ABSTRACT. Results of this study provided data on new qualitative and quantitative characters of the lemma and palea epidermis in amphiploids of Aegilops kotschyi, Ae. biuncialis × Secale cereale and their parental species using scanning electron microscopy. Several micromorphological characters of both long and short cells (cork cells, crown cells, prickles and macrohairs), stomata and their types, wax and the cuticle were observed. All characters were treated as separate, although crown cells, prickles and macrohairs are treated as single groups (exodermic cells) owing to their protrusion from the periclinal wall. All observed micromorphological characters of lemmas and paleae in amphiploids are inherited after Ae. kotschyi and Ae. biuncialis, irrespective of the method in which these hybrids were produced. Hexaploid (2n = 6x = 42, x = 7) amphiploids in many features exhibit an extended range of variation in comparison to their parental species. In amphiploids in the palea a new shape type is observed in crown cells wide and oblate, not found in either parent form. Moreover, certain dependencies were observed between the method in which amphiploids were produced and their micromorphological characters. The genera Aegilops and Secale cereale and their amphiploids may be identified using the micromorphological characters of lemmas and/or paleae.

KEY WORDS: Aegilops kotschyi, Aegilops biuncialis, amphiploids, lemma, palea, micromorphology, Secale cereale, SEM

INTRODUCTION

Intergeneric hybrids are interesting in the theoretical aspect in evolutionary, taxonomic and cytological studies. Cytological analysis proved that the U genome of Aegilops sp. polyploids is very closely related to the U diploid genome of Aegilops umbellulata and it has not been substantially modified during their evolution, whereas the S genome of Ae. kotschyi (UUSS) and the M genome of Ae. biuncialis (UUMM) have undergone substantial changes (Kimber et al. 1988, Kimber and Yen 1989, Yen and Kimber 1990). For these reasons combinations of two different genomes of Aegilops sp. with the Secale cereale genome (RR) seemed to be an interesting material for molecular and micromorphological investigations.

Micromorphological characters are valuable for systematic studies in the family Poaceae. For example, the absence of microhairs is characteristic in Pooideae (Clayton and Renvoize 1986). Micromorphological characters of floral bracts in grasses have been used to assess systematic relationships, as well as evolutionary trends. Nevertheless, the micromorphology of lemmas and paleae has not been widely studied in the Poideae, except for some particular genera, such as Stipa (Thomasson 1978), Melica and selected species of Briza, Catabrosa, Glyceria, Neostepha, Pleuro pogon and Schizachne (Thomasson 1986), Bromus (Acedo and Llamas 2001), Koeleria (Klimko and Czarna 2001) and Calamophila (Klimko et al. 2007).

Investigations presented in this paper are a continuation of studies on amphiploids of Ae. kotschyi, Ae. biuncialis × Secale cereale and their parental species. To date studies have been conducted on the morphology and proteins of the pollen coat and protoplast in amphiploids of Ae. kotschyi × Secale cereale (Kalnowski et al. 2004), leaf isoenzymes (Kalnowski and Wojciechowska 2004) and peptides of pollen grain in Ae. biuncialis × S. cereale (Kalnowski et al. 2003). The spikelet and its parts are considered as diagnostic characters which show a wide variation between taxa, especially in relation to the lemma and palea.

The aim of the present report was to conduct detailed observations of micromorphological characters of lemmas and paleae in Ae. kotschyi and Ae. biuncialis × S. cereale amphiploids and their parents.
MATERIAL AND METHODS

Plant material consisted of the parental species form of *Ae. kotschyi* (accession 14 408, in this paper referred to as AK-3), *Ae. biuncialis* (accession 14 716, referred to as Ab 7), and the *S. cereale* self-compatible line (WS 79N/85, denoted as S 14).

Amphiploids 84 C (*Ae. kotschyi × S. cereale*) and 122 A (*Ae. biuncialis × S. cereale*) were produced by colchicine treatment of F₁ hybrids. Amphiploids 84 E (*Ae. kotschyi × S. cereale*), and 122 A (*Ae. biuncialis × S. cereale*) were obtained by chromosome doubling via *in vitro* culture propagation (WOJCIECHOWSKA and PUDIELSKA 2002).

