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Stem anatomy and cell wall autofluorescence during growth of three maize (*Zea mays* L.) cultivars

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SUMMARY

The anatomy of the outer zone of the stem of the *Zea mays* cultivars 'Markant', 'Protasil' and 'LG11' was studied. The autofluorescence of the parenchyma cell walls of the stem and of the leaf sheath cuticle in developmental stages of the cultivars were quantitatively analysed.

The anatomical study reveals that 'Markant' possesses more sclerenchyma tissue and smaller ground tissue parenchyma cells than 'Protasil' and 'Lg11', especially in the older stem parts. The cell walls in both tissues, however, are slightly thinner than in the other two cultivars. Anatomically, the stem of all three cultivars is fully developed in 70 days.

Quantification of the autofluorescence shows that the content of phenolics in stem parenchyma cell wall and leaf sheath cuticle of the upper part of the plant increases till about 70 days of culture. In the fourth stem internode, near the ground level, the content of phenolics increases gradually during 160 days of culture. In 'Lg11' the intensity of autofluorescence is low compared to that in 'Markant' and 'Protasil'.

Key-words: anatomy, autofluorescence, lignin, maize, phenolics.

INTRODUCTION

The suitability of maize cultivars for cattle fodder production depends on several characteristics, e.g. stem anatomy, cell wall content and degree of cell wall impregnation with lignin. The knowledge of these characteristics is a prerequisite for improvement of the nutritional value of maize and valuable for genetic engineering.

In combination with chemical treatments, histochemical tests, and *in vitro* fungal degradation of lignin, a relationship has been demonstrated between a change in autofluorescence and lignin degradation (Willemse 1981; Akin *et al.* 1985). Autofluorescence of cell walls is probably due to both the presence of the phenolics polymerized as lignin and of phenolic acids attached to hemicelluloses. Through microspectrophotometry of autofluorescence the emission spectrum of the phenolic substances, especially of the aromatic rings, can be represented in terms of the wave length of maximum emission, of the intensity of that wave length and of the decrease in intensity with time (Willemse 1981). Autofluorescence can be used as an indicator of the changes of cell wall phenolics during the development.

In *Zea mays*, as in other Poaceae, nearly all cell walls of the plant autofluoresce (Willemse 1981). In the stem cell walls, the phenolic acids such as ferulic acid and *p*-coumaric acid are known to be the main causes of autofluorescence (Harris & Hartley 1976; Hartley & Haverkamp 1984). The amount of lignin in maize cell walls depends on the developmental stage of the plant, but also on external factors such as temperature (Struik *et al.* 1985). The developmental pattern of *Zea mays* has been the subject of extensive studies by others (Hayward 1938).

In the present study the anatomy of the outermost stem layers is analysed to evaluate the differences among tissues and genotypes. In addition, a comparative study was made of the changes in autofluorescence of ground tissue parenchyma cell walls and of the leaf sheath cuticle of developmental stages of three *Zea mays* cultivars.

MATERIALS AND METHODS

Three cultivars of *Zea mays* ('Markant', 'Protasil' and 'Lg11') were grown in a greenhouse. The anatomical analysis is based on a 2-month-old (56 days) plant, sown on 8th August, harvested 3rd October and on about 3-month-old (87 days) plants, sown on 8th July and harvested 3rd October. Also 65- and 91-day-old plants, sown 23rd April and 28th March, respectively, both harvested 27th June were used.

Measurements were made using a light microscope with a calibrated ocular micrometer at a magnification of 1000 ×. Only the outermost 1 mm of the stem was analysed. Sections of about 15 μm thick at fixed places along the plant stem, i.e. the morphological 4th, 9th and flag internode (last formed internode before the male influorescence), each about 2 cm beneath the nodal point, were made with a sledge microtome and embedded in Kaiser's gelatin–glycerin.

