Theor. Appl. Genet. 50, 137-146 (1977)

Regulation of Internodal Lenght by Peroxidase Enzymes in Grain Sorghum*

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Summary. A relationship between height genes (dw locus) and perioxidase was demonstrated by extracting and determining peroxidase specific activity in internode tissue from different height isogenic lines of sorghum [*Sorghum bicolor* (L.) Moench]. Tall plants (2 dwarf) had less peroxidase per gram tissue than their short counterparts (3 dwarf); their F_1 offspring internodes were closer but had more peroxidase than the tall parent. Peroxidase in the F_2 offspring was inversely related to their height and followed a simply-inherited pattern similar to that for height.

Among different tissues analyzed, peroxidase concentration in roots was higher than in leaves and internodes, whole internode higher than in pith, and seed embryo higher than in endosperm. Peroxidase activity of nonviable seeds was negligible.

Isoelectric focusing provided a more detailed peroxidase zymogram than did gel electrophoresis. Differences in peroxidase bands among tall and short parental plants, F_1 and F_2 segregating groups all appear to be reflected by intensity differences rather than by position or number of bands.

Activities of nitrate reductase and acid phosphatase did not correlate with height. That finding provides a control and suggests that peroxidase activity is not associated with height by chance but may have a functional relationship.

Key words: Sorghum - Height - Regulation - Peroxidase

Introduction

The availability of height isogenic lines of grain sorghum, *Sorghum bicolor* L. (Moench), provides a unique opportunity to study the effects of a single allele on different traits and to specify gene-controlled enzyme activities in flowering plants. Peroxidase (EC 1.11.1.7), a multifunctional enzyme, in various plant species is associated with the length of internodes or plant height (McCune and Galston 1959; Evans and Alldridge 1965; Schertz et al. 1971; Cunningham et al. 1975). The height gene-peroxidase relationship can be demonstrated with height isogenic lines, without interference from heterogeneous genetic backgrounds at other loci, and paired isogenic-lines can be directly compared. The isoelectric focusing technique in this study revealed peroxidase isoenzymes not apparent in disc or zone electrophoresis gels.

Acid phosphatase was chosen as a control enzyme because of its association with genetic variability (Brown and Allard 1969) and tissue specificity (Efron 1970) as well as its assay simplicity. Nitrate reductase was also studied, to determine whether it had any indirect relation to the genes that control height. There is evidence that short sorghum yields less grain but higher seed protein. Nitrate reductase is a possible control point to introduce nitrogen ultimately incorporated into proteins.

Our study reports on peroxidase, acid phosphatase, and nitrate reductase activities in four sets of sorghum-height isogenic lines. We examined the relationship between peroxidase and internodal length in detail using enzyme specific activity measurements and electrophoresis patterns.

Materials and Methods

Four sets of height isogenic pairs of grain sorghum, F_1 hybrids derived from each pair, and the segregat-

