Annals of Botany **79**: 41–46, 1997

Characterization of the Leaf Epidermis of Barley (*Hordeum vulgare* L. 'Himalaya')

C. L. WENZEL*, P. M. CHANDLER†*, R. B. CUNNINGHAM‡, and J. B. PASSIOURA†*

** Cooperative Research Centre for Plant Science, GPO Box 475, Canberra, ACT 2601, † CSIRO Division of Plant Industry, GPO Box 1600, Canberra, ACT 2601 and ‡ Statistics Department, Australian National University, Canberra, ACT 0200, Australia*

Received: 1 August 1995 Accepted: 29 July 1996

The cell types of mature leaves of barley (*Hordeum vulgare* L. 'Himalaya') are described. Blade and sheath epidermal cell types were characterized according to their position relative to the veins, stomatal rows, and sclerenchyma cells. Cells over veins were further classified according to the size of vein. Cell lengths of the approx. 15 different cell types ranged from approximately 50 μm to over 2 mm. The principal difference in cell length between the abaxial and adaxial surfaces was seen for cells lying between the veins; on the adaxial surface these cells (bulliform cells) were about 200 μm long whereas those on the abaxial surface were over 2 mm in length. Total file number across the blade width was the same on the abaxial and adaxial surfaces. However, there were more cells lying over veins than between veins on the adaxial surface, and *vice versa* for the abaxial surface. The detailed description of leaf epidermal cell types of barley in this study provides the basis for comparison with mutants which differ in leaf length (Wenzel *et al.*, *Annals of Botany* **79**: 45–50, 1997).

Key words: Barley (*Hordeum vulgare* L. 'Himalaya'), blade, sheath, epidermis.

INTRODUCTION

We are interested in comparing the epidermal anatomy of a number of barley (*Hordeum vulgare* L. 'Himalaya') mutants which differ from the parent in their final leaf length. The epidermis is considered one of the factors affecting the rate of growth in plants by restraining expansion of the internal tissues (Taiz, 1984; Kutschera, 1992; Bret–Harte and Talbott, 1993).

Previous descriptions of the epidermis of barley leaf blades (Zeiger, 1971; Silvy, 1982) and the closely related wheat leaf blade (Percival, 1921; Metcalfe, 1960) provided the basis for this study. However, there are no reports describing the epidermal anatomy of variety 'Himalaya' barley, nor do previous studies provide a comprehensive description of all cell types observed in the epidermis of the blade. Furthermore there is very little information in previous studies on sheath epidermal cell types. Hence we considered it necessary to characterize the blade and sheath epidermal cells for 'Himalaya' barley according to their length, and their positions relative to different vein types, stomatal rows and sclerenchyma.

This paper provides a detailed description of the mature blade and sheath epidermis of the first leaf of the parent variety 'Himalaya'. The data form the basis for comparisons with mutants in this genetic background. The accompanying paper compares epidermal anatomies of mutants varying in mature leaf length (Wenzel *et al.*, 1996).

Correspondence to: Dr P. M. Chandler, CSIRO Division of Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia.

MATERIALS AND METHODS

Plant material and growth conditions

Barley (*Hordeum vulgare* L. 'Himalaya') plants were grown in pots containing soil in a growth cabinet. A 12 h daylength was maintained (photosynthetic photon flux density 400–450 μmol m^{-2} s^{-1}) with temperatures of 17 °C day, 15 °C night. The plants were grown until leaf one (L1) was fully expanded.

Preparation of leaves for microscopy

L1 was removed from five plants and quickly photocopied. The leaves were cut into 4 cm lengths, cleared in absolute methanol at about 60 °C and then immersed in 85% aq DL-lactic acid (Aldrich Chemical Co.; modified from Kevekordes, McCully and Canny, 1988). Randomly selected cleared leaves were compared to the photocopied image to show that there was no reduction in leaf length during clearing.

Measurements of leaves

The photocopied images were used to determine the length of the blade and sheath, and width of the blade. A scale of 0% to 100% blade (ligule to tip) or sheath (base to ligule) length was used to standardize the positions of analysis along the leaves of the different plants. Blade width was measured at 33% and 66% blade length. Sheath width was not measured since the sheath was often torn by the growth of subsequent leaves.

