ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 50, nr 4: 567—574
1981

Genetical and anatomical analysis of brittlenses of stems in rye (*Secale cereale* L.)

HELENA KUBICKA, BOGUSŁAW KUBICKI

Institute of Genetics and Plant Breeding, Agricultural University at Warsaw, Nowoursynowska 166, 02-766 Warszawa, Poland

(Received: July 30, 1980)

Abstract

Brittlenes of the stem was found to be one of the more frequently segregating traits in inbred rye lines. In dependence on the outset cultivated variety this trait appears in the S_2 generation in 4.0-0.6 per cent of the inbred lines. Genetical analysis demonstrated that the trait of brittlenes is determined by one recessive gene denoted by the symbol *bs* (brittle stem). The *bs* gene exerts a strong pleiotropic effect on the whole plant, beside brittleness of the stem it causes fragility of the roots, heads and leaves and depresses the general viability of the plants. Anatomical observations of the stem and root showed that the *bs* gene causes disturbances in the normal lignification of the sclerenchyma cells both in shoots and roots, so that these cells are thin-walled. The thickness of the sclerenchyma layers and the number and size of the vascular in the brittle forms are significantly smaller than in the normal ones. It was found that these changes appear in the brittle forms at the phase of heading of the plants.

INTRODUCTION

Several cases of brittleness of the stem have been described so far (Davidson et al. 1924, Brewbaker 1926, Hornburg 1929, Łada 1934, Jermoljew 1942, Sybenga and Prakken 1962).

It was found that this characteristic is conditioned by one recessive gene designated by Łada (1934) by the symbol *g* and by Sybenga and Prakken (1962) by *b*. These studies have not been more precisely documented by anatomical observations.

In our work on selfpollination of various rye varieties the trait of brittleness of the stem frequently segregates and is variably expressed therefore we decided to analyse accurately both from the anatomical and the genetical view point.

Fig. 1. Cross section of II internode of normal line stem as seen in the Docuval microscope. × 420
1 — epidermis; 2 — sclerenchyma zone with greatly thickened cells; 3 — parenchyma

Fig. 2. Cross section of II internode of normal line stem inspected in Docuval microscope. × 420
1 — epidermis; 2 — sclerenchyma zone; 3 — vascular bundle; a — intercellular space; b — protoxylem; d — metaxylem; e — sieve tubes; f — cells surrounding sieve tubes; g — fibres

Fig. 3. Cross section of II internode of normal line stem inspected in Docuval microscope. Vascular bundle in parenchyma: × 280
a — intercellular space; b, c — protoxylem; d — metaxylem; e — sieve tubes; f — cells surrounding sieve tubes; g — fibres

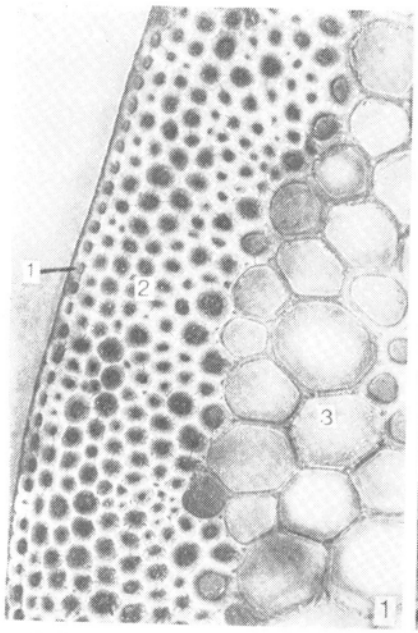
Fig. 4. Cross section of II internode of brittle line stem inspected in Docuval microscope. × 340
1 — epidermis; 2 — sclerenchyma zone with thin-walled cells; 3 — parenchyma