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Set		Genotype				No. of internodes	Height (cm)
1	'Redlan' 2-dwarf	dw_1/dw_1	Dw_2/Dw_2	Dw_3/Dw_3	dw_4/dw_4	11.7	167.5
	'Redlan' 3-dwarf	"	"	dw_3/dw_3	"	12.0	82.1
	F_1	"	"	Dw_3/dw_3	"	11.7	135.7
	F_2 Short	"	"	dw_3/dw_3	"	11.7	84.6
	F_2 Medium	"	"	Dw_3/dw_3	"	11.0	127.1
	F_2 Tall	"	"	Dw_3/Dw_3	"	13.0	165.1
2	'Plainsman' 2-dwarf	"	"	Dw_3/Dw_3	"	11.3	122.7
	'Plainsman' 3-dwarf	"	"	dw_3/dw_3	"	11.3	70.0
	F_1	"	"	Dw_3/dw_3	"	11.0	90.0
	F_2 Short	"	"	dw_3/dw_3	"	11.3	66.3
	F_2 Medium	"	"	Dw_3/dw_3	"	10.7	94.7
	F_2 Tall	"	"	Dw_3/Dw_3	"	11.0	127.0
3	'Martin' 2-dwarf	"	"	Dw_3/Dw_3	"	10.7	116.0
	'Martin' 3-dwarf	"	"	dw_3/dw_3	"	10.0	64.2
	F_1	"	"	Dw_3/dw_3	"	10.6	101.7
	F_2 Short	"	"	dw_3/dw_3	"	10.7	67.3
	F_2 Medium	"	"	Dw_3/dw_3	"	10.5	92.7
	F_2 Tall	"	"	Dw_3/Dw_3	"	10.5	118.8
4	'Tx403' 3-dwarf	"	dw_3/dw_3	Dw_3/Dw_3	"	9.0	53.5
	'Tx403' 4-dwarf	"	"	dw_3/dw_3	"	8.0	37.5
	F_1	"	"	Dw_3/dw_3	"	8.0	47.5
	F_2 Short	"	"	dw_3/dw_3	"	8.5	34.1
	F_2 Medium	"	"	Dw_3/dw_3	"	8.0	46.7
	F_2 Tall	"	"	Dw_3/Dw_3	"	9.0	54.0

ing F_2 plants were used. Their genotypes in terms of height, number of internodes, and average height (ground level to peduncle) were as follow:

In a preliminary study we measured peroxidase activities of different sorghum tissues, especially internodes. The parental lines and their F_1's were grown in nutrient solution in a growth chamber. Parental lines were also grown in field plots. Roots, leaves, and internodes were sampled from growth chamber plants - four plants from each line. Temperature in the reach-in growth chamber was maintained at 26-28°C (12 hr) and 24°C night. Light intensity was 27 K lux at the source. From the plot materials (May-June 1972) samples of leaves and internodes were taken at different stages during growth and analyzed for peroxidase activity. Single seeds of the parental lines were also analyzed (dormant or germinated 40 hours) on blot paper in petri dishes.

One year after the preliminary study, the parental lines and F_1 of each set were planted in 3-row plots and F_2 of each set was planted in 5-row plots at the Agricultural Experimental Farm, Manhattan, Kansas. Four upper internodes were taken from two plants in each plot at each of the three stages: heading, flowering, and soft dough.

To extract peroxidase, 1 g of tissues was ground with 2g sand in 4 ml of cold 30 % saturated $(NH_4)_2SO_4$ (175 g ammonium sulfate added to 1 liter distilled water) and centrifuged at 20,000 × g (3°C) for 10 minutes. The supernatant fraction, stored at 0°C, was assayed for peroxidase the same day or was frozen and stored at -20°C for assay later. For single-seed analyses, 1 ml 30 % saturated $(NH_4)_2SO_4$ and 0.2 g sand were used.

The internode extracts were concentrated by adding $(NH_4)_2SO_4$ to near saturation at 4°C (0.4 g added to each ml of extract with continuous stirring). After 8 hours, samples were centrifuged, the precipitate dissolved in 0.02 ionic strength pH 8 Tris buffer, and dialyzed against that buffer. Aliquots were then used directly for zone electrophoresis or isoelectric forcusing.

Peroxidase activity was measured with guaiacol at pH 6 by the method of Johnson and Cunningham (1972).

Zone electrophoresis

A thin, rectangular slab of agarose (1% in 0.02 ionic strength Tris/HCl buffer pH 8) was the matrix. Sample application, electrophoresis, and diaminobenzidine staining were by the method of Cunningham et al. (1975) adapted from Cawley (1969).

