

Genotype Differences in the Proportion of Different Tissues in the Leaves of Spring Barley

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Abstract. A number of markers characterizing the quantitative anatomical structure of the blade of the first and the third leaf were measured in young plants of eight cultivars of spring barley, *Hordeum vulgare* L.

The number of vascular bundles was in significant negative correlation with the mean distance between two adjoining bundles ($r = -0.8$) and also with their area in the cross section ($r = -0.82$). The proportion of the thickness of the epidermis in the thickness of the leaf and the proportion of the volume of the epidermis in the total volume of the leaf was in the order of 0.25 to 0.30. The proportion of the volume of the photosynthetic tissues in the total volume of the first (or the third) leaf fluctuated from 0.51 (0.53) to 0.58 (0.63). The thickness of the leaf blade and the thickness of the particular leaf tissues and their relative voluminal proportion significantly differed in the basal (B), middle (M), and apical (A) parts. No significant correlations were found between the values of the parameters of the first and the third leaf. Significant differences between genotypes concern most of the measured characters. No tendency to unequivocal changes between older and new genotypes could be inferred from the results obtained.

The results are discussed with regard to the necessity of a quantitative description of anatomical structures for the elucidation of physiological processes.

The leaf blade which is an important anatomical unit of the plant is a very complicated system. Its deeper understanding requires the analyses of tissues, cells and subcellular and molecular structures (PASSIOURA 1979). Whereas most investigations of processes at the level of membranes and organelles were accompanied by studies of the structures, such studies were rather exceptional when investigations were carried out at the level of tissues or whole leaves. It can be stated with some degree of simplification that the basic data on the functional structure of the leaf were already obtained in the last century (HABERLANDT 1918). But only the 1980's have brought some "renaissance" of interest of plant physiologists in the "dynamic and quantitative" anatomy of the leaf, that is quantitative description of structures including their development and changes in dependence on environmental factors (e.g. NOBEL 1977, 1980, JELLINGS and LEECH 1982, JURIK *et al.* 1982, PARKHURST 1982).

The growing interest in the relationship between the anatomical structure and physiological processes may be exemplified by studies of connections

between the rate of photosynthesis and different anatomical characteristics (EL-SHARKAWY and HESKETH 1965, WILSON and COOPER 1969a,b, EVANS and DUNSTONE 1970, SASAHARA 1982 a, b).

Of course, we are only at the beginning of our understanding: for example the influence of water deficit (NOBEL 1980) or nitrogen deficiency (PAZOUREK and NÁTR 1981) on the anatomical structure of the leaf was described. But the mechanism which would enable the description of the entire sequence of reactions starting with changes in root substrate and ending with changes in the rate of cell wall growth and of its type in particular tissues is nearly or completely unknown to us.

At the present time, quantitative data on the basic anatomical structure of the leaf are still lacking. Because there are significant differences not only among plant genera and species (JELLINGS and LEECH 1982) but also among cultivars, we report in this paper on a set of parameters describing some structures of the leaf blades of young plants of eight genotypes of spring barley.

MATERIAL AND METHODS

Young plants of spring barley *Hordeum vulgare* L. grown in Hoagland's nutrient solution 3 were employed in the experiments. The following genotypes were selected for investigation: 1. Selekční hanácký; 2. Proskovcův hanácký; 3. Valtický; 4. Favorit; 5. Korál; 6. HE 2553; 7. HE 2107; 8. HE 1562.

Description of the genotypes, of their cultivation, sampling and sample analyses was reported earlier (NÁTR and PAZOUREK 1986). Vascular bundles, similarly as the other measured characters of the leaf, were measured in cross sections of the leaf in its middle part. Measurements were carried out using an eye piece micrometer, as usually. The proportion of particular tissues in the leaf was determined in cross sections of the leaf in the basal, middle and apical parts of the leaf by means of the stereological point counting method with the interval of points $a = 0.1$ mm.

RESULTS

Vascular bundles are a very important element of the leaf structure. Their number fluctuated in the first leaf of the genotypes under investigation between 13.6 (cv. 6) and 17.0 (cv. 1). The number of vascular bundles was indirectly proportional ($r = -0.848$) to the distance between them (Table 1).

In the third leaf, the number of vascular bundles was higher, with the boundary values of 17.2 (cv. 8) and 22.2 (cv. 2). Negative correlation between the number of vascular bundles and average distance between them was also found in the third leaf: $r = -0.803$.