For the autofluorescence studies 'Markant', 'Protasil' and 'Lg11' sown on 27th January, 28th February, 28th April and 28th May, and all harvested on 3rd June, were used; the plants were then 160, 130, 70 and 40 days old, respectively. One-millimetre thick transverse sections were made from the same stem areas as indicated for the anatomical study. After 70 as well as 130 and 160 days of growth the fourth internode of all three cultivars had a position of 4–10 cm above ground level, the ninth 88–106 cm and the flag internode 225–270 cm. After 40 days of growth the positions were 4–10 cm, 42–45 cm and 46–49 cm, respectively. The collected transverse sections were placed on a slide, no cover-glass was used. Subsequent measurements were performed in the open air according to Willemse (1981). The autofluorescence of an area of $10 \times 10\ \mu m^2$ of the parenchyma cell walls neighbouring an intercellular space was measured, at 1000 or 1200 V using a 40 × objective and 6 × ocular at a fixed spectral maximum of 475 nm. The intensity of autofluorescence was measured in millivolts in at least five different areas with parenchyma cell walls. This value was corrected for background effects by subtracting the value of autofluorescence of a $10 \times 10\ \mu m^2$ area without cell walls. In a similar way the leaf cuticle was studied at a maximum of 490 nm.

RESULTS

Stem anatomy

Based on sections as shown in Fig. 1, Table 1 shows a summary of characteristics of 3-month-old stems of the *Zea mays* cultivars 'Markant', 'Protasil' and 'Lg11' sown on 8th July. Comparable figures resulting in the same tendencies were found for 3-month-old

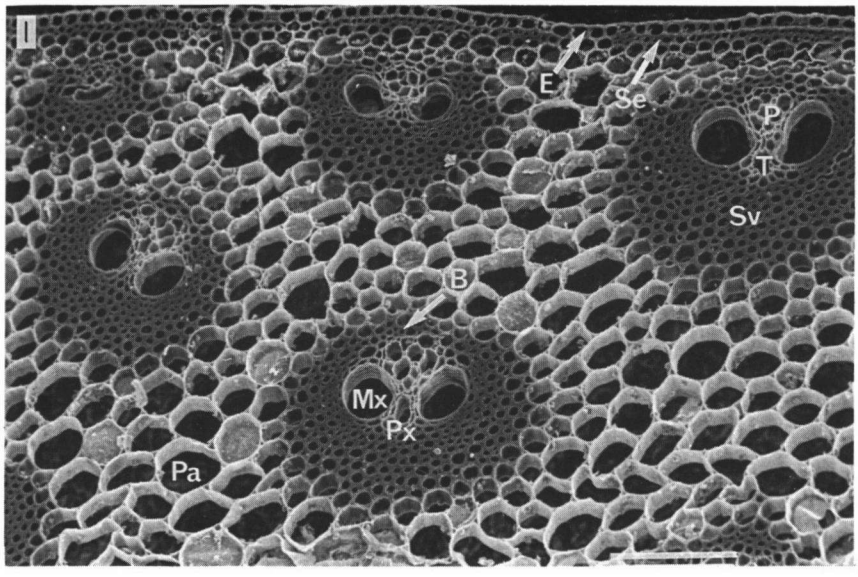

Fig. 1. SEM of an old maize stem cross-section. The figure represents the analysed zone. E = epidermis, Se = sclerenchyma below the epidermis, Sv = sclerenchyma around the vascular bundle, P = phloem, Px = protoxylem, Mx = metaxylem vessels, T = metaxylem tracheids, B = bundle sheath, Pa = parenchyma. Bar represents 100 μm.

plants sown on 28th March. The differences between the analyses of 2- and 3-month-old plants are very small.

If we consider only the more pronounced differences between the figures presented in Table 1, the results can be summarized as follows. The number of vascular bundles decreases from the ninth to the flag internode and is positively correlated with the diameter of the stem. The number of vascular bundles per cross section of the older internodes is higher in 'Markant' than in 'Protasil' and 'Lg11'. The greater distance between the epidermis and the outermost vascular bundles in the fourth internode of 'Markant' and 'Lg11', indicates that root characteristics in the stem persist longer than in 'Protasil'. The surface area of the epidermis in cross-section is related to the diameter of the stem. Since the cell size remains the same, this means that in internode 4 a higher number of epidermis cells is present than in internode 9 and the flag internode. The surface area of the primary phloem and the metaxylem vessels is always highest in internode 9; in this region the spadix is present.

In the flag internode, the sclerenchyma is not fully differentiated. The surface area of sclerenchyma decreases towards the top of the stem, that is from old to young tissue. The amount of sclerenchyma tissue, especially in internode 4, is somewhat higher in 'Markant' than in 'Protasil' and 'Lg11'. In contrast, the sclerenchyma cell walls in internode 4, subepidermal as well as in the bundle sheaths, are slightly thinner in 'Markant' than in 'Lg11' and 'Protasil'.