Isoelectric focusing

Our method was based on descriptions by Bours (1973), Vesterberg and Svenson (1966), and Awdeh et al. (1968). The apparatus was built with plexiglas following, in general, the model of Vesterberg and Svenson (1966). Polyacrylamide gel slabs (5% w/v acrylamide, 2% w/v LKB ampholine) were made according to Bours (1973). A gel mold was prepared with two glass plates (one treated with dichlorodimethylsilane) clamped over a U-shaped gasket of flat 1.5 mm Tygon. Polymerization was for 1-2 hr at 24°C with two 20 W daylight fluorescent lamps about 10 cm on each side of the gel. Sample (2λ of dialyzed concentrate) was applied by pipetting onto small filter paper squares and laying them on the gel surface. The platinum electrodes contacted filter paper strip reservoirs flat on the gel ends, one soaked with 0.5 M NaoH, the other with 0.5 M H_3PO_4. Focusing was at 15 v/cm overnight, with circulating ice water coolant. Peroxidase detection was by immersing the gels in diaminobenzidine stain as described for agarose zone electrophoresis experiments. pH gradient was measured with a 6 mm diameter flat electrode (Ingold 403-30-M8). In contrast to agarose slabs, the polyacrylamide slabs required special treatment to preserve clarity. We adapted the method of Dangerfield and Faulkner (1963), using 1% agarose and 5% glycerol. A hard, transparent layer was obtained after drying two days.

Acid Phosphatase

A method modified from Uehara et al. (1974) was used. The reaction mixture contained 720 μmoles sodium acetate buffer, pH 5.8, 3.6 μmoles p-nitrophenylphosphate, and 0.5 ml appropriately diluted enzyme or distilled water, in a final volume of 3.0 ml.

After incubating 10 min at 35°C, the reaction was stopped by adding 1.0 ml of 0.4 N NaOH. Absorbance at 410 nm was converted to μmoles substrate hydrolyzed/min/ml enzyme taking $\varepsilon_{410\,nm}$ = 17 $mM^{-1}cm^{-1}$ for p-nitrophenol.

Nitrate reductase

Nitrate reductase activity in intact leaf tissue was determined by the method of Jaworski (1971). Approximately 100 mg of leaf tissue was suspended in a stoppered test tube containing 5 ml of extraction medium (0.1 M phosphate buffer, pH 7.5; 0.02 M KNO_3; 5% propanol; and two drops of chloramphenicol), incubated at 25°C for 1 hr, then 1 ml of 1% sulfanilic acid in 3 N HCl and 1 ml of 0.02% N-1-Naphthyl ethylenediamine hydrochloride were added. Absorbance was read at 540 nm.

Results

1. Single Seed Analysis

Peroxidase activities in single dormant and germinating seeds and those of endosperm, endosperm plus embryo, and nonviable seeds are shown in Table 1. Enzyme activities did not differ significantly among the three genotypes within each set. Thus peroxidase activities or amount of peroxidase in dormant and germinating seeds did not reflect differences in plant height that are apparent between tall and short genotypes at maturity. Seed embryo is the primary site of peroxidase and only viable seeds show the enzyme activity. Peroxidase activity of dormant seeds was lower and varied less than that of germinating seeds. Greater variation in enzyme activity of germinating seeds is expected because of differences in age, rate of water absorption, storage conditions, etc. Such variation in single seed peroxidase activities points out that many seeds should be analyzed when investigating enzyme differences among genotypes.

Table 1. Peroxidase activity (μmole H_2O_2/min/g tissue) in single seeds

Genotype	'Redlan'				'Plainsman'				'Martin'				'Tx403'			
	2dw	3dw	F_1	LSD	2dw	3dw	F_1	LSD	2dw	3dw	F_1	LSD	2dw	3dw	F_1	LSD
Dormant seeds	4.8	5.2	4.8	0.6	4.9	4.4	4.4	0.6	5.6	5.6	5.6	0.5	11.2	11.3	10.7	1.3
Germinating Seeds	7.7	8.4	7.1	1.6	7.6	7.6	6.9	1.5	8.8	9.5	6.7	3.1	14.3	13.3	19.2	6.8
Endosperm	-	-	-	-	-	-	-	-	-	0.8	-	-	-	-	-	-
Endosperm + embryo	-	-	-	-	-	-	-	-	-	5.0	-	-	-	-	-	-
Nonviable Seeds	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-