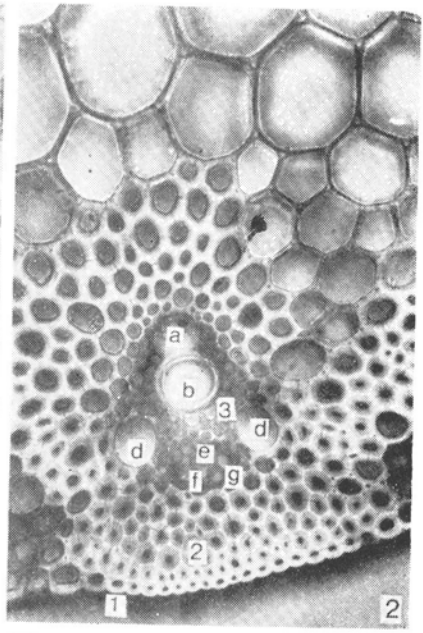
MATERIAL AND METHODS

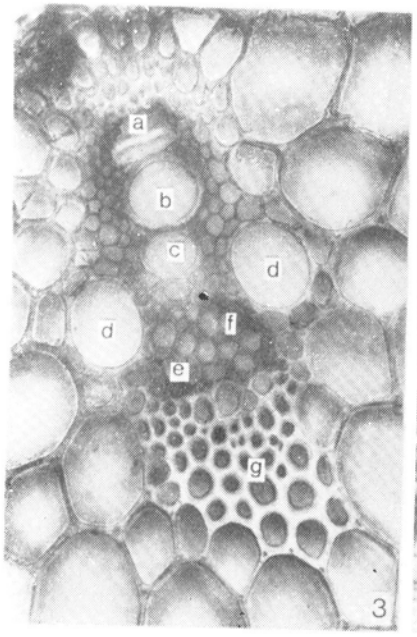
Jointly 17 independent cases of brittleness were detected in the inbred generations S_1 and S_2 of various rye varieties. The largest number, as many as 6 independent cases of brittleness, was noted in the variety 'Pancerne', 3 in the variety 'Garczyńskie', 2 in each of the varieties 'Chrobre' and 'Perl' and 1 case in each of the varieties 'Dańkowskie Złote, 'Dańkowskie Selekcyjne', 'Smolickie', 'Dołgoletnaja'. After selfpollination of the brittle segregates, 17 homozygous lines were developed and subjected to anatomical analysis and crossed with normal in order to perform genetical analysis.

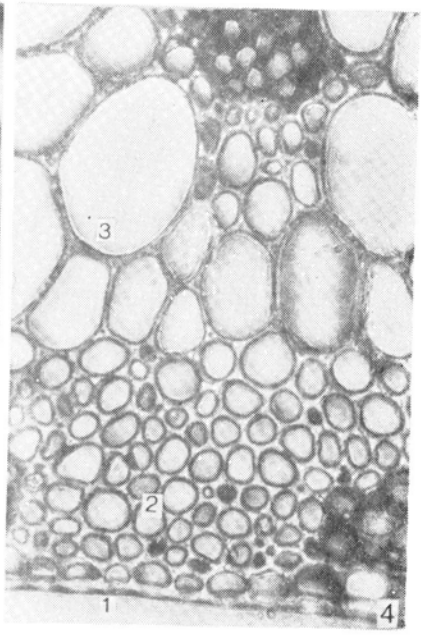
For anatomical studies 2-cm segments were collected from roots and all internodes of stems at the phase of flowering when brittleness is most pronounced. The samples were fixed in 70% ethyl alcohol. The cross sections were prepared with a razor blade and stained with iodine green and carmine, and embedded in gelatin-glycerin. Anatomical measurements were performed under a light microscope and statistical significance was established by Student's test.

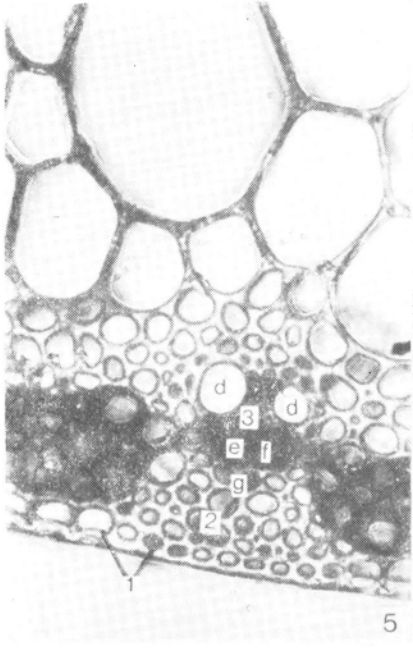
Additional preparations were made of the second internode of the brittle and normal lines and studied under a JEOL ISM-U3 scanning electron microscope. Since all the brittle lines showed similar anatomical changes, only results of analysis of one brittle line L-303 and one normal one L-105 derived from a common outset plant of the variety 'Chrobre' are given here. Additionally photographs are shown of a very interesting case of brittleness in one line characterised by elongated thin-walled sclerenchyma cells.