The average surface area of an individual ground tissue parenchyma cell about 1 mm below the epidermis is smallest in 'Markant', followed by 'Protasil' and 'Lg11', respectively. In general, 'Markant' possesses more sclerenchyma tissue and smaller ground tissue parenchyma cells especially in the older stem parts, but the cell walls are slightly thinner than those in similar tissues in the other two cultivars.

Table 1. Stem characteristics of three *Zea mays* cultivars

	'Markant'			'Protasil'			'Lg11'		
	Int. 4	Int. 9	Flag Int.	Int. 4	Int. 9	Flag Int.	Int. 4	Int. 9	Flag Int.
Stem diameter (mm)	17·1	14·4	4·5	16·7	13·1	5·6	14·4	14·9	4·7
Average number of vascular bundles									
Per cross section	486	546	135	337	380	178	328	338	141
Per square millimetre cross section	2·12	3·35	8·49	1·55	2·84	7·16	2·01	1·95	8·04
Average distance from epidermis to outermost vascular bundles (μm)	267	55	60	153	93	55	300	71	62
Average surface area (mm^2) and percentage (—):									
Epidermis	0·860 (1·7)	0·547 (1·3)	0·231 (2·1)	1·031 (2·1)	0·606 (1·6)	0·248 (1·7)	0·758 (1·8)	0·914 (2·1)	0·234 (2·0)
Phloem	0·506 (1·0)	0·716 (1·7)	0·440 (4·0)	0·295 (0·6)	0·720 (1·9)	0·540 (3·7)	0·168 (0·4)	1·088 (2·5)	0·455 (3·8)
Protoxylem	0·101 (0·2)	0·126 (0·3)	0·231 (2·1)	0·098 (0·2)	0·644 (1·7)	0·263 (1·8)	0·042 (0·1)	0·522 (1·2)	0·257 (2·2)
Metaxylem (vessels)	0·911 (1·8)	1·179 (2·8)	0·297 (2·7)	1·424 (2·9)	1·592 (4·2)	0·438 (3·0)	0·547 (1·3)	1·523 (3·5)	0·316 (2·7)
Metaxylem (tracheids)	0·304 (0·6)	0·253 (0·6)	0·121 (1·1)	0·246 (0·5)	0·303 (0·8)	0·175 (1·2)	0·168 (0·4)	0·305 (0·7)	0·140 (1·2)
Non-sclerified bundle sheath	—	—	0·110 (1·0)	—	—	0·234 (1·6)	—	—	0·316 (2·7)

Continued

Table 1. (*Continued*)

	'Markant'			'Protasil'			'Lg11'		
	Int. 4	Int. 9	Flag Int.	Int. 4	Int. 9	Flag Int.	Int. 4	Int. 9	Flag Int.
Sclerenchyma (subepidermis and bundle sheath)	10·879 (21·5)	7·831 (18·6)	1·353 (12·3)	8·052 (16·4)	6·026 (15·9)	1·533 (10·5)	6·904 (16·4)	7·743 (17·8)	1·193 (10·2)
Parenchyma ground tissue	37·039 (73·2)	31·449 (74·7)	8·217 (74·7)	37·954 (77·3)	28·008 (73·9)	11·169 (76·5)	33·512 (79·6)	31·407 (72·2)	8·798 (75·2)
Total	50·546 (100)	42·101 (100)	11·000 (100)	49·100 (100)	37·900 (100)	14·600 (100)	42·099 (100)	43·502 (100)	11·699 (100)
Average surface area of a ground tissue cell $(\mu m)^2$ and average tangential × radial cell diameter (μm) (—)									
±8 cell layers below epidermis	1998 (53 × 48)	748 (34 × 28)	2111 (56 × 48)	828 (31 × 34)	1133 (37 × 39)	755 (31 × 31)	3695 (84 × 56)	828 (34 × 31)	950 (39 × 31)
±1 mm below epidermis	8884 (112 × 101)	5938 (90 × 84)	7389 (84 × 112)	15 339 (126 × 155)	9698 (98 × 126)	6362 (90 × 90)	18 583 (140 × 169)	11 172 (112 × 127)	12 843 (112 × 146)
Average cell wall thickness (μm)									
Sclerenchyma cells of subepidermis	2·3	2·8	2·3	4·5	2·7	2·3	2·8	2·8	2·3
Sclerenchyma cells of bundle sheath	3·4	2·5	2·3	4·5	2·8	2·5	3·7	2·8	2·8
Parenchyma cells ground tissue	2·0	0·8	1·0	1·7	1·1	0·6	2·5	1·7	0·8

Figures given in Table 1 are based on 3-month-old plants sown on 8th July. Except for vascular bundle numbers, the figures refer to the outermost 1 mm of the cross-section, (Int. = internode).