The shape of the cross section of the bundles is only moderately elliptical. The lengths of the maximal diameters of the cross sections are very similar in two directions perpendicular to each other. If the area of the cross section is calculated as that of a circle with a diameter that equals the mean value of the measured axes (Table 1), the areas obtained correspond in the first leaf to values ranging from 2.5 % (cvs. 1 and 6) to 3.3 % (cv. 8) of the total area of the cross section of the leaf. These values are somewhat lower than the data on the proportion of the volume of conductive tissues in the total volume of the leaf (Table 3).

TABLE 1

Basal parameters of leaf blade and of vascular bundles of the first and the third leaf of eight genotypes of spring barley. $\bar{x}$ represents the value of the mean of all genotypes, % min (% max) represents the lowest (the highest) value of the genotype in % of the mean of all genotypes (= 100 %), respectively

Genotype no.	Number per leaf width	Distance between bundles [mm]	Vascular bundles Dimensions of cross sections of vascular bundles in two directions perpendicular to each other	Area of cross section of vascular bundles [mm ² × 10 ⁻³]	Leaf width [mm]	Area of cross section of the leaf [mm ²]	Proportion of bundle cross section area in leaf cross section area [%]	
1	17.0	0.29	0.044	0.043	25.262	5.07	1.01	2.50
2	15.8	0.32	0.043	0.045	24.032	5.11	0.91	2.64
3	16.2	0.31	0.042	0.040	21.380	4.67	0.84	2.55
4	16.8	0.31	0.043	0.041	23.285	5.01	0.85	2.74
5	16.4	0.33	0.048	0.047	29.061	4.94	0.98	2.97
6	13.6	0.42	0.056	0.055	32.898	5.84	1.34	2.46
7	16.0	0.33	0.044	0.045	24.880	4.75	0.95	2.62
8	14.0	0.34	0.047	0.049	25.340	3.89	0.77	3.29
$\bar{x}$	15.73	0.33	0.046	0.046	25.767	4.91	0.96	2.72
% min	86.46	87.88	91.30	86.96	83.01	79.23	80.21	90.44
% max	108.07	127.27	121.74	119.57	127.69	118.94	139.58	120.96
First leaf								
1	20.2	0.29	0.056	0.056	49.753	5.53	1.21	4.11
2	22.2	0.26	0.052	0.053	48.063	6.09	1.15	4.18
3	17.8	0.30	0.051	0.052	37.077	4.68	0.98	3.78
4	21.0	0.24	0.044	0.040	29.085	3.87	0.81	3.59
5	19.2	0.28	0.048	0.049	35.482	4.00	0.92	3.86
6	18.0	0.28	0.052	0.054	39.708	3.60	0.82	4.84
7	19.0	0.29	0.047	0.046	32.262	3.90	0.66	4.89
8	17.2	0.32	0.056	0.057	43.120	4.99	0.94	4.39
$\bar{x}$	19.33	0.28	0.051	0.051	39.320	4.58	0.94	4.23
% min	88.98	85.71	86.27	78.43	74.00	78.60	70.21	84.87
% max	114.85	114.29	109.80	111.76	126.53	132.97	128.72	115.60
Third leaf								

TABLE 2

Thickness of the leaf and of different leaf tissue types in the first and the third leaf of eight genotypes of spring barley. For other details ($\bar{x}$, % min, % max) see Table 1

	Genotype no.	Thickness of the leaf [mm]	Thickness of the epidermes [mm]		total	Thickness of the photosynthetic parenchyma + conductive tissues	
			AD	AB		[mm]	[% of leaf thickness]
First leaf	1	0.20	0.028	0.026	0.054	0.146	73.0
	2	0.18	0.027	0.031	0.058	0.122	67.8
	3	0.18	0.026	0.028	0.054	0.126	70.0
	4	0.17	0.025	0.042	0.067	0.103	60.6
	5	0.20	0.031	0.028	0.059	0.141	70.5
	6	0.23	0.028	0.031	0.059	0.171	74.3
	7	0.20	0.025	0.029	0.054	0.146	73.0
	8	0.20	0.028	0.031	0.059	0.141	70.5
	$\bar{x}$	0.195	0.027	0.031	0.058	0.137	69.96
	% min	87.18	92.59	83.87	93.10	75.18	86.62
	% max	117.95	114.81	135.48	115.52	124.82	106.20
Third leaf	1	0.22	0.024	0.027	0.051	0.169	76.8
	2	0.19	0.027	0.026	0.053	0.137	72.1
	3	0.21	0.023	0.026	0.049	0.161	76.7
	4	0.21	0.022	0.027	0.049	0.161	76.7
	5	0.23	0.022	0.023	0.045	0.185	80.4
	6	0.23	0.027	0.028	0.055	0.175	76.1
	7	0.17	0.023	0.024	0.047	0.123	72.4
	8	0.19	0.024	0.027	0.051	0.139	73.2
	$\bar{x}$	0.206	0.024	0.026	0.050	0.156	75.55
	% min	82.52	91.67	88.46	90.00	78.85	95.43
	% max	111.65	112.50	107.69	110.00	118.59	106.42

The proportion is somewhat higher in the third leaf, ranging from 3.6 % (cv. 4) to 4.9 % (cv. 7).