Table 2. Peroxidase activity (μmole H_2O_2/min/g tissue) in leaf, stem, and root of five-week-old sorghum plants grown in growth chambers

	'Redlan'				'Plainsman'				'Martin'				'Tx403'			
Tissues	2dw	3dw	F_1	LSD	2dw	3dw	F_1	LSD	2dw	3dw	F_1	LSD	3dw	4dw	F_1	LSD
Leaf	1.4	1.2	1.5	0.3	1.0	1.4	1.2	0.4	1.0	1.5	1.5	0.4	0.9	1.0	1.2	0.4
Stem	1.7	1.4	1.2	0.6	1.3	1.4	1.1	0.4	1.2	1.4	1.7	0.6	1.8	1.5	1.8	0.4
Root	6.7	6.7	6.4	1.2	9.2	7.3	10.6	2.5	12.1	9.2	8.3	3.4	5.8	9.1	11.4	3.8

Table 3. Peroxidase activities (μmole H_2O_2/min/g tissue) in internodes and in flag leaf at heading stage (8 weeks after germination) for sorghum isogenic lines

	'Redlan'				'Plainsman'				'Martin'				'Tx403'			
Tissues	2dw	3dw	F_1	LSD	2dw	3dw	F_1	LSD	2dw	3dw	F_1	LSD	3dw	4dw	F_1	LSD
Growth chamber:																
Internodes	15.20	23.46	16.50	5.89	6.50	11.68	8.42	3.74	5.47	11.58	5.79	3.32	-	-	-	-
Flag leaf	11.52	11.20	11.52	2.75	11.20	12.48	11.60	2.27	11.20	12.16	13.12	2.85	-	-	-	-
Field:																
Internodes	1.86	3.17	-	1.06	1.44	2.88	-	0.93	1.15	2.18	-	0.80	1.79	2.53	-	0.90
Flag leaf	10.76	12.48	-	1.86	11.07	10.56	-	2.37	14.88	15.36	-	2.34	13.44	13.60	-	6.69

Table 4. Peroxidase activities (μmole H_2O_2/min/g/tissue) in whole section and in inner section (pith) of internodes in sorghum variety 'Martin'

				F_2		
Internode number		2dw	3dw	F_1	Short	Medium	Tall
Inner section	1	1.28	2.48	1.70	2.59	1.55	1.76
	2	1.22	3.12	2.82	2.35	1.52	1.41
	3	0.93	1.90	2.70	2.85	1.15	2.16
	4	0.82	2.10	1.26	1.60	1.54	1.17
	Avg	1.06	2.39	2.12	2.35	1.44	1.63
Whole section	1	1.38	2.53	2.50	2.56	1.47	1.31
	2	1.49	4.66	2.96	2.91	1.54	1.65
	3	2.02	3.47	2.74	2.93	1.62	1.34
	4	1.65	3.38	1.63	3.12	2.00	1.22
	Avg	1.63	3.51	2.46	2.88	1.66	1.38

2. Peroxidase Activities in Different Tissues

The average peroxidase activities in leaf, stem, and root of five-week-old plants grown in growth chamber are shown in Table 2. None of the enzyme activites showed any pattern indicating height differences. That was probably because internodes of young plants are not well differentiated nor elongated to their maximum length; expression of differential peroxidase acitivity depends on age. Root tissue had the highest peroxidase activity in these materials.