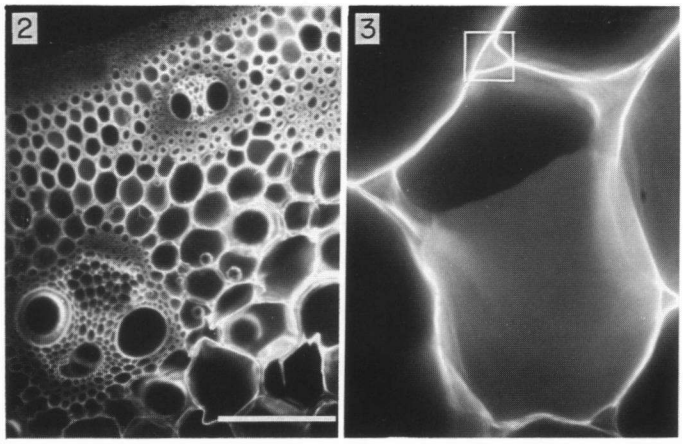

Fig. 2. Autofluorescence of the tissues in the stem. Note the difference in intensity of the cell walls of the different tissues. Bar represents 100 μm.

Fig. 3. Autofluorescence of the parenchyma cell walls. The square indicates the measured area of 10 × 10 μm² around an intercellular space.

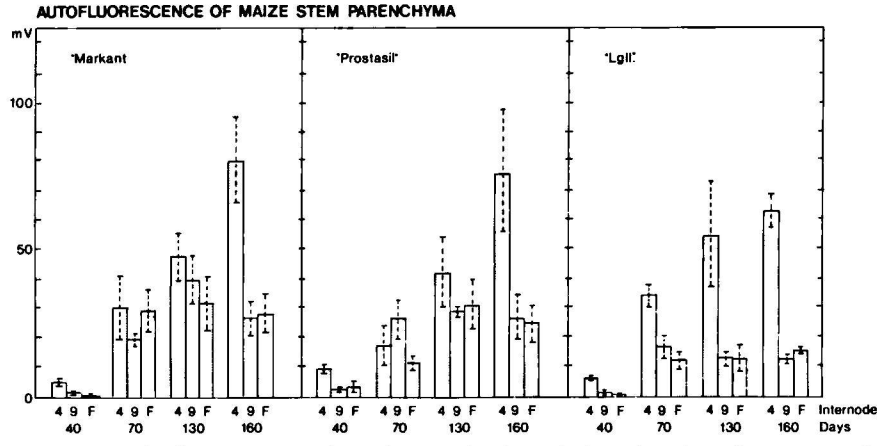

Fig. 4. Histograms of different maize cultivars showing the change in intensity of autofluorescence of parenchyma cell walls. The internodes 4, 9 and flag internode were measured in plants 40, 70, 130 and 160 days old, respectively. Vertical dotted bars indicate standard errors of means ($n = 3$).

The data of this study suggest an interdependence between parenchyma and sclerenchyma. Apparently a one-directional transition from parenchyma cells into sclerenchyma cells occurs. Thinner sclerenchyma cell walls are compensated for by a higher surface area of the total sclerenchyma tissue.

Autofluorescence of stem parenchyma during development of different cultivars of Zea mays

The photographs (Figs 2 and 3) show autofluorescent cell walls.

The results of the measurements of parenchyma cell wall autofluorescence at 1·0 kV over an area of 10 × 10 μm² parenchyma cells, ± 1 mm below the epidermis, are presented in Fig. 4.

In 40-day-old plants only the internode 4, located just above ground level, shows a weak autofluorescence. From day 70 onwards internode 9 and flag internode reach a level

Table 2. Cuticular autofluorescence; mean value of five measurements in millivolts of the leaf sheath covering internode 4, measured at an emission maximum of 490 nm

Days of growth	'Lg11'	'Markant'	'Protasil'
160	3·9±0·5	4·9±0·3	5·2±0·7
130	4·0±0·1	4·4±0·3	3·4±0·2
70	3·8±0·2	3·8±0·7	3·1±0·4
40	1·9±0·1	1·1±0·3	2·0±0·2

of autofluorescence which remains nearly constant during the remaining growth period. The autofluorescence intensity from day 70–160 differs for the used cultivars. For 'Lg11' the mean is about 15±2 mV; for 'Protasil' 26±7 mV and for 'Markant' 30±6 mV. The last two cultivars are about the same.