0305-7364/97/010041+06 $25.00/0 bo960300

All cell measurements were determined using bright field optics (100 × magnification) on a Zeiss compound microscope fitted with an ocular scale. Preliminary studies of the cell lengths of selected cell types measured along the blade or sheath length showed that cell length of some cell types varied with position along the leaf. However, measurements taken at 33% and 66% blade or sheath length adequately represented the average cell length of the different cell types. Hence for each plant the lengths of 25 cells of each different cell type (see Results for description of cell types) were measured at 33% and 66% blade or sheath length on both surfaces of the blade, and on the adaxial surface of the sheath. Cells on the abaxial surface of the sheath were not measured since trichomes and sclerenchyma occurred on this surface apparently at random, preventing consistent anatomical observations of similar cell types. The numbers of files of the different cell types and the numbers of veins across the blade were determined at 33% and 66% blade length. The number of cell files over the midrib was not determined.

Statistical analyses

For each variable the appropriate analysis of variance was carried out according to the experimental design described above. From these analyses particular summary statistics have been selected and examined. Means are shown with the 95% confidence interval. In some instances it was necessary to take logarithms of the data to ensure that the assumptions which underlie the analysis of variance were satisfied.

Photography

A dental impression material (3M IMPRINT, 3M Australia Pty. Ltd.) was applied to both surfaces of the mid regions (approx. 50% length) of the blade and sheath of mature first leaves. The material was left for 5 min to set, peeled off and sonicated in concentrated sodium hypochlorite for 30 min to remove all plant tissue. These negative moulds were washed in water, air-dried and filled with Spurr's resin which was polymerized at 70 °C overnight. The negative moulds were peeled from the positive resin replicas. The positive replicas were mounted on stubs with conductive paint, sputter coated with gold and examined using a JEOL JSM-6400 scanning electron microscope.

TABLE 1. *Explanation of abbreviations defining cell types and positions*

Abbreviation	Definition
S	small vein
L	large vein
MR	mid rib
ov	over vein
ovS	over small vein
ovL	over large vein
ovMR	over mid rib
ovs	over vein and next to sclerenchyma
ovn	over vein and next to lateral cell
sc	sclerenchyma
scS	sclerenchyma over small vein
scL	sclerenchyma over large vein
ovsS	adjacent to sclerenchyma over small vein
ovsL	adjacent to sclerenchyma over large vein
bv	between veins
bvn	between veins and next to lateral cell
s	stomata (guard cell and lateral subsidiary cells)
g	guard cell
sr	stomatal row
is	interstomatal cell
lc	lateral cell
sh	sheath

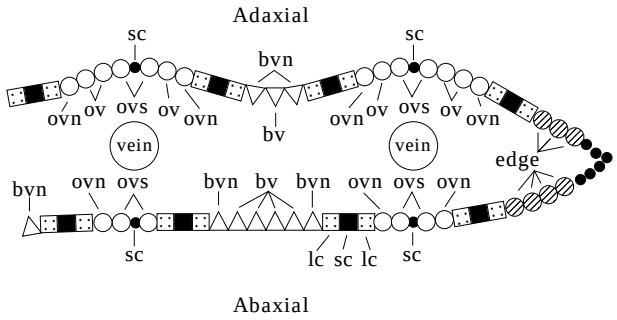

FIG. 1. Diagrammatic representation of a cross section through the blade of L1 showing different epidermal cell types. The majority of cells were initially defined as over veins (ov), between veins (bv), stomatal rows (sr) with lateral cells (lc), sclerenchyma (sc), and edge cells. Cells over and between veins were subdivided according to adjacent cells: bvn and ovn cells are next to lc cells; and ovs cells are adjacent to sclerenchyma. The cells over the veins would be further characterized according to their position over small (S), large (L) or midrib (MR) veins. The bv and bvn cells on the adaxial surface are bulliform cells. Not drawn to scale. ⊡ ■ ⊡, Stomatal row (sr) and the lateral cells (lc); △, cells between vein (bvn, bv); ○, cells over veins (ovn, ovs, ov); ●, sclerenchyma (sc); ▨, edge cells.