At heading stage (8 weeks after germination), peroxidase activities were determined for three sets of growth-chamber and four sets of field-grown height isogenic lines. Internode activities are averages from all individual internodes in a single stalk. Flag leaf

Table 5. Peroxidase activities (μmole H_2O_2/min/g tissue) of individual internodes

Internode number	'Martin'			'Plainsman'		
	2 dw	3 dw	F_1	2 dw	3 dw	F_1
1	6.34	9.22	7.68	3.94	4.16	7.84
2	5.31	10.05	9.79	3.52	10.56	15.68
3	5.44	12.29	6.72	8.32	10.56	8.32
4	6.08	8.26	5.92	9.41	10.24	6.72
5	4.86	11.52	4.48	11.52	11.20	6.08
6	9.60	18.24	5.28	6.08	16.32	6.40
7	4.74	5.89	4.80	6.72	12.48	6.72
8	6.34	3.46	5.70	6.40	12.00	5.76
9	4.54	3.52	5.31	5.92	11.52	8.96
10	3.46	6.53	4.80	5.12	9.38	6.72
11	5.06	6.40	5.60	5.60	11.52	7.04
12	3.84	5.92	4.48	5.60	-	6.72
13	4.61	-	5.60	-	-	-
14	3.58	-	4.29	-	-	-
Avg	5.27	8.44	5.75	6.51	10.90	7.75

activity is from an aliquot of total leaf homogenate. The results are presented in Table 3.

For growth chamber material the 3-dwarf plants had significantly higher peroxidase activity in internodes than the corresponding 2-dwarf and the F_1's. The 2-dwarf and F_1 plants did not differ significantly. For field grown material, all 3-dwarf types had higher peroxidase activity in the internodes than corresponding 2-dwarf plants. Differences observed between internodes of 3-dwarf and 4-dwarf were not statistically significant. None of the peroxidase activities in flag leaf differed significantly between height types within any set.

For internodes there was somewhat more peroxidase in the peripheral region than in the central region. When internode outer layers were removed, peroxidase activity was lower than for intact internodes, except for F_2 tall plants (Table 4).

Peroxidase activity also varied from top internodes to lower ones. Usually we used the average activity of the top four to six internodes to represent overall enzyme activity for the plant. A good correlation exists between total peroxidase and that for the top 4-6 internodes ($r = 0.94$). The peroxidase activity for individual internodes is shown in Table 5 ('Martin' and 'Plainsman' cultivars only).

3. Internodal Peroxidase Activities of Parental Lines and Their Offspring

Average internode number, plant height of two parental lines, their F_1, and three types of F_2 plants of each set, and peroxidase activities measured at three different stages are given in Table 6.

Number of internodes in the parental lines, F_1, and segregating F_2 plants did not differ significantly. However, plant height or internodal length was notably different between tall and short parents. The F_2 medium-height plants were, similar to F_1 plants, intermediate between the two parental lines. Peroxidase activity was similar for the three maturity stages measured in each plant type but differed significantly between tall and short plants. The F_1 and F_2 medium-height plants had intermediate peroxidase activity. Those results showed that the tall plants were lowest in peroxidase activity, while short plants consistently had more peroxidase activity. Medium-height plants had intermediate activities. The only exception to the inverse relationship between height and peroxidase activity was the F_2 population of 'Tx403' where peroxidase activities of medium and short plants appeared to be similar.

Although 'Redlan', 'Plainsman', and 'Martin' carry the same height genes, their absolute heights differed and the peroxidase activity did not correspond exactly to those heights. For example, 'Martin' 2-dwarf and 3-dwarf types are the shortest among the three varieties but the peroxidase activity was the lowest among the three varieties instead of being the highest as anticipated. Quinby (1975) postulated that height difference results from allelic differences in various cultivars and the allelic difference may also cause variations in the absolute peroxidase activities of the cultivars.