The mean thickness of the first leaf is 0.195 mm, whereas the mean value of the third leaf of all genotypes is 0.206 mm (Table 2). Both epidermes occupy a large proportion of the total thickness of the leaf. The sum of the adaxial and abaxial epidermis in the first leaf amounts to 0.054 mm to 0.067 mm. The epidermis is somewhat thinner in the third leaf. The above values show that the proportion of both the epidermes in the total thickness of the leaf is nearly one third. The thickness of the other tissues (including intercellular spaces) between the two epidermes is generally the same when expressed in relative terms (on the average 70 to 75 %), but its absolute values vary from 0.103 mm (cv. 4) to 0.171 mm (cv. 6) in the first leaf, and from 0.123 mm (cv. 7) to 0.185 mm (cv. 5) in the third leaf.

The proportion of the particular tissues in the leaf can best be estimated when their volume is expressed in terms of relative values of the total volume of the leaf (Table 3). In agreement with the data on the thickness of the epidermis, its proportion calculated as the mean of all cultivars is 32.8 % in the first leaf and 25.4 % in the third leaf. The largest proportion of the leaf volume is occupied by the photosynthetic tissue, the proportions of the

TABLE 3

Proportions of different leaf tissues in % of the total leaf blade volume of the first and the third leaf

	Genotype no	Epidermis	Photo-synthetic parenchyma	Conductive tissues	Sheaths of the vascular bundles	Sclerenchyma
First leaf	1	34.5	52.0	5.5	7.0	0.9
	2	32.3	55.0	4.1	7.7	0.9
	3	32.0	57.3	3.9	6.4	0.6
	4	31.7	54.5	4.6	7.8	1.4
	5	32.6	55.4	4.0	6.6	1.3
	6	31.2	56.5	3.2	8.1	1.0
	7	36.1	51.3	4.0	8.3	0.3
	8	31.8	57.7	3.5	6.3	0.7
Third leaf	1	25.2	60.1	4.8	7.7	2.3
	2	23.0	60.8	5.6	8.6	2.0
	3	24.8	60.6	5.5	8.1	1.1
	4	28.3	56.3	8.3	6.3	0.9
	5	21.9	62.7	6.1	7.2	2.2
	6	23.8	60.9	5.7	8.1	1.5
	7	31.6	52.9	5.8	7.9	1.8
	8	24.9	60.2	5.0	8.5	1.4

conductive tissues, the sheaths of vascular bundles and the sclerenchyma being only small. The proportion of the photosynthetic tissue ranges in the first leaf from 51.3 % (cv. 7) to 57.7 % (cv. 8), and in the third leaf from 52.9 (cv. 7) to 62.7 % (cv. 5).

Absolute values of the volume of conductive tissues are very small and their proportion in the total volume of the leaf, when expressed in relative terms, is on the average 4.1 % in the first leaf of all cultivars and 5.85 % in the third leaf. However, there are marked differences among cultivars in this character; for example, the volume of conductive tissues in the first leaf of cultivar 6 is only 69 % of that of cv. 4. With cultivar 4 also the highest value of the volume of conductive tissues was recorded in the third leaf, which was nearly twice as high as the lowest value found in cv. 1.

Whereas the above data on the proportions of tissues were related to the average of the entire leaf blade, Table 4 gives values for the basal (B), middle (M), and apical (A) parts of the blade. A marked heterogeneity can also be observed in this case, with different gradients occurring in different genotypes. The proportion of the epidermis in the total volume is in most cases highest in the apical part and lowest in the basal part, both in the first and in the third leaf. The absolutely highest value was found in the apical part of the first leaf of cv. 7, namely 43.8 %. That means that almost one half of the volume of the leaf is occupied by the epidermis. In contrast, in a number of cultivars the epidermis occupies as little as 20 % of the volume of the leaf in different parts of the third leaf, that is only one fifth of the volume of the leaf. There are marked differences in the gradients among genotypes also in this case.