1
2
3
1

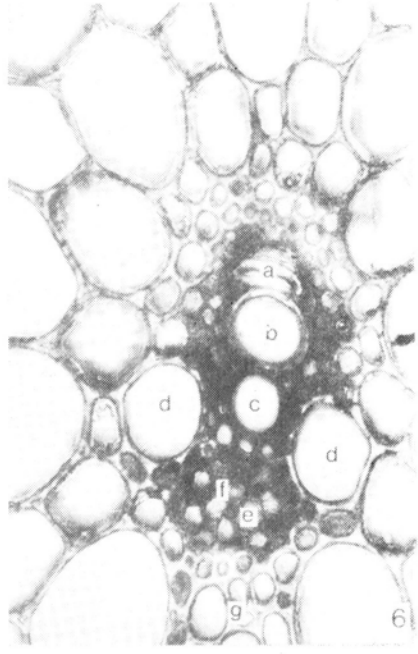

a
b
3
d
d
e
f
g
2
1
2


a
b
c
d
d
f
e
g
3

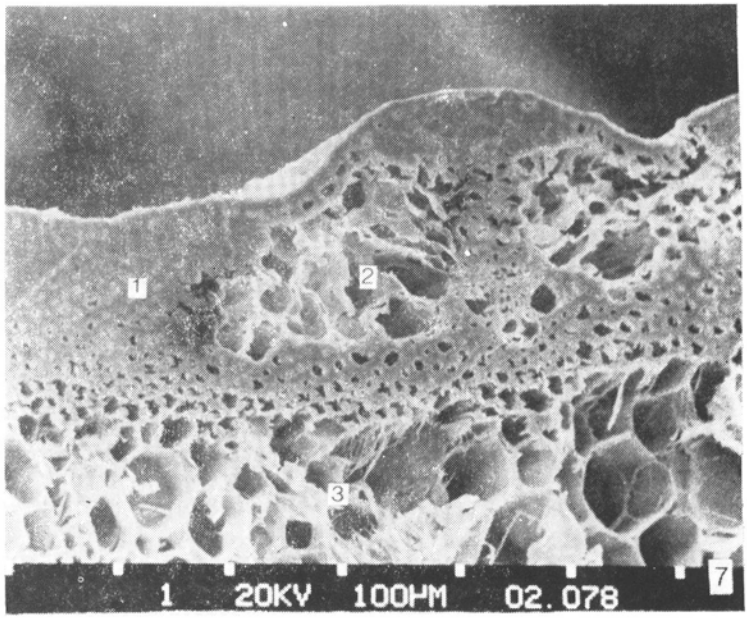

3
2
1
4



5



6



7

Fig. 5. Cross section of II internode of brittle line stem inspected in Docuval microscope. × 280
1 — epidermis; 2 — sclerenchyma zone; 3 — vascular bundle in sclerenchyma; d — metaxylem; e — sieve tubes; f — cells surrounding sieve tubes; g — fibres

Fig. 6. Cross section of II internode of brittle line stem inspected in Docuval microscope. Vascular bundle in parenchyma. × 340
a — intercellular space; b, c — protoxylem; d — metaxylem; e — sieve tubes; f — cells surrounding sieve tubes; g — fibres

Fig. 7. Cross section of II internode of normal line stem seen in scanning microscope
1 — parenchyma zone with greatly thickened cell walls; 2 — metaxylem; 3 — parenchyma

RESULTS

ANATOMICAL STRUCTURE OF THE STEM IN THE BRITTLE AND THE NORMAL LINES

The normal line exhibited stems 145 cm tall and thickness of the 3rd internode 7000 μm. It tillered up to 16 shoots. The brittle line as compared with the normal one had shorter (100 cm) and thinner stems (5485 μm), showed weaker tilling (10 shoots) light green leaves and supple shoots bending outwards. Leaves stems and roots exhibited an unusual brittleness and broke easily.

The essential difference in the anatomical structure of the stems of the normal and the brittle lines consisted in the thickness of the sclerenchyma cells walls. In the normal line the sclerenchyma had strongly lignified thick cell walls, whereas in the brittle line it was composed of thin-walled cells (Figs. 1, 2, 4, 5).

In both lines the epidermis cells outside the mesophyll islets were distinctly larger and they became smaller outside the outer bundles. In the normal line, they were however more lignified (Figs. 2, 4). Four layers of thin-walled cells originating from the degenerated mesophyll occured between the vascular bundles in the first and second internode of the normal line. These cells were additionally supported from the inside by four layers of thick-walled sclerenchyma cells. In the brittle line there were only two layers of thin-walled cells derived from the mesophyll. They were supported by only three layers of thin-walled sclerenchyma cells. In III, IV, V internodes of both lines there normal mesophyll islets occurred. Their number and shape were similar in both lines, but in the normal form they were significantly larger (Table 1).