4. Correlation between peroxidase activity in different tissues and plant height

Peroxidase activities of dormant and germinating seeds, leaves, roots, and internodes from four sets of height-isogenic lines were determined and evaluated for correlation with height. The correlation coefficients are shown in Table 7. The association between

Table 6. Number of internodes, plant height, and internodal peroxidase activities (μmole H_2O_2/min/g tissue) of

Characteristics	'Redlan'						'Plainsman'					
	2 dw	3 dw	F_1	F_2			2 dw	3 dw	F_1	F_2		
				Tall	Med	Short				Tall	Med	Short
No. of internodes	11.7	12.0	11.7	13.0	11.0	11.7	11.3	11.3	11.0	11.0	10.7	11.3
Height (cm)	167.5	82.1	135.7	165.1	127.1	84.6	122.7	70.0	90.0	127.0	94.7	66.3
Peroxidase activity at stage of												
Flowering	2.35	3.92	2.35	2.02	3.49	4.19	2.59	4.45	2.82	2.59	2.61	3.87
Dough	2.08	4.40	1.86	1.74	2.00	3.98	2.66	5.06	2.58	2.14	2.90	3.55
Hard dough	1.81	3.34	3.04	2.18	2.34	2.94	2.48	3.71	3.10	2.21	2.32	4.18
Mean	2.08	3.89	2.42	1.98	2.61	3.71	2.58	4.40	2.83	2.32	2.61	3.87

Table 7. Correlation coefficients between peroxidase activity and plant height in seeds, roots, and internodes for height isogenic pairs and their F_2 generations of four sorghum varieties

Genotypes	Seeds		Leaves		Roots (Growth chamber grown)	Internodes		
	Dormant	Germinating	Growth chamber grown	Field grown		5-wk old growth chamber grown	Field grown parents	Field grown F_2
'Redlan'	-0.21	-0.22	0.18	-0.31	0.14	0.11	-0.61	-0.78
'Plainsman'	0.22	-0.01	0.37	0.22	0.20	-0.24	-0.64	-0.69
'Martin'	0.02	-0.19	-0.41	-0.18	0.31	-0.25	-0.59	-0.71
'Tx403'	-0.10	0.11	-0.35	-0.17	-0.29	0.06	-0.77	-0.74

The values must exceed 0.58 to be significant ($P < 0.05$).

seed peroxidase and height was low and variable; the same was true for leaves and roots; also for internode tissue from 5-week old plants, where elongation of internodes is not complete. Only in fully elongated internodes of tall and short height isogenic pairs and their F_2 height segregates was peroxidase negatively associated with height consistently. Peroxidase is clearly higher in internodes of short plants than in internodes of their taller isogenic counter-parts.

5. Acid Phosphatase Activity (APA)

Acid phosphatase was chosen as a neutral enzyme (a control) for comparing enzymes that may correlate with internode length. Acid phosphatase and peroxidase were extracted and assayed from height isogenic plants grown in the same plots. Height differences produced significant differences in peroxidase but not phosphatase activities; also phosphatase varied significantly from internode to internode while peroxidase did not (Table 8).

Our analyses showed that plant height differences are linked to peroxidase rather than the association

Table 8. Analyses of variance for peroxidase and acid phosphatase activities in top four internodes of 'Martin' height isogenic lines and their derivatives

Sourve of variation	df	Mean squares	
		Peroxidase	Acid phosphatase
Height (H)	5	26.1**	2.0
Plants within height	12	2.7	2.6
Internode (I)	3	2.6	1.2**
H × I	15	1.6	0.2
Error	36	1.3	0.2

** $P < 0.01$

four sets of grain sorghum

'Martin'						'Tx403'					
2 dw	3 dw	F_1	F_2			3 dw	4 dw	F_1	F_2		
			Tall	Med	Short				Tall	Med	Short
10.7	10.0	11.0	10.5	10.5	10.7	9.0	8.0	8.0	9.0	8.0	8.5
116.0	64.2	101.7	118.8	92.7	67.3	53.5	37.5	47.5	54.0	46.7	34.1
2.26	3.58	1.82	2.16	2.16	3.23	2.30	6.54	3.41	3.28	5.36	5.44
1.63	3.50	2.46	1.38	1.66	2.48	-	-	-	-	-	-
1.60	3.04	2.19	1.78	2.32	3.25	3.02	4.72	3.71	3.89	5.66	5.54
1.82	3.38	2.16	1.78	2.05	2.99	2.67	5.63	3.57	3.58	5.52	5.49

being a chance event. In marked contrast, the phosphatase seemed not to be influenced by height at all. However, the positive correlation ($r = 0.54$) between peroxidase and phosphatase indicates that they are associated in some manner, not necessarily related to the height gene.