RESULTS

Leaf dimensions

The mature blade of L1 was approx. 10 cm long and the sheath 3.5 cm long. The blade was approx. 9.5 mm wide at both 33% and 66% blade length.

Classification of blade epidermal cell types

The veins provided the framework upon which the characterization of epidermal cell types was based. The veins were classified as the midrib (MR), and as small (S) or large (L) veins with diameters, including the mestome sheath cells, of < 40 μm and > 40 μm, respectively. This division of veins into S and L types is an oversimplification (Kuo, O'Brien and Canny, 1974; Dannenhoffer, Ebert Jr, and Evert, 1990), but was felt to be adequate for use in these studies. In general, our 'small veins' correspond to the small veins and small intermediate veins described previously, and 'large veins' to the large intermediate veins and large veins (Kuo *et al.*, 1974; Dannenhoffer *et al.*, 1990). There were more small veins (approx. 12) than large veins (approx. five) across the barley blade.

The blade epidermal cells were classified according to their positions relative to the veins, stomatal rows and sclerenchyma cells. Figure 1 and Table 1 outline the blade

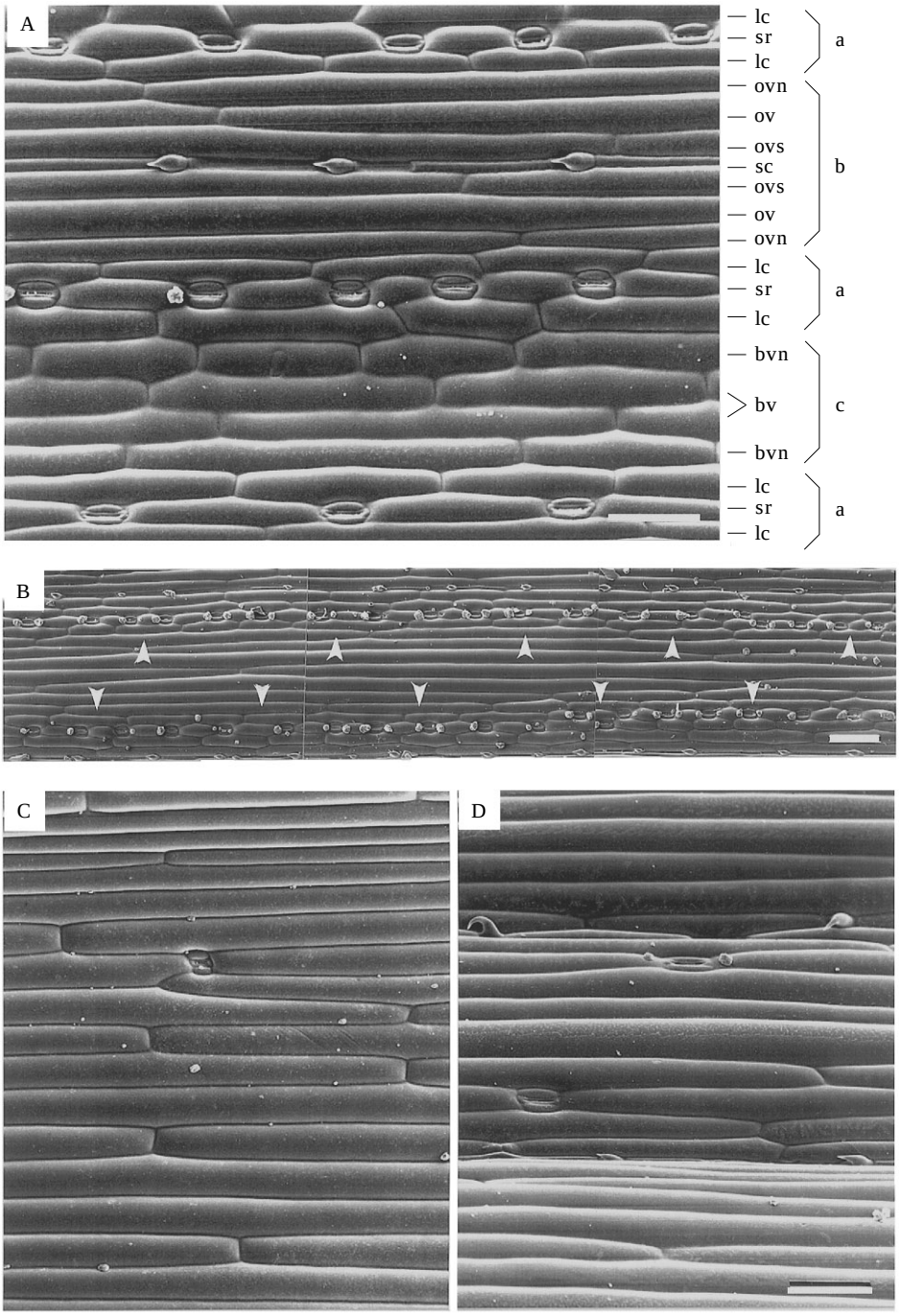

FIG. 2. SEM micrographs at approximately 50% blade (Fig. 2A, B) or sheath (Fig. 2C, D) length of mature L1 of 'Himalaya' barley. Fig. 2A shows the different cell types of the adaxial blade epidermis. The cells are grouped together as (a) sr and lc, (b) cells over the veins, and (c) cells between veins. The cells over the veins could not be characterized according to vein types since the replicas show epidermal details only. Bar = 100 μm. Fig. 2B shows the abaxial blade epidermis. There are stomatal rows changing files on either side of the cells lying between veins. When lc and bvn cells occurred within the same file, the bvn cells were always progressively shorter with increasing proximity to the lc cells (arrows). Bar = 200 μm. For Fig. 2C and D the cell types could not be labelled since these surface replicas do not show the position of veins. Bar = 100 μm. Fig. 2C is the adaxial surface showing only one stoma. Fig. 2D is the abaxial epidermis showing stomata and sclerenchyma and trichomes.