The number of vascular bundles of the sclerenchyma was equal in both lines. In the normal one they were, however, significantly larger and surrounded by many layers of strongly lignified sclerenchyma cells (Figs. 2, 5, Table 1). Table 1 shows that the number of vascular bundles

Table 1

Anatomical traits of normal /a/ and brittle /b/ lines ; cross section of internodes at two dates June 2 /I/ and July 14 /II/ - data in micrometres /µm/

Trait		First internode peduncle		Second internode		Third internode		Fourth internode		Fifth internode		$\bar{x}$ for date		Mean value	$x_1 - x_2$	LSD
		I	II	I	II	I	II	I	II	I	II	I	II			
Stem diametre	a	4820	4850	5720	5790	6980	7020	8010	8040	-	-	6380	6420	6400	520+	152.60
	b	4320	4270	4630	4610	5500	5470	6590	6600	8380	8470	5880	5880	5880		
Thickness of sclerenchyma layer without vascular bundles	a	-	-	98	90	114	104	117	120	-	-	110	106	107	46+	15.90
	b	41	51	55	49	52	51	73	69	79	89	60	62	61		
Thickness of sclerenchyma layer with bundles	a	226	257	172	170	147	153	159	180	-	-	176	190	183	72+	26.30
	b	104	109	108	111	105	102	116	119	114	126	109	113	111		
Thickness of sclerenchyma layer with parenchyma bundles in peduncle	a	480	524	-	-	-	-	-	-	-	-	480	524	502	199+	116.00
	b	301	305	-	-	-	-	-	-	-	-	301	305	303		
No. of bundles in sclerenchyma	a	35	33	37	35	38	36	41	40	-	-	38	36	37	0	2.12
	b	38	37	40	37	37	34	39	34	37	39	38	36	37		
Length of bundles in sclerenchyma	a	68	68	55	58	61	62	69	69	-	-	63	65	64	17+	4.81
	b	42	43	45	44	46	42	48	48	55	52	47	47	47		
Width of bundles in sclerenchyma	a	68	69	57	58	57	60	84	89	-	-	67	69	68	15+	12.65
	b	41	44	41	39	55	49	60	59	71	74	53	53	53		
No. of bundles in parenchyma	a	31	42	31	42	36	40	42	42	-	-	35	41	38	10+	4.97
	b	19	22	25	25	29	30	29	30	33	33	28	28	28		
Length of bundles in parenchyma	a	298	305	234	232	248	240	241	226	-	-	255	251	253	51+	4.24
	b	193	194	195	197	214	192	195	204	215	220	205	199	202		
Width of bundles in parenchyma	a	175	181	152	154	161	163	183	185	-	-	168	171	169	28+	17.72
	b	122	126	113	115	154	145	149	156	164	167	140	142	141		
No. of metaxylem islets	a	43	45	56	58	-	-	-	-	-	-	49	53	51	2	2.12
	b	41	46	56	53	-	-	-	-	-	-	49	49	49		
Length of metaxylem islets	a	159	165	202	218	-	-	-	-	-	-	181	191	186	59+	33.88
	b	128	132	125	121	-	-	-	-	-	-	127	127	127		
Width of metaxylem islets	a	84	82	88	78	-	-	-	-	-	-	86	80	83	26+	
	b	56	58	54	60	-	-	-	-	-	-	55	58	57		
Thickness of thin-walled cell layer in lower internodes	a	-	-	-	-	57	52	90	78	-	-	73	65	69	20	25.40
	b	-	-	-	-	38	40	35	40	68	72	44	50	49		