Material was collected from greenhouse-grown plants from the Institute of Plant Genetics, Polish Academy of Sciences in Poznań. The lemmas and paleae were analysed in the lowest floret of the spikelet in the middle parts of the spike. Data were gathered from the middle part and along veins (Fig. 1 A), while due to the considerable variation of characters in the paleae near the apex, observations were conducted on the middle part and along veins (Fig. 1 B).

Observations were carried out first with a stereo microscope, a light microscope (LM) and finally a scanning electron microscope (SEM). Stains such as Sudan LM. Samples were sonicated in xylene for at least thirty minutes to remove epicuticular wax that may obscure surface features (ACEDO and 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 paleae, because they show similar epidermal characteristics.

The shortest Euclidean distances were calculated in an agglomerative grouping by the nearest neighbour method and dendrograms were constructed to examine the relationships between amphiploids and parental species (SOKAL and RÖHLF 2003).

RESULTS

Description of micromorphological characters

**Long cells**

Long cells are the dominant element in the epidermis of lemmas and paleae, which, despite their name, vary notably in length. There are rectangular, long and narrow cells having convex periclinal walls. In contrast, the anticlinal walls are parallel and highly sinuous, with W-shaped waves. The ends are often angular, ranging in length from 24.71 to 211.8 µm.

**Short cells**

Short cells are in general nearly equal-sided, although cells with the width exceeding their length are also relatively frequent. Short cells alternate with long cells, or occur solitarily or in pairs. The frequency of short cells results to some degree from the length of long cells and shows variations between species and amphiploids. Anticlinal cell walls are straight or sinuous. Two types of short cells have been observed: cork cells and exodermic cells.

**Cork cells**

They are short cells containing solid deposits of organic substances and suberified cell walls (KAUFMAN et al. 1970). Their periclinal walls are usually slightly convex and collapse upon dehydration. The most frequent shape is elliptic, reniform and semicircular.

**Exodermic cells**

These cells protrude beyond the general surface of the epidermis and have periclinal walls with differing degrees of convexity. Depending upon the size and shape of the processes, three subtypes may be distinguished:

- **Crown cells**: short exodermic cells with small convex or conical protrusions, which occur frequently on the lemma and palea. This term was used by PRAT (1932) and revived by WATSON and DALLWITZ (1992) in order to characterize the leaf epidermis in some genera of grasses. Many authors include all exodermic cells under the name of macrohairs. ELLIS (1979) referred to these cells as hooks, using the term as a synonym of crochets and crown cells, although he only presented figures with hooked protrusions, thereby excluding those with rounded protrusions. The protrusions of the periclinal wall of crown cells vary in shape and size. In general they have crenulate walls, with pronounced waves similar to those of long cells.
• **Prickles**: Exodermic cells with elongated tips and swollen bases arising directly from the epidermis. They occur frequently especially on veins and the marginal zone, although in some groups they occur between long cells with a comparable frequency, and a high silica concentration in this structure.

• **Macrohairs**: Unicellular structures, soft or rigid, long, often with a bulbous base. They occur between other epidermal cells, the marginal zone and in some cases they have an associated small cork cell.

**Epicuticular wax**

Epicuticular waxes are deposits, formed mainly by a large mixture of different chemical compounds.

**Micromorphological description of lemma and paleae**

*Aegilops kotschyi* (AK-3)

Lemma. Long cells 56.5-74.1 μm. Cork / silica – cell pairs reniform / elliptic 7.1-17.6 μm, density 11-20 cork cells per 1 mm². Crown cells rounded, 24.7-49.4 μm long, with a conical protrusion (Fig. 2 A), density 204-226 crown cells per 1 mm². Prickles in the middle part, near the apex and the marginal zone absent or scarcely distributed mainly on veins. Macrohairs 0-1 mm², up to 176.5 μm long. Stomata 31.7-63.5 μm long. Wax granulate, the cuticle smooth and irregularly, longitudinally striated (Fig. 2 B).