cell types and their abbreviations used throughout this paper. Epidermal cells were initially classified as those lying over (ov) or between (bv) veins (Figs 1 and 2A). The cells lying between the veins (bv) on the adaxial surface of the blade were the bulliform cells (Figs 1 and 2A). The bv cells on the abaxial surface were long cells about 2 mm in length (see later). The cells lying over the veins (ov) on both abaxial and adaxial surfaces were further classified since there were different vein types, and since files of sclerenchyma were present over veins. Hence they were characterized according

to the type of vein (i.e. ovS, ovL, ovMR), or as sclerenchyma cells (e.g. scS or scL, sclerenchyma cells lying over small or large veins respectively) or as cells directly adjacent to the sclerenchyma cells over small and large veins (ovsS or ovsL respectively) (Figs 1 and 2A). The distinction is made between ov cells next to sclerenchyma (ovs) compared with other ov cells on the basis of differences in average cell length (see later). Sclerenchyma cells (sc) were also present at the edges of the blade (Fig. 1). The cells between these sc and the lc (see below) nearest the edge of the blade were termed 'edge' cells (Fig. 1). There were never any trichomes or sclerenchyma for blade epidermal cells between veins.

For the blade epidermis there was generally a stomatal row on both sides of each vein. The stomatal rows (sr) consisted of the stomata (s, i.e. guard cells and lateral subsidiary cells) and interstomatal cells (is), and had a single file of lateral epidermal cells (lc) on either side (Figs 1 and 2A). These lateral epidermal cells had previously been part of the subsidiary mother cell which divided to produce the lateral subsidiary cell. [For terminology of lateral subsidiary cell see Stebbins and Jain, 1960; Tomlinson, 1974. Zeiger (1971) called the lateral subsidiary mother cell a 'lateral cell'. However here we define a lateral cell as one of the two progeny of the lateral subsidiary mother cell]. On the abaxial surface one or both stomatal rows were occasionally absent from over the outermost veins. In this case the distinction between edge cells and those lying over the veins was arbitrary. Infrequently there were intervals of about five to ten cells within a sr with no stomata, or two sr were separated by a single common lc file.