/+/ - differences significant at $P_{0,05}$

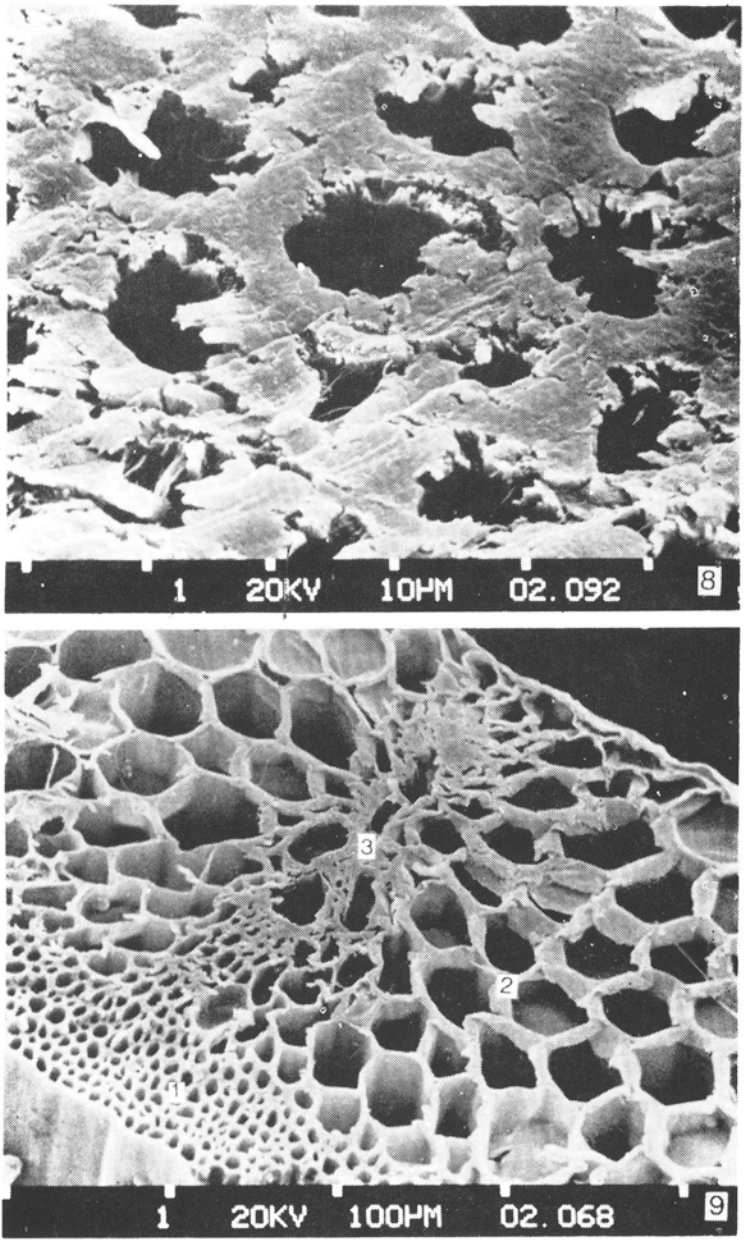
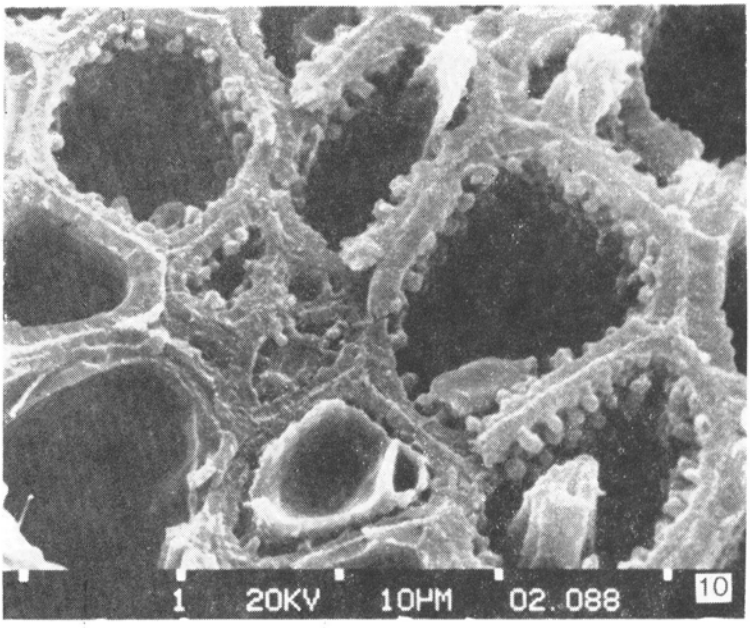
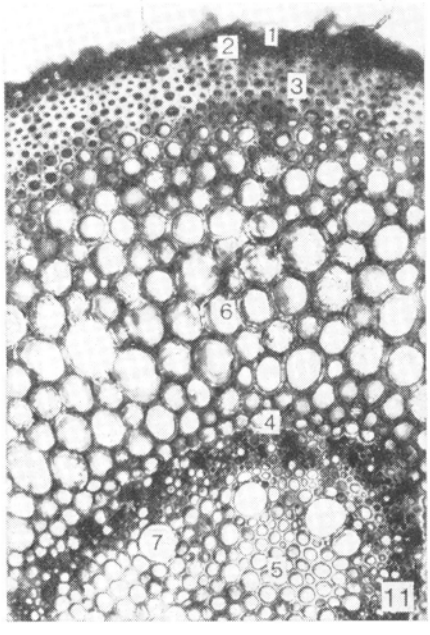


Fig. 8. Thickness of sclerenchyma cells in II internode of normal line stem seen in scanning microscope

Fig. 9. Cross section of II internode of brittle line stem seen in scanning microscope

1 — sclerenchyma zone with thin-walled cells; 2 — parenchyma with vascular bundle