Palea. Long cells 49.4-169.4 μm. Cork / silica cell – pair reniform / elliptic 12.1-17.6 μm, density 20-30 cork cells per 1 mm². Crown cells rounded 24.7-38.8 μm, density 148-168 crown cells per 1 mm². Crown cells in the middle part rounded (Fig. 3 A), with a conical protrusion. Closer to the apex a considerable prevalence of crown cells with pointed protrusions (Fig. 3 B). Prickles present at the margin and in the area of the apex. Macrohairs absent in the abaxial epidermis. Stomata 28.2-38.8 μm long, wax absent, the cuticle smooth and nodular (along anticlinal walls of long cells) (Fig. 3 C).

*Secale cereale* (S 14)

Lemma. Long cells 77.6-112.9 μm. Cork / silica – cell pairs semicircular / elliptic 10.6-21.2 μm, density 141-189 cork cells per 1 mm². Crown cells rounded, 24.7-28.3 μm long, with pointed protrusions (Fig. 2 C), density 17-28 crown cells per 1 mm². Prickles generally absent in most of the lemma, sometimes several prickles may be found on veins. Macrohairs absent. Stomata 31.7-42.4 μm long. Abundant accumulations of mixed wax (flaky-filiform), masking the cuticle, which is striated transversely and longitudinally (Fig. 2 D).

Palea. Long cells 24.7-176.5 μm. Cork / silica cell – pairs semicircular / elliptic 14.1-17.6 μm long, density 177-236 cork cells per 1 mm². Crown cells rounded with a pointed protrusion (Fig. 3 D), generally absent in most of the palea, although sometimes several may be found near the base or beside the veins. Macrohairs absent. Stomata 31.7-38.8 μm long. Wax mixed (granulate-filiform), abundant. The cuticle smooth and striated longitudinally (Fig. 3 E).

*Aegilops biuncialis* (Ab 7)

Lemma. Long cells 42.4-211.8 μm. Cork / silica – cell pairs reniform / elliptic 10.6-35.3 μm, density 13-27 cork cells per 1 mm². Crown cells rounded with a spherical and conical protrusion (Fig 2 E), density 131-140 crown cells per 1 mm². Prickles, macrohairs in the middle part absent. Macrohairs present very rarely at the margins and at the base of the lemma, 45.9-70.6 μm long. Stomata 31.7-45.9 μm long. Wax dense, filiform, covering almost all the surface, the cuticle irregularly, longitudinally striated and nodular (along anticlinal walls of long cells) (Fig. 2 F).

Palea. Long cells 70.6-158.8 μm. Cork / silica cell – pairs reniform / elliptic 10.6-21.2 μm long, density 16-38 cork cells per 1 mm². Crown cells rounded, 10.6-17.6 μm long, density 217-281 crown cells per 1 mm². Crown cells in the middle part of the surface differ considerably in terms of their shape: with conical and pointed protrusions (Fig. 3 F). Prickles and macrohairs distributed mainly in the marginal zone. Stomata 31.7-38.8 μm long, wax absent, the cuticle smooth, striated transversely and longitudinally striated, nodular (along anticlinal walls of long cells and stomata) (Fig. 3 G).

*Aegilops kotschyi × S. cereale* (84 C)

Lemma. Long cells 38.8-130.6 μm. Cork / silica – cell pairs reniform / elliptic, 14.12-21.18 μm, density 64-84 cork cells per 1 mm². Crown cells rounded 35.3-52.9 μm long, with a conical protrusion (Fig. 4 A), density 103-121 crown cells per 1 mm². Prickles in the middle part absent; single, wide prickles present on veins. Macrohairs absent. Stomata 31.7-52.95 μm long. Wax dense, granulate, covering almost all the surface. The cuticle striated longitudinally, masked by wax (Fig. 4 B).