6. Nitrate Reductase Activity

The protein content and that of seven essential amino acids were determined for seeds from the paired isogenic lines (Table 9). Analyses of variance in protein content revealed that the genetically shorter lines had significantly more protein; protein percentage was consistently higher in shorter plants than their tall counterparts for all isogenic pairs. Amino acid content did not differ between seed protein in short and tall isogenic plants except for minor variations in arginine and leucine.

Tall isogenic plants yield more grain (Cadady 1965) but have less protein than their short counterparts. Difference in protein may result from differences in such enzymes as nitrate reductase or low protein in taller plants may result from a diluting effect as more grain is produced. Nitrate reductase activity was assayed in seedling and fully-grown plants for all the paired isogenic lines. The results are presented in Table 10.

There is no obvious pattern for nitrate reductase activities in short plants versus tall ones in any of the isogenic pairs. Statistical analyses showed no significant differences for nitrate reductase among short, tall, or their F_1 plants at any developmental stage.

Table 9. Seed protein percentage of total amino acid percentage in protein for nine amino acids (corrected to 100 % recovery) in sorghum 2-dwarf and 3-dwarf isogenic lines

	'Redlan'		'Plainsman'		'Martin'		'Tx403'	
	Short	Tall	Short	Tall	Short	Tall	Short	Tall
Protein (%)	12.9	12.5	13.9	12.6	14.5	13.2	10.2	9.5
Lysine	1.643	1.651	1.801	1.826	1.486	1.758	2.377	2.311
Methionine	1.166	1.159	1.197	1.143	1.322	1.382	1.277	1.254
Histidine	2.106	2.019	1.958	2.023	2.635	2.027	3.083	2.310
Phenylaline	4.954	4.955	5.194	5.177	5.077	4.965	4.844	4.891
Valine	4.495	4.651	4.146	4.256	4.711	4.570	5.060	4.989
Arginine	3.094	3.167	3.000	3.306	2.899	3.343	3.755	3.878
Leucine	14.042	13.866	13.814	14.144	14.175	14.352	13.212	12.960
Isoleucine	3.602	3.630	3.737	3.715	3.698	3.667	3.612	3.504
Threonine	3.062	3.189	3.084	3.495	3.048	3.153	3.202	3.227

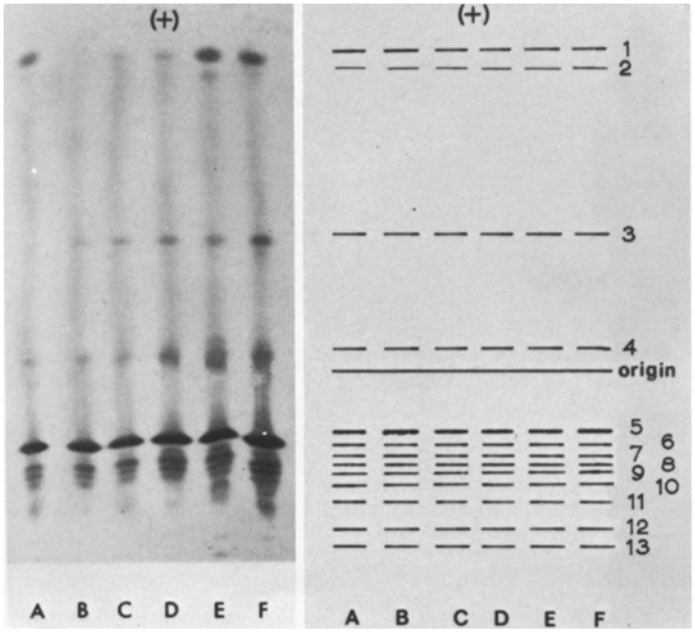

Fig. 1. Isoelectric focusing electrophoresis showing peroxidase isozymes (left) and diagrammatic presentation of the bands (right). A. 'Martin' 3-dwarf, B. 'Martin' 2-dwarf, C. F_1 (3 dw × 2 dw), D. F_2 tall, E. F_2 medium, F. F_2 short