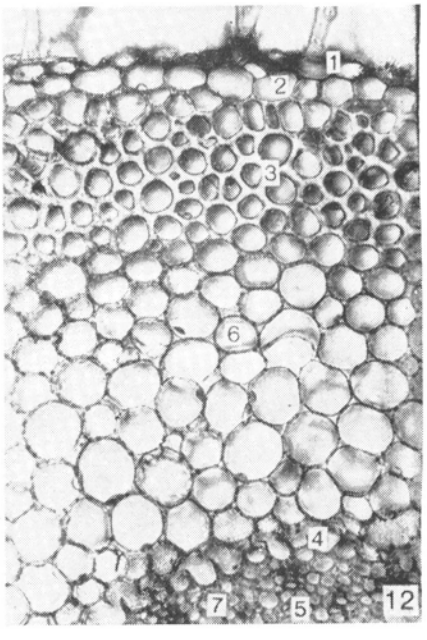


Fig. 10. Thickness of sclerenchyma cells in II internode of brittle line stem seen in scanning microscope

Fig. 11. Cross section through root of normal line plant seen in Docuval microscope. × 350

1 — rhizodermis; 2 — exodermis; 3 — primary cortex sclerenchyma; 4 — endodermis; 5 — central cylinder sclerenchyma; 6 — primary cortex; 7 — vessels

Fig. 12. Cross section through root of brittle line plant seen in Docuval microscope. × 420