Palea. Long cells 42.4-112.9 μm. Cork / silica cell – pairs reniform / elliptic 17.65-21.18 μm, density 8-23 cork cells per 1 mm². Crown cells rounded 24.71-45.89 μm long with conical and pointed protrusions (wide and oblate) (Fig. 5 A), density 121-149 crown cells per 1 mm². This is a new character. Prickles and macrohairs absent in the middle part, found only on veins and crenations and the margin of the palea. Stomata 24.71-52.95 μm long. Wax granulate, irregularly distributed, scarce. The cuticle finely, longitudinally striated and nodular along anticlinal walls of long cells (Fig. 5 B).

*Aegilops kotschyi × S. cereale* (84 E)

Lemma. Long cells 24.71-134.14 μm. Cork / silica – cell pairs reniform / elliptic 14.12-21.18 μm, density 43-86 cork cells per 1 mm². Crown cells rounded and irregular 28.24-49.42 μm long, with conical protrusions, density 64-84 crown cells per 1 mm². Not very numerous prickles on middle veins (Fig. 4 C) and the marginal zone. Macrohairs absent. Stomata 31.77-52.95 μm long. Wax granulate, abundant. The cuticle smooth, transversely striated and nodular along anticlinal walls of long cells and stomata (Fig. 4 D).

Palea. Long cells 77.6-134.14 μm. Cork / silica – cell pairs reniform / elliptic 17.65-24.81 μm long, density 12-14 cork cells per 1 mm². Crown cells rounded and irregular 31.77-45.89 μm long, with conical and pointed protrusions, caucused (Fig. 5 C), density 96-107 crown cells per 1 mm². Prickles in the middle part absent, distributed mainly do 1/2 length of the palea on veins. Stomata 35.30-88.25 μm long. Wax granulate, the cuticle smooth, irregularly, longitudinally and transversely...
Fig. 2. SEM. Lemma surface: A, B – *Aegilops kotschyi* (AK-3); C, D – *Secale cereale* (S 14); E, F – *Aegilops biuncialis* (Ab 7)
Fig. 3. SEM. Palea surface: A, B, C – *Aegilops kotschyi* (AK-3). Note A in the middle part and B near the apex; D, E – *Secale cereale* (S 14), F, G – *Aegilops biuncialis* (Ab 7)
FIG. 4. SEM. Lemma surface of amphiploids: A, B – *Aegilops kotschyi* × *Secale cereale* (84 C); C, D – *Ae. kotschyi* × *S. cereale* (84 E); E, F – *Ae. biuncialis* × *S. cereale* (122 A); G, H – *Ae. biuncialis* × *S. cereale* (122)
Variation of micromorphological characters of lemma and palea in Aegilops kotschyi ...

Fig. 5. SEM. Palea surface of amphiploids: A, B – *Aegilops kotschyi* × *Secale cereale* (84 C); C, D – *Ae. kotschyi* × *S. cereale* (84 E); E, F – *Ae. biuncialis* × *S. cereale* (122 A); G, H – *Ae. biuncialis* × *S. cereale* (122)
striated and papillated along anticalinal walls of long cells (Fig. 5 D).

*Aegilops biuncialis* × *S. cereale* (122 A)

Lemma. Long cells 60-169.44 μm. Cork / silica – cells pairs or solitary reniform / elliptic, 17.65-24.71 μm long, density 33-37 cork cells per 1 mm². Crown cells rounded or irregular 35.3-49.42 μm long, with conical and pointed protrusions (wide, oblate), caouched (Fig. 4 E) density 74-85 crown cells per 1 mm². Prickles distributed mainly on veins and awns. Stomata 38.83-52.95 μm long. Wax mixed (granulate–flaky), irregularly distributed. The cuticle smooth and finely striated, nodular along anticalinal walls of long walls and stomata (Fig. 4 F).

Palea. Long cells 35.30-144.70 μm. Cork / silica cells – pairs, reniform / elliptic – oval, 17.65-24.71 μm long, density 18-27 cork cells per 1 mm². Crown cells rounded or irregular 21.18-31.77 μm long, density 114-120 crown cells per 1 mm². Crown cells with conical and pointed protrusions (wide, flattened), caouched (Fig. 5 E). Prickles and macrohairs distributed mainly in the marginal zone. Stomata 35.30-49.42 μm long. Wax granulate, accumulated mainly around cork cells. The cuticle smooth, longitudinally striated and nodular along anticalinal walls of long cells and stomata (Fig. 5 F).