After 70 days of growth most of the internodes have reached their maximal value. In internode 4 the autofluorescence value increases during the whole growth period, notwithstanding the fact that plants, especially those of 'Markant' and 'Lg11' start to dry-out after about 130–160 days of culture. Particularly for 'Markant' and 'Protasil', the autofluorescence of internode 4 increases in intensity after 130 days of growth. This implies that in the basal part of the plant the phenolics in the cell walls increase over a longer period.

As mentioned before, the differences between the analyses of 2- and 3-month-old plants are very small. This means that the average cell wall thickness of the parenchyma cells in the ground tissue remains the same. The increase in autofluorescence in the basal part of the plant may indicate an increase in the phenolic content in the cell walls.

During development the cuticle of the leaf thickens. With incident light the irregular surface layer shows a variation in autofluorescence from blue to yellow. Table 2 summarizes the results of the autofluorescence measurements of the different cultivars in an area of $10 \times 10\ \mu m^2$ during the development of the leaf sheath belonging to internode 4. From these values it is clear that after 70 days few differences exist between the cuticular autofluorescence of the different cultivars. Forty-day-old plants provide a value about half or a quarter of that of plants at day 70–160. At day 40 'Markant' shows a low autofluorescence intensity compared with 'Lg11' and 'Protasil'.

The stem cuticle covered by the leaf sheath of a 'Markant' plant compared with the abaxial cuticle of the covering leaf sheath, shows a lower stem cuticle autofluorescence of 0·7±0·1 mV at internode 4 after 70 days.

DISCUSSION AND CONCLUSION

From the anatomical studies it is clear that the stem of maize is fully developed at day 70. The anatomy reveals the interdependence between the following items: number of vascular bundles and stem diameter from the ninth internode onwards, epidermis surface area in cross-section and stem diameter, a high surface area in the cross-section of phloem as well as metaxylem vessels and position of spadix, and, finally, parenchyma and sclerenchyma. This last mentioned observation means that a shift towards a certain tissue is at the cost of another tissue. The greater surface area

of the sclerenchyma tissue of 'Markant' is accompanied by a smaller surface area of parenchyma and at the cost of the cell wall thickness of the sclerenchyma cells.

Autofluorescence of the stem parenchyma and stem and leaf sheath cuticle shows that the amount of phenolics increases during plant growth and that there are quantitative differences among the cultivars 'Lg11', 'Markant' and 'Protasil'.

The method of measuring autofluorescence is relatively quick and fresh sections can be used. The differences in cell wall dimension, possible changes in chemical and structural composition in the cell wall during growth, as well as the anatomical construction of the internode, however, introduce considerable variation. Therefore, the measurements have to be executed carefully. The method offers only relative data on the amount of phenolics in the plant. The relationship between autofluorescence of phenolics and the presence of lignin was not clear until now. It is not possible to distinguish between the fluorescence of the middle lamella and the other parts of the cell wall.

Because of the change in composition and thickness of the cuticle during growth and the irregular structure of this layer, autofluorescence measurements of the cuticle should be interpreted carefully. In the leaf sheath it is clear that after 70 days of culture autofluorescence changes little. Cuticular autofluorescence shows some differences between the cultivars, which are partly similar to the differences found for the parenchyma autofluorescence.

For maize the difference in the autofluorescence of parenchyma cell walls means that the upper part of the stem reaches its maximal content of phenolics within about 70 days of culture. This is in contrast to internode 4, which accumulates phenolics over a longer period and by doing so the basal part of the plant is consolidated. Because of the differences in parenchyma cell wall thickness between the cultivars, as well as between the basal part of the plant and its top, the measured autofluorescence seems to be influenced more by an increase in phenolic content rather than in cell wall thickness.

The stem anatomy of maize is completed within 2–3 months. The content of phenolics, perhaps partly related to the content of lignin, shows the same pattern except for the basal internode 4. The content of phenolics in 'Lg11' is lower compared with 'Protasil' and 'Markant'.

Anatomical analysis can help state differences between maize cultivars. The measurement of autofluorescence can indicate the content of the phenolics of the plant, which may be related to the digestibility of the fodder.

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