Other explanations as in Fig. 11

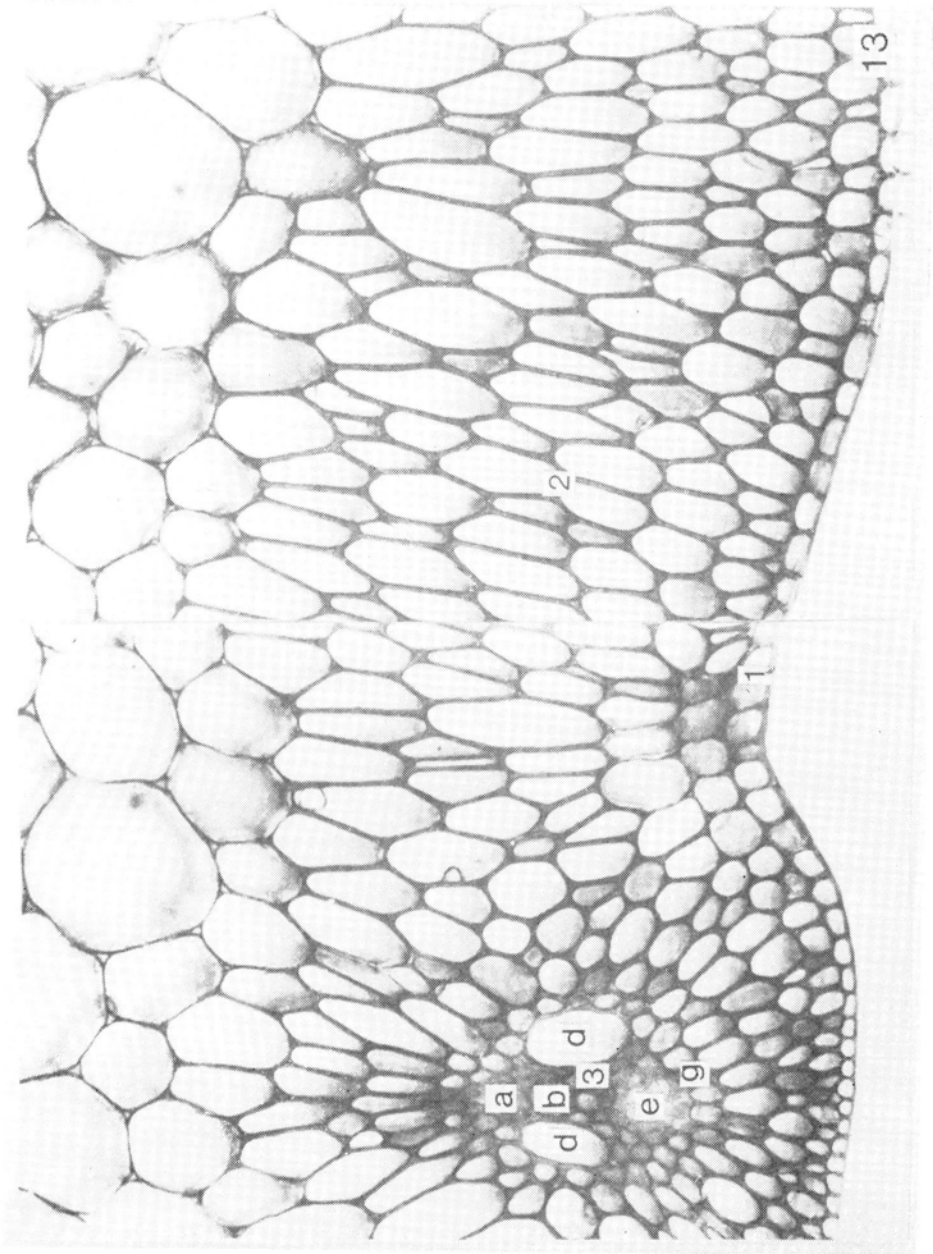


Fig. 13. Cross section of II internode of brittle line stem with elongated sclerenchyma cells seen in Docuval microscope. × 380

1 — epidermis; 2 — sclerenchyma zone; 3 — vascular bundle in sclerenchyma; a — intercellular space; b — protoxylem; d — metaxyiem; e — sieve tubes; g — fibres

in the parenchyma was markedly higher in the normal line. The bundles were here significantly longer and wider. They were additionally supported on the side facing the epidermis by 4 layers of sclerenchyma cells with highly thickened walls (Figs. 3, 6). The brittle line was also characterised by a singnificantly thinner sclerenchyma layer without vascular bundles and a thinner sclerenchyma layer with parenchyma bundles in the peduncle (Table 1).

It is noteworthy that there were no major differences in the examined characteristics between both lines in dependence on the date of observation (Table 1). This indicates that in June 2, about one week after flowering the characteristics typical for the brittle plants were fully manifested. Since preliminary anatomical and morphological observations performed before heading did not demonstrate differences between plants of both lines, it would seem that these lines begin to differentiate at the time of heading and reach the widest differences after flowering.

Observations in the scanning microscope confirmed that the essential difference between the brittle and normal lines concerne the degree of lignification of the sclerenchyma cells (Figs. 7, 8, 9, 10). Beside the differences in anatomical structure different staining intensity was noted between sclerenchyma cells of the brittle (dark-green) and the normal (light-green almost celadon) plants. The same is suggested by the differences in their chemical composition.

Finally worth describing is a peculiar case of brittleness detected in one line denoted L-7-254. This line exhibits particularly thin-walled radially elongated sclerenchyma cells both in the main subepidermal and in the vascular bundle zone (Fig. 13). Since all the plants of this line exhibit the shape of sclerenchyma cells shown in Fig. 13, it appears that it must be the results of a different genetic character which will require a special study.

ROOT ANATOMICAL STRUCTURE IN NORMAL AND BRITTLE LINES

The normal root (Fig. 11) is usually built of a layer rhizoderm and a layer exoderm. Under that lie 5 layers of sclerenchymatous cortex cells with greatly thickened walls, (cortex parenchyma) of 8 layers of thin-walled cells. Under the primary cortex (cortex parenchyma) there is an endodermis with thickened cell walls. The main tissue of the central cylinder is sclerenchyma. Alternate sieve and vascular strands lie under the pericycle. Metaxylem vessels are situated in the central part (Fig. 11).

The differences in the anatomical structure between roots, similarly as those between stems lie the different thickness of the sclerenchyma

cell walls (Figs. 11, 12). In the brittle line there were sclerenchymatous primary cortex cells under the exoderm. Their walls were much thinner as compared with those in the normal line in which there were two more layers of these cells (Figs. 11, 12). The situation is similar as regards the cells of the central cylinder cells where the walls of the brittle line cells were less lignified.

GENETICAL ANALYSIS

The F_1 generation of the hybrid between the brittle and the normal line was quite normal. This was confirmed by both morphological and anatomical observations. In the F_2 generation of 170 plants segregation occurred into 129 normal and 41 brittle plants. This is in agreement with the ratio 3 : 1 ($x^2 = 0.097$ at $P = 0.80$). These data are evidence that brittleness is conditioned by one recessive gene, which was denoted by the symbol *bs* (brittle stem).

DISCUSSION

Stem brittleness in the one of the most frequently segregating traits in inbred rye lines (K u b i c k i and K u b i c k a 1980). In the present study 17 independent cases of brittleness were detected. Their frequency varied in the particular varieties within the limits of 6-1, that is 4.0-0.6 per cent of the outset plants producing seeds after selfpollination. In a great majority, that in 16 cases the trait of brittleness appeared in the inbred lines of the second generation (S_2), this being probably connected with the larger number of plants of this generation as compared with the less numerous plants of the first generation (S_1).