*Aegilops biuncialis* × *S. cereale* (122)

Lemma. Long cells 28.24-84.72 μm. Cork / silica – cell pairs reniform / elliptic – oval, 17.65-24.71 μm long, density 71-99 cork cells per 1 mm². Crown cells rounded 35.30-42.36 μm long, with conical protrusions (Fig. 4 G), density 58-63 crown cells per 1 mm². Prickles distributed mainly in the marginal zone and on awns. Stomata 42.36-56.48 μm long. Wax mixed (granulate–flaky–filiform), abundant. The cuticle smooth, longitudinally striated and nodular along stomata (Fig. 4 H).

Palea. Long cells 63.54-155.32 μm. Cork / silica cell – pairs, reniform / elliptic – oval, 17.65-24.71 μm long, density 17-35 cork cells per 1 mm². Crown cells rounded or irregular 24.71-31.77 μm long, with spherical and pointed protrusions (wide, flattened), caouched (Fig. 5 G), density 68-72 crown cells per 1 mm². Prickles and macrohairs present on veins and in the marginal zone. Stomata 35.30-45.89 μm long. Wax granulate, scarce. The cuticle transversely and longitudinally striated, nodular along anticalinal walls of long walls (Fig. 5 H).

**DISCUSSION AND CONCLUSIONS**

One of the main problems with micromorphological studies is to determine the optimal site of observation. The lemma shows variations in the expression of micromorphological characters between the apex, base and margins, whereas the palea varies mostly near the apex and the marginal zone. Thus the middle part of the lemma side and the central part of the palea body were selected for analyses. These regions appear to be quite constant in their micromorphological characteristics when studied in different taxa. However, this area does not fully illustrate the entire variation in the analysed micromorphological characters in the palea of *Aegilops* sp. For this reason observations were conducted near the apex and along veins.

On the basis of the epiderma micromorphology of the lemma and palea in the studied taxa and *Ae. variabilis, Ae. ovata, Ae. cylindrica* (Klimko, unpublished data) it may be inferred that species of the genus *Aegilops* have the same epidermal cell types, differing markedly from those in *S. cereale*. In *Aegilops* and *Secale* there are two characters that in general show a positive correlation. They are the length of long cells (in the lemma and palea) and the number of short cells. More differences occur in short cells than long ones. Thus, crown cells show considerable variation in density and their type of protrusions, whereas cork cells exhibit variation in their density and size. Exodermic cells, such as e.g. cork cells, are distributed in longitudinal patterns, alternating with long cells and other epidermal cells. The density of cork and crown cells in relation to the other epidermal elements is also variable.

Leommas of parental species *Ae. biuncialis* and *Ae. kotschyi* differ in terms of the density of cork cells per 1 mm², which amounts to 16.6 and 21.7, respectively. Much bigger interspecific differences were observed in the density of crown cells per 1 mm², which was 216 in *Ae. kotschyi* and 136.3 in *Ae. biuncialis*. No significant differences were observed in this trait in the palea.

*Secale cereale* (S14) is characterised by a considerable prevalence of the density of cork cells per 1 mm², amounting in the lemma to 189 and in the palea to 202.3 cells, respectively. The density of crown cells per 1 mm² in the lemma is 22.3, while in the palea it is only 3.66.

Crown cells are generally rounded, although sometimes they are irregular in shape as in the palea of amphiploids 122 and 122 A. The protrusion or protuberance may be conical or pointed and vary in its relative size to the cell supporting it. The protrusion is formed by most of the external periclinal wall of the cell.

