variation of micromorphological characters of lemma and palea in *Aegilops kotschyi* and *Aegilops biuncialis* × *Secale cereale* hybrids, amphiploids and parental forms. *Roczniki Akademii Rolniczej w Poznaniu* 388, Botanika Steciana 13: 167–176.
- MAIRE R. (1955): Flore de l’Afrique du Nord, vol. 3. Le Chevalier, Paris: 65–69.
- MEJIA-SAULES T., BISBY F.A. (2003): Silica bodies and hooked papillae in lemmas of *Melica* species (Gramineae: Pooideae). *Botanical Journal of Linnean Society* 141: 447–463.
- METCALFE C.R. (1960): Anatomy of the Monocotyledons, vol. 1. Gramineae. Clarendon Press, Oxford.
- MHAIDAT R., AL-JASSABI S., EL-OGLAH A.A., JARADAT A.A. (1998): Glume morphology and comparative culm anatomy of *Aegilops* species from Jordan. In: A.A. Jaradat (ed.). *Triticeae III*. Science Publishers Inc., New Hampshire, USA: 135–147.
- ORTÚÑEZ E., DE LA FUENTE V. (2010): Epidermal micromorphology of the genus *Festuca* L. (Poaceae) in the Iberian Peninsula. *Plant Systematics and Evolution* 284: 201–218. DOI 10.1007/s00606-009-0248-7.
- PALMER P.G., TUCKER A.E. (1981): A scanning electron microscope survey of the epidermis of East African Grasses. I. *Smithsonian Contributions to Botany* 49: 1–84.
- PALMER P.G., TUCKER A.E. (1983): A scanning electron microscope survey of the epidermis of East African Grasses. II. *Smithsonian Contributions to Botany* 53: 1–120.
- PAZY B., ZOHARY D. (1965): The process of introgression between *Aegilops* polyploids. Natural hybridization between *A. varabilis*, *A. ovata*, and *A. biuncialis*. *Evolution* 19: 385–394.
- PERRINO P., LAGHETTI G., CIFARELLI S., VOLPE S., SPAGNOLETTI-CHICHESTER P.L., ZEULI U.K. (1993): Wild wheats in southern Italy. In: A.B. Damania (ed.). *Biodiversity and wheat improvement*. Willey, Chichester: 361–368.
- PETERSON P.M. (1989): Lemma micromorphology in the annual *Muhlenbergia* (Poaceae). *The Southwestern Naturalist* 34(1): 61–71.
- QUEZEL P., SANTA S. (1962): Nouvelle flore de l’Algérie et des régions désertiques méridionales. Tome 1. Edition du Centre National de la Recherche Scientifique, Paris.
- SHAW R.B., SMEINS F.E. (1981): Some anatomical and morphological characteristics of the North American species of *Eriochloa* (Poaceae: Paniceae). *Botanical Gazette (Crawfordsville)* 142(2): 534–544.
- SNOW N. (1996): The phylogenetic utility of lemmatal micromorphology in *Leptochloa* s.l. and related genera in subtribe Eleusininae (Poaceae, Chloridoideae, Eragrostidae). *Annals of the Missouri Botanical Garden* 83: 504–529.
- THOMASSON J.R. (1978): Epidermal patterns of the lemma in some fossil and living grasses and their phylogenetic significance. *Science* 199: 975–977.
- THOMASSON J.R. (1986): Lemma epidermal features in the North American species of *Melica* and selected species of *Briza*, *Catabrosa*, *Glyceria*, *Neostapfia*, *Pleuropogon* and *Schizachne* (Gramineae). *Systematic Botany* 11: 253–262.
- THOMASSON J.R., NELSON M.E., ZAKRZEWSKI R.J. (1986): A fossil grass (Gramineae: Chloridoideae) from the Miocene with Krantz Anatomy. *Science* 233: 876–878.
- WINKELMANN T., GRUNEWALDT J. (1995): Analysis of protoplast-derived plants of *Saintpaulia ionantha* H. Wendl. *Plant Breeding* 114: 346–650.
- WOJCIECHOWSKA B., PUDELSKA H. (1999): Production, morphology and fertility of the amphiploids *Aegilops variabilis* × *Secale cereale* and *Ae. kotschyi* × *Secale cereale*. *Cereal Research Communications* 27: 79–82.
- WOJCIECHOWSKA B., PUDELSKA H. (2002a): Hybrids and amphiploides of *Aegilops ovata* L. with *Secale cereale* L.: production, morphology and fertility. *Journal of Applied Genetics* 43: 415–421.
- WOJCIECHOWSKA B., PUDELSKA H. (2002b): Production and morphology of the hybrids *Aegilops kotschyi* × *Secale cereale* and *Ae. biuncialis* × *S. cereale*. *Journal of Applied Genetics* 43: 279–285.
- ZAHARIEVA M., DAVID J., THIS D., MONNEVEUX P. (1999): Analyse de la diversité génétique d’*Aegilops geniculata* Roth en Bulgarie. (In French, with English abstract). *Cahiers Agricultures* 8: 181–188.
- For citation: KLIMKO M., WYSAKOWSKA I. (2015): Epidermal features of glumes and florets in *Aegilops geniculata* Roth and *Aegilops peregrina* (Hack.) Maire et Weiller × *Secale cereale* L. hybrids, amphiploids and parental forms. *Steciana* 19(1): 13–24. DOI 10.12657/steciana.019.003