TABLE 4
Proportions of leaf tissues in the basal (B), middle (M), and apical (A) parts of the leaf in % of the total volume of leaf in each respective part of the leaf

Genotype no.	Part of the leaf	First leaf				Third leaf					
		Epidermis	Photo-synthetic parenchyma	Conduc-tive tissues	Sheats of the vascular bundles	Sele-ren-chyma	Epidermis	Photo-synthetic parenchyma	Conduc-tive tissues	Sheats of the vascular bundles	Sele-ren-chyma
1	A	33.9	53.8	4.7	6.4	1.2	35.6	49.0	7.4	6.7	1.3
	M	33.7	54.9	5.2	5.8	0.4	25.3	61.7	4.4	6.8	1.8
2	B	36.1	47.0	6.3	9.1	1.6	22.6	60.8	4.5	8.9	3.0
	A	42.6	43.7	5.6	8.1	0.0	32.5	51.3	8.8	7.5	0.0
3	M	31.3	58.3	3.8	6.0	0.7	22.2	62.1	5.4	9.2	1.2
	B	28.2	57.0	3.7	9.5	1.6	22.3	61.0	5.3	8.0	3.5
4	A	30.6	58.8	5.3	4.7	0.6	21.7	56.6	6.6	14.6	0.5
	M	34.7	54.7	3.5	6.7	0.5	27.4	59.2	4.9	7.6	0.9
5	B	28.9	59.3	3.6	7.2	0.9	23.5	63.0	5.6	6.6	1.4
	A	32.2	54.2	3.4	8.5	1.7	33.5	52.6	7.5	6.4	0.0
6	M	35.6	52.4	5.1	6.0	0.9	29.8	55.1	8.7	5.6	0.8
	B	26.5	56.8	5.0	9.7	1.9	23.3	60.1	8.1	7.1	1.4
7	A	33.1	55.7	4.3	5.9	1.0	24.0	60.1	6.6	6.6	2.7
	M	33.9	55.9	3.7	5.3	1.2	21.4	64.6	5.0	7.5	1.4
8	B	30.7	54.6	4.1	8.9	1.7	21.5	61.4	7.1	7.1	2.8
	A	38.6	53.3	4.2	3.9	0.0	28.1	55.4	6.6	7.4	2.5
9	M	30.1	59.0	2.7	7.4	0.9	22.8	62.6	6.3	6.7	1.7
	B	28.3	55.1	3.2	11.5	1.8	23.7	60.6	4.6	10.1	1.0
10	A	43.8	43.8	4.5	7.6	0.4	35.5	49.4	7.2	7.2	0.6
	M	32.9	54.6	3.9	8.3	0.2	35.5	48.3	5.5	8.6	2.1
11	B	35.1	52.1	3.6	8.9	0.3	26.3	58.6	5.6	7.5	1.9
	A	36.5	53.2	2.7	7.6	0.0	21.5	58.1	8.6	11.8	0.0
12	M	30.9	59.2	2.7	6.8	0.5	26.3	59.2	4.4	9.2	0.9
	B	29.6	58.7	4.9	4.9	1.8	24.2	61.6	4.9	7.1	2.2

For example, only minute differences in the volumes of different parts of the first and the third leaf were found in cv. 5. By contrast, the proportion of the epidermis in the first leaf of cv. 2 is 28.2 % in B, 31.3 % in M and 42.6 % in A. The third leaf of cv. 4 can also be mentioned in which the values for B, M, and A were 23.3, 29.8, and 33.5 %, respectively.

The heterogeneity of the leaf in the proportion of the photosynthetic tissue can be characterized similarly (Table 4). The gradients between B, M and A do not show a uniform pattern. Among the eight cultivars under investigation, the values recorded in the basal part of the first leaf are the lowest in two cultivars (cvs. 1, 5) and the highest in other two cultivars (cvs. 3, 4). Thus, the proportion of the photosynthetic tissue is usually the highest in the middle part of the blade of the first leaf. A similar pattern was recorded in the third leaf. It can be stated that the relative volume of the photosynthetic tissue is higher in the third leaf than in the first leaf.

The proportion of the sclerenchyma is highest almost without any exception in the basal part of both leaves in all genotypes, the occurrence and proportion of this type tissue being negligible in a number of cultivars in the apical part of the first and the third leaf.