The file of cells next to the lc, excluding the sr, was classified as either 'bvn' or 'ovn' cells (Figs 1 and 2A). These cells were distinguished from bv or ov cells which would be situated at least one cell file away from the lc (Figs 1 and 2A). The distinction was made because of differences in average cell length (see later), and because of the greater variability in cell length for the ovn and bvn cells compared to that for ov and bv cells, respectively. Variation in cell length occurred when the cells within such a file changed from one cell type to another associated with a sr occasionally changing to an adjacent file (Fig. 2B). The cells within the ovn or bvn files reached progressively shorter final cell lengths prior to the formation of lc cells (and *vice versa* for the opposite transition; Fig. 2B). The only exception to this was for bvn cells on the adaxial surface which were about the same size as the lc cells (Fig. 3).

If there was only one cell between an sc file lying over a vein and the lc of the adjacent sr, then this cell was arbitrarily classified as ovs rather than ovn (see abaxial surface of left hand vein in Fig. 1). Sometimes there were no bv or bvn cells between the MR and the adjacent vein.

Classification of sheath epidermal cell types

The sheath adaxial epidermis had very few stomata and hence no sr. Also, there were neither bulliform cells nor any sclerenchyma (Fig. 2C). The adaxial sheath cells were classified as either shbv, shovS, or shovL. If stomata were present, cell length measurements were taken on cells separated by at least two cell files from the stomata. The long, thin cells in the region where the longitudinal splitting of the tubular leaf sheath occurred were not examined. The abaxial epidermis had many trichomes and sclerenchyma lying both over veins and between veins which resulted in irregular epidermal cell lengths (Fig. 2D).

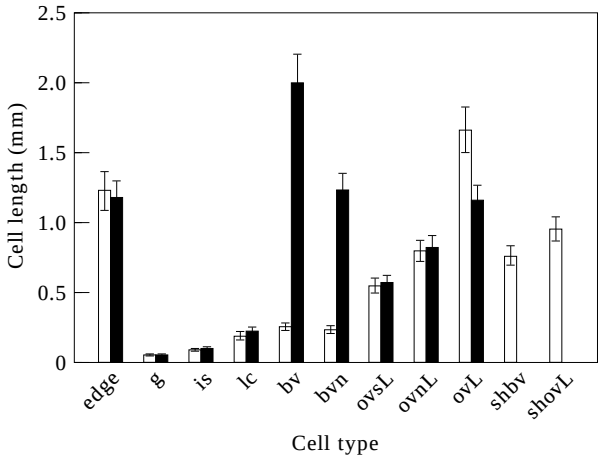

FIG. 3. Lengths of different cell types on the adaxial (□) or abaxial (■) surfaces of the blade, and the adaxial surface of the sheath. Average of measurements made at 33% and 66% blade or sheath length. $n = 250$, mean and 95% confidence limits are shown.

Cell lengths

At any given position along the blade the different cell types varied in length from about 50 μm to over 2 mm (Fig. 3). The cells in, or directly adjacent to, the stomatal rows (g, is, lc) were amongst the shortest, from 50 μm to about 200 μm in length. The edge cells were about 1 mm in length (Fig. 3).

The main difference between the adaxial and abaxial surfaces of the blade was that the bulliform cells (bv and bvn) on the adaxial surface were much shorter than the bv and bvn cells on the abaxial surface of the blade (Fig. 3). This difference made it easy to distinguish between the two surfaces of the blade. Also, the ovS and ovL cells were generally longer on the adaxial than on the abaxial surface (e.g. Fig. 3).

The bvn cells on the abaxial surface varied in length from about 200 μm to over 3 mm because the cells in these files were shorter prior to the formation of lc associated with occasional changes of stomatal rows from one file to another (Fig. 2B). In contrast, the bv cells on the abaxial surface were never shorter than 1 mm. Hence on the abaxial surface the bv cells were on average longer than the bvn cells (Fig. 3). There was no obvious corresponding difference between bv and bvn cells on the adaxial surface since they were already about the same length (200 μm) as the lc (Fig. 3).