The density of cork cells per 1 mm² in *Ae. kotschyi, Ae. biuncialis* and *S. cereale* is higher in the palea than the lemma. In amphiploids the trend is opposite. In AK-3 and S 14 the density of crown cells per 1 mm² is higher in the lemma than the palea. Ab 7 and all amphiploids have highest values for the mean number of crown cells per 1 mm² in the palea. Amphiploid 84 C in comparison 84 E has a higher density of cork and crown cells per 1 mm² in the lemma and palea. In amphiploid 122 A in comparison to 122 only the density of crown cells in the lemma and palea is higher. Prickles on the lemma in studied taxa occur especially above the veins. Long macrohairs in amphiploids and parent species were found very rarely in the abaxial epidermis. Their presence or absence is of little taxonomic significance. It is in contrast to the glume, where the type of prickles in *Ae. kotschyi* (semi-erect prickles), *Ae. biuncialis* (caouched prickles) and their distribution effectively differentiate both species (MHAIDAT et al. 1998).

All studied taxa have macrohairs and prickles restricted to the margin of the palea. Sometimes prickles are longer and then it is difficult to differentiate between prickles and macrohairs (METCALFE 1960, ELLIS 1979, SNOW 1996).
The size of stomata is considered to be an indirect indicator in studies on polyploidy (Winkelmans and Grunwaldt 1995). On the lemma and palea stomata are arranged in rows along veins. The mean length of stomata in the abaxial epidermis of the lemma ranged from 38.87 μm (Ae. biuncialis) to 44.27 μm (Ae. kotschyi) and 36.93 μm (S. cereale). The length of stomata in amphiploid 84 C was 45.89 μm. Stomata in amphiploid 84 E were much shorter, of 35.30 μm. The length of stomata in amphiploids 122 A and 122 was 45.59 μm and 47.40 μm, respectively.

In the palea mean differences in the analysed trait between parental species were smaller, as the value was 35.74 μm (Ae. kotschyi), 36.35 μm (Ae. biuncialis) and 34.79 μm (S. cereale). The length of stomata in amphiploid 84 C was 39.88 μm, while it was 35.59 μm in amphiploid 84 E. The mean value of this character in amphiploids 122 A and 122 (Ae. biuncialis × S. cereale) was 39.98 μm and 41.35 μm, respectively.

In studied taxa the length of stomata was higher in the lemma than the palea in the parental species and their amphiploids, except for amphiploid 84 E, where these differences were slight. The interesting aspect of the epicuticular wax residues, according to some authors, lies in the fact that the configuration of wax deposits seems to have a genetic basis (Baum and Hadland 1975). On the lemma the morphology is different in Ae. kotschyi, S. cereale and Ae. biuncialis (Figs 2B, D, F). In amphiploids 84 C, 84 E and 122 A wax granulate is found, in 122 mixed (granular-flaky-filiform). In analysed taxa granular wax predominated on the palea, in S 14 it was mixed, while in AK-3 and Ab 7 it was not observed. Detailed observations of epicuticular wax on the abaxial surface provided new data on the subject. The cuticle on the lemma and palea may be smooth, papillated and striated (nomenclature following Barthlott et al. 1998). The cuticle sculpture in the lemma and palea of the parental species and amphiploids did not vary. In contrast, marked differences were observed in a thick cuticle layer of the culm: smooth in Ae. kotschyi and striated in Ae. biuncialis (Mhydodat et al. 1998).

Cluster analysis using Euclidean distances segregated all the taxa compared within the one group (Fig. 6). It was formed by parental species (Ae. kotschyi, Ae. biuncialis) and their amphiploids. In terms of analysed characters S. cereale showed the biggest differences (S 14).

On this basis the following conclusions may be inferred. For the micromorphological studies it is necessary to carefully select the site for observations. All micromorphological characters in the lemma and palea in the amphiploids are inherited after Ae. kotschyi or Ae. biuncialis, irrespective of the method in which they were produced. Hexaploid amphiploids in terms of analysed characters exhibit extensive variation in comparison to the parental species. Moreover, near the apex on the palea of amphiploids a new shape of crown cells was observed, a wide and oblate, not found in the parental species. A certain variation was also found in the relationship between the method of amphiploid production (colchicine treatment or in vitro propagation) and their micromorphological characters, similarly as it was the case in studies on pollen grain sculpture (Kalinowski et al. 2004). Selected species of Aegilops, Secale cereale and their amphiploids may be identified using the micromorphological characteristics of the lemma and/or palea.

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