DISCUSSION

The physiological significance of the thickness of the first leaf of spring barley for the formation of grain yields was dealt with in full detail by APEL (1978). The mean thickness of the first leaf of 240 cultivars reported by this author was 0.2641 mm, that is higher than the values obtained in our investigations. The difference is obviously a result of the fact that APEL measured the virtually maximal thickness in the middle of the leaf blade, whereas our data rather correspond to the average thickness along the entire length of the leaf blade.

From the experiments of APEL (1978) it also follows that there are marked cultivar differences in leaf blade thickness. The possibility of unequivocal and generally valid interpretation of the relationship between the thickness of the leaves and their physiological properties, or even the formation of the yield, is also in substance excluded by the experiments conducted by APEL. Simultaneously, great influence of ecological factors must be taken into account, especially the effect of irradiation on leaf thickness (NOBEL 1977). And again, lack of sufficient understanding of the problem is apparent. CHABOT *et al.* (1979) found in *Fragaria* leaves that not the maximal values of irradiance are the factor determining the adaptation of the leaf but the total daily sum of radiation energy. We agree with the statement of the above authors that we know very little about the fact of "how does a leaf know where it is".

APEL (1979) reported the value of average density of vascular bundles in leaves of middle insertion of spring barley to be 3.13 mm^{-1} . That is in good agreement with our data, *i.e.* 3.02 mm^{-1} in the first leaf and 3.54 mm^{-1} in the third leaf.

JELLINGS and LEECH (1982) characterized the anatomical structure of leaves of a number of species of cereals also by the number of cells in particular types of tissues. They found in barley leaves 306 000 cells cm^{-2} in the mesophyll, and 46 000 cells cm^{-2} in the epidermis. The percentage proportion

of mesophyll cells and of epidermis cells in the cross section of barley leaf was 44 % and 23 %, respectively, from the total area of these cross sections. JURIK *et al.* (1982) reported for *Fragaria virginiana* leaves that the proportion of the epidermis in the total volume of the leaf was 21.4 to 28.4 % in dependence on irradiance and mineral nutrition, the proportion of the mesophyll (without intercellular space) 30.9 to 42.5 %, and the proportion of the intercellular space 15.1 to 28.1 %. AZCÓN-BIEITO *et al.* (1981) similarly found that the proportion of the epidermis in the cross section of the leaf of *Lolium perenne* L. equalled 29.6 %. Our results also show a high proportion of the epidermis in the total volume of the leaf, on the average about 30 % (Tables 3, 4). The so-called cellulae bullatae which are an important constituent of grass leaves and which do not occur in the leaves of dicotyledons certainly contribute to the high proportion of the epidermis. If we take into consideration that intercellular spaces are also included in the total volume of the leaf and that epidermal cells have only thin cell walls, it can be inferred that the epidermis of the leaf blades may contain some 50 % of the total amount of water present in this organ. It may be presumed that much of present-day knowledge of the influence of water deficit on leaf physiology need verification with respect to the likelihood of great differences between mean values of the entire leaf blade and of the particular tissues.

NOBEL (1977, 1980) also analyzed the dependence of some parameters on the leaf structure and gas exchange. He emphasized species- and genus-dependent differences in the thickness and in the specific dry mass of leaves. However, he devoted most attention to the development of the "inner assimilative area", *i.e.* to the area of all cell walls adjoining the intercellular space.

Its significance for the transpiration and especially for the diffusion of CO₂ in the process of photosynthesis is indisputable. Not only the proportion of the particular tissues participates here quantitatively, but especially the number and size of particular cells determining the relative inner surface of the leaf.

To conclude the paper, we would like to point out some relationships between individual parameters of the leaf structure. In addition to the above mentioned negative correlation between the number of vascular bundles and their mutual distances ($r = -0.8$ both for the first and the third leaf), significant correlation was also determined between the number of vascular bundles and their size expressed in terms of their area in the cross section: $r = -0.82$. A significant positive correlation was found in the first leaf between the size of the vascular bundle and the thickness of the leaf ($r = 0.89$) or the thickness of the photosynthetic and conductive tissues ($r = 0.80$). Further, there is a very clear relationship between the thickness of the photosynthetic tissue and the leaf thickness in both leaves ($r = 0.98$). By contrast, no significant correlation was determined between the values of the particular parameters in the first and the third leaf. This fact is somewhat surprising, because the existence of similar relationships was proved for stomatal frequency (NÁTR and PAZOUŘEK 1986).

We assume that the above-mentioned correlations reflect certain relationships which can take part in the growth of the leaf and in the formation of particular structures. In our future work we will try to comprehend the mechanisms including the effects of environmental factors determining quantitative anatomical structures.

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