Generally the cells next to the sclerenchyma (ovs) and the ovn cells were shorter than the ov cells (Fig. 3). The ovn cells also varied greatly in length from 200 μm to approx. 2 mm because, as with the bvn cells, some were shorter prior to the formation of lc cells (Fig. 2B). Vein size (small *vs.* large) made no difference to the cell lengths of the ovs, ovn and ov

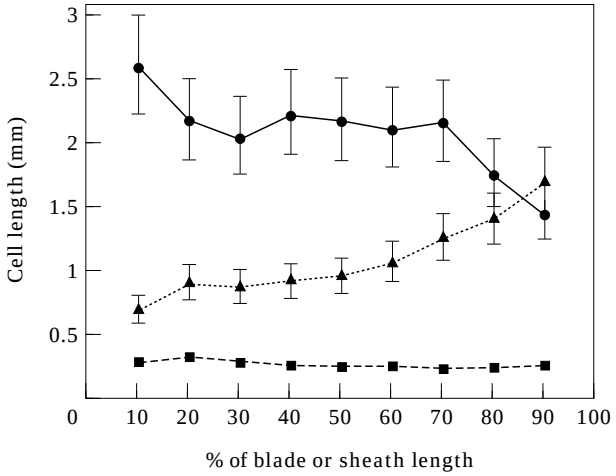

FIG. 4. Variation in cell length with position along the blade or sheath of L1 'Himalaya' barley for three cell types on the abaxial (ab) or adaxial (ad) surface. $n = 125$, mean and 95% confidence limits are shown. (■) bv(ad); (▲) shbv(ad); (●) bv(ab).

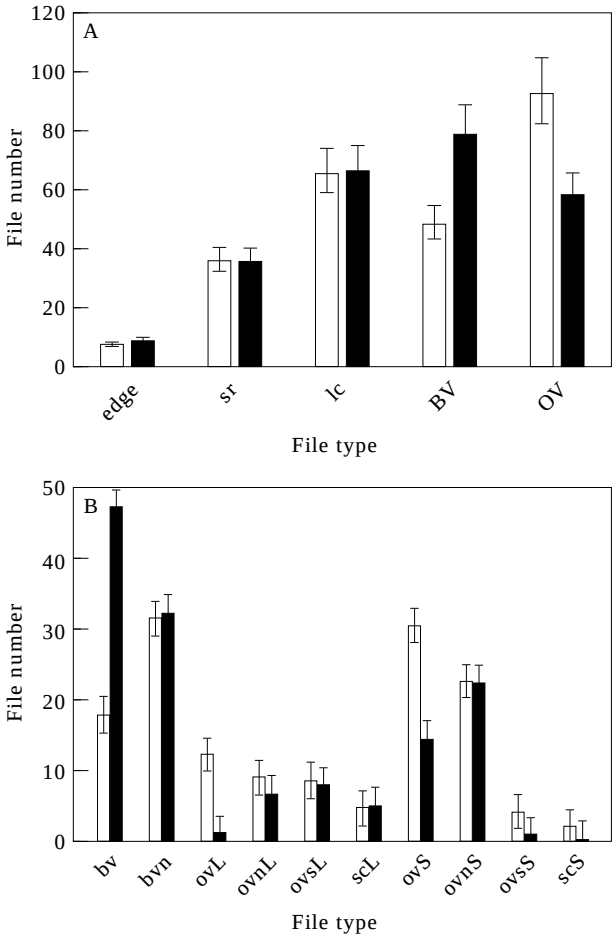

FIG. 5. Number of cell files across the blade of L1 of 'Himalaya' barley for the adaxial (□) and abaxial (■) surfaces. A, Cell types lying between or over the veins were grouped together, i.e. (BV = bv + bvn) and (OV = ov + ovn + ovs). Average of measurements made at 33% and 66% blade length. $n = 10$, mean and 95% confidence limits are shown. B, Individual cell types lying over and between the veins are shown. Average of measurements made at 33% and 66% blade length. $n = 10$, mean and 95% confidence limits are shown.

cells of the blade (data not shown). However, since there were often fewer files over large than small veins (see later) this resulted in most cells over large veins being either ovsL or ovnL cells, which are shorter than ovL cells. Hence cells over large veins appeared to have a shorter average cell length (of all cell types) than over small veins because in general the small veins had more of the longer ov cells.