As demonstrated by Ł a d a (1934), and S y b e n g a and P r a k k e n (1962) the trait of brittleness is conditioned by recessive gene. Ł a d a (1934) was the first to denote the brittleness gene by the symbol *g*, but this was not in agreement with the principles of genetic nomenclature and was not used in the literature. S y b e n g a and P r a k k e n (1962) designated this gene by the symbol *b* (brittle). The same symbol, however, had been earlier adopted by D u m o n (1947) for the pair of alleles *B* and *b* conditioning the colour of caryopses. For these reasons the authors decided to denote the brittleness gene by a new symbol *bs* (brittle stem), since, in spite of its pleiotropic effect, this gene is most distinctly manifested in the plant shoots.

As demonstrated by the present anatomical and morphological observations the *bs* gene exerts a deep pleiotropic influence on the plant as a whole. Its main effect seems to consist in disturbances of cell

wall lignification of the sclerenchyma, both in shootes and roots, owing to which they are markedly thinner than in the normal forms. This main effect is accompanied by secondary changes such as a thinner zone of thin-walled subepidermal sclerenchyma, smaller dimensions of the vascular bundles and their distinctly lower number in the parenchyma. These changes are directly associated not only with shoot brittleness, but with the general brittleness of the remaining parts of the plant such as the head, leaves and roots, a general suppleness (limpness) and lodging and also probably a reduced viability manifested in lower length, poorer tillering and thinner light-green shoots. This abnormal colour of the shoots may be explained by the smaller mesophyll islets in the brittle line.

As demonstrated in the present study the action of the *bs* gene becomes fully manifest just after flowering because the plants of the brittle line already exhibit at this time traits characteristic for it. Since the changes characteristic for the brittle line are not noticeable before heading, it may be supposed that the main effect the *bs* gene begins to manifest itself in the phase of heading when in normal plants an intensive process of sclerenchyma cell lignification occurs.

There is no doubt that the changes described here caused by the *bs* gene are very unfavourable for the development and reproduction of the plant. This explains why they occur relatively seldom in populations of cultivated varieties, most frequently in the heterozygotic state and are detectable usually only after selfpolination of the plants.

The present paper does not elucidated all the problems connected with brittleness of rye plants. The nature of this defect remains unkown at the chemical level and so are its manifold pleiotropic effects. The case detected in the present study of brittleness combined with greatly elongated thin-walled sclerenchyma cells and high degree of self-fertility of all the brittle lines also seems interesting.

These problems require further studies.

REFERENCES

Brewbaker H., 1926. Univ. Minnesota, Agr. Exp. St. Techn. Bull. 40.

Davidson F. R., Brewbaker H. R., Thompson N. A., 1924. J. Agr. Res. 28: 169-172.

Dumon A. G., 1974. Agricultura Bull. Ass. Anc. Etud. Inst. Agr. Univ. Louvain 45: 213-223

Hornburg P., 1929. Z. Pflanzenzüchtung 14: 509-512.

Jermoljew E., 1942. Z. Pflanzen 24: 59-70.

Kubicki B., Kubicka H., 1980. Genetyka populacyjna i osobnicza. Zeszyty Naukowe Uniwersytetu Śląskiego, Katowice.

Łada P., 1934. Imprim. Univ. Cracovie. No. 7-10 II: 183-194.

Sybenga J., Prakken R., 1962. Genetica 33: 95-105.

Analiza genetyczna i anatomiczna łamliwości źdźbła u żyta (Secale cereale L.)

Streszczenie

Stwierdzono, że łamliwość źdźbła jest jedną z częściej segregujących cech w liniach wsobnych żyta. W zależności od wyjściowej odmiany uprawnej cecha ta ujawnia się w pokoleniu S_2 u 4,0-0,6% linii wsobnych. Cecha łamliwości źdźbła warunkowana jest jednym recesywnym genem oznaczonym symbolem *bs* od "brittle stem". Gen *bs* wywiera silny efekt plejotropowy na całą roślinę gdyż oprócz łamliwości źdźbła powoduje kruchość korzeni, kłosów i liści oraz obniża jej ogólną żywotność. Obserwacje anatomiczne budowy źdźbła i korzeni wykazały, że gen *bs* wywołuje zaburzenia w normalnym drewnieniu komórek sklerenchymatycznych zarówno pędów jak i korzeni na skutek czego są one wyraźnie cienkościenne. Niezależnie od tego zarówno grubość warstw sklerenchymatycznych jak i liczba oraz wielkość wiązek naczyniowych form łamliwych są w porównaniu z normalnymi istotnie mniejsze. Stwierdzono, że powyższe zmiany u form łamliwych zaczynają się ujawniać w fazie kłoszenia roślin.