At any given position along the sheath there was little difference in length of the different cell types (shbv, shovS and shovL) for the adaxial sheath (e.g. Fig. 3). On average all adaxial sheath epidermal cells were about 1 mm long.

Lengths of some cell types varied with position along the blade or sheath (Fig. 4). For example, the bv cells on the abaxial surface of the blade were shorter near the tip than at the ligule, whereas the bulliform cells on the adaxial surface were the same length at all positions. All cell types of the adaxial surface of the sheath tended to increase in length from 0% to 100% sheath length. Although some cell types did vary in length at different positions along the leaf, average cell lengths taken at 33% and 66% blade or sheath length still provided a statistically valid measure for comparison between cell types.

Cell file number across the blade

There was no significant difference in total file number across the adaxial or abaxial surface of the blade at 33% or 66% blade length (data not shown). The only difference between the two surfaces of the blade was that the adaxial surface had fewer files between the veins than over the veins and *vice versa* for the abaxial surface (Fig. 5A). This difference resulted mostly from changes in the number of bv, ovS and ovL file types (Fig. 5B). Since there were fewer files between veins on the adaxial surface there were more bvn than bv files (Fig. 5B) and often no bv files lying between two veins. Since there were fewer large veins than small veins, there was a smaller total number of files over large veins than over small veins (Fig. 5B). Frequently the abaxial surface did not have any ovL but only ovnL and often scL and ovsL as well (Fig. 5B).

DISCUSSION

Previous studies characterized blade epidermal cell types in relation to the veins. Metcalfe (1960) distinguished between costal (over the vein) and intercostal (between the vein) cells. Percival (1921) described the cells on the wheat adaxial blade epidermis as either cells lying over the ridges (over veins) or cells in the furrows between ridges. The latter description proved inadequate for the abaxial blade epidermis because it is relatively flat. These reports did not further characterize the cell types except for mentioning the presence of stomatal rows, sclerenchyma files and bulliform cells.

Silvy (1982) characterized the barley leaf epidermis in terms of regions of cells lying between stomatal rows, instead of using venation as the basis for classification of cell

types. The adaxial epidermis was described as having files of long cells alternating with files of short cells. These would correspond to cells lying over veins (ovs, ovn, ov) and the bulliform cells (bv, bvn) between the veins, respectively. We would also further characterize the cells lying over veins according to vein type (S, L, MR). For the abaxial blade epidermis Silvy (1982) described only long cells in the positions which we would distinguish as lying over or between veins. Silvy also mentions epidermal cells bordering the stomatal rows (here called lateral cells, lc) as well as stomatal and interstomatal cells. Where comparisons can be made, the measurements of the lengths of the cell types reported by Silvy were similar to those found in this study indicating that the two barley varieties were similar in many respects. However, our study has distinguished many more cell types over and between veins. Hence Silvy's measurements of cell lengths, particularly for the abaxial surface for which all cells lying over and between veins were grouped together, are not as precise as those reported here.

This study has revealed several novel features of epidermal anatomy in barley. Previous reports have not described the blade cell types lying next to the stomatal rows, i.e. the bvn and ovn cells. These cells differed from bv and ov cells since they varied greatly in length and were, on average, shorter than the bv and ov cells (except for the bv cells on the adaxial surface, i.e. the bulliform cells). Classifying epidermal cells over veins as ov, ovs or ovn cells with further distinction made according to vein type (S, L, MR), provides a more detailed description of these cells than in previous studies. To our knowledge, there are also no previous descriptions or measurements of the barley blade edge cells nor of the barley sheath epidermal cell types.

We are also unaware of other reports showing the variation in file number across the blade for the different cell types. This study showed how the adaxial blade had fewer cells between the veins than over the veins and *vice versa* for the abaxial surface (Fig. 5A). Consequently there were almost no ovL cells on the abaxial surface (Fig. 5B). There were also very few ovsS and sclerenchyma files on either surface of the blade (Fig. 5B). Hence these cell types would not be convenient for comparing epidermal cell lengths between different barley lines or treatments.

Overall, the four major surfaces of the barley leaf (sheath/blade, abaxial/adaxial) had four different epidermal patterns. The blade adaxial epidermis had alternating intervals of predominantly long cells over the veins and much shorter cells (bulliform cells) between veins. The blade abaxial epidermis had mostly long cells lying both over and between veins. The blade cells lying over and between veins were easily distinguished since the blade sclerenchyma cells only occurred over veins and at the edge of the blade. In addition, on the abaxial surface there were fewer files over the veins than between veins. The sheath epidermis, unlike the blade, had no stomatal rows and very few stomata especially on the adaxial surface. The adaxial surface of the sheath had cells of fairly uniform length at any given position giving it a smooth homogenous appearance, and had no sclerenchyma. In contrast, the abaxial sheath epidermis had many schlerenchyma cells and trichomes which resulted in a greater heterogeneity of cell length (since cells next to sclerenchyma were generally short) compared with the sheath adaxial epidermis. These general features gave each part of the barley leaf epidermis a distinct pattern.

In conclusion, we have extended the work of previous investigators in characterizing barley leaf epidermal cell types and described in detail the L1 epidermis of 'Himalaya' barley. This detailed description was essential before comparisons could be made with mutants generated in a 'Himalaya' background that potentially differ in epidermal cell length and number (Wenzel *et al*., 1996).

ACKNOWLEDGEMENTS

We thank C. Donnelly for help with statistical analyses, J. Whitehead for Fig. 1, S. Craig, A. Lewis and C. Miller for Fig. 2, and D. Bittisnich, M. J. Canny, A. L. Cleary, M. E. McCully, and R. E. Williamson for useful comments.

LITERATURE CITED

Bret–Harte MS, Talbott LD. 1993. Changes in composition of the outer epidermal cell wall of pea stems during auxin-induced growth. *Planta* **190**: 369–378.

Dannenhoffer JM, Ebert W Jr, Evert RF. 1990. Leaf vasculative in barley, *Hordeum vulgare* (Poaceae). *American Journal of Botany* **77**: 636–652.

Kevekordes KG, McCully ME, Canny MJ. 1988. The occurrence of an extended bundle sheath system (paraveinal mesophyll) in the legumes. *Canadian Journal of Botany* **66**: 94–100.

Kuo J, O'Brien TP, Canny MJ. 1974. Pit-field distribution, plasmodesmatal frequency, and assimilate flux in the mestome sheath cells of wheat leaves. *Planta* **121**: 97–118.

Kutschera U. 1992. The role of the epidermis in the control of elongation growth in stems and coleoptiles. *Botanica Acta* **105**: 246–252.

Metcalfe CR. 1960. *Anatomy of the monocotyledons. I. Gramineae*. London: Oxford University Press.

Percival J. 1921. *The wheat plant. A monograph*. London: Duckworth & Co.

Silvy A. 1982. La premiere feuille des plantules d'orge. I. Morphogenese, les epidermes. *Canadian Journal of Botany* **60**: 2864–2876.

Stebbins GL, Jain SK. 1960. Developmental studies of cell differentiation in the epidermis of monocotyledons. I. *Allium*, *Rhoeo*, and *Commelina*. *Developmental Biology* **2**: 409–426.

Taiz L. 1984. Plant cell expansion: Regulation of cell wall mechanical properties. *Annual Review of Plant Physiology* **35**: 585–657.

Tomlinson B. 1974. Development of the stomatal complex as a taxonomic character in the monocotyledons. *Taxon* **23**: 109–128.

Wenzel CL, Chandler PM, Cunningham RB, Passioura JB. 1997. Comparative leaf epidermal anatomy of mutants of barley (*Hordeum vulgare* L. 'Himalaya') which differ in leaf length. *Annals of Botany*, **79**: 45–50.

Zeiger E. 1971. Organelle distribution and cell differentiation in the formation of stomatal complexes in barley. *Canadian Journal of Botany* **49**: 1623–1625.