Table 10. Nitrate reductase activity (μmole NO_2/g/hr) in leaves of sorghum height-isogenic pairs at different developmental stages

Development stage	'Redlan'			'Plainsman'			'Martin'			'Tx403'		
	Short	Tall	F_1	Short	Tall	F_1	Short	Tall	F_1	Short	Tall	F_1
Growth-chamber grown												
4-leaf stage	2.07	1.55	-	1.85	2.10	-	2.31	1.97	-	2.10	1.76	-
5-leaf stage	0.82	0.84	-	0.94	1.57	-	0.73	1.05	-	1.52	0.76	-
6-leaf stage	2.09	2.31	-	2.72	3.51	-	3.89	3.03	-	5.15	6.09	-
10 leaf stage	1.94	1.91	2.17	4.60	4.39	4.80	2.48	2.90	3.49	2.55	3.00	2.67
Field-grown												
Half bloom	6.34	8.20	6.17	6.47	7.54	7.03	4.51	5.80	4.51	7.36	8.24	7.01
Soft dough	8.09	7.30	6.17	9.90	11.21	7.03	6.87	7.10	6.40	5.02	6.30	5.62
Dough	6.95	7.50	7.69	8.55	6.76	7.81	7.35	5.45	5.02	5.98	5.05	5.47
Hard dough	4.05	5.90	3.70	5.85	5.95	5.70	7.10	7.20	6.30	4.86	5.91	6.41

7. Electrophoresis

The peroxidase isoenzyme-banding patterns of 'Martin' 2- and 3-dwarf, their F_1, and F_2 tall, medium, and short plants are shown in Fig. 1. Although the intensity of some bands varies depending on height differences, the number and position of bands are essentially the same for tall and short plants. Isoelectric focusing shows four bands (1 through 4) moving anodic and nine bands (5 through 13) moving cathodic. Bands 1, 2, 12, and 13 are very light for tall plants (B and D), band 5 is very dark and wide for all plants. The pKi values of all bands appear similar in each channel. The intensity difference between C and E band (medium height) is from concentration differences in the extracts. Similar extracts were applied

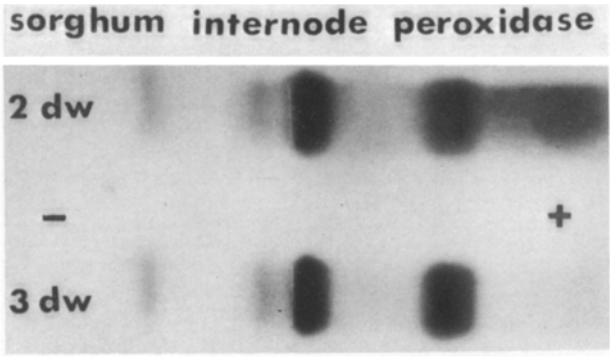

Fig.2. Zone electrophoresis showing peroxidase isozymes of 'Martin' 2-dwarf and 3-dwarf plants

in zone electrophoresis (agarose slab) experiments as shown in Fig.2. Again, similar qualitative banding patterns were observed but fewer bands were seen because this technique has lower resolving power.

Discussion

The pathway from gene to character in flowering plants is long and complex. Important steps include those that link gene-controlled enzyme activity to histological and morphological phenomena. The association between enzyme activity differences during development and morphological expression of height in a mature plant permits evidence to be gathered concerning events leading from the gene to the height character. The genetic background of height-isogenic pairs being uniform except for the locus regulating plant height allows us to determine the gene-enzyme-character association.

Our genetic analyses of the peroxidase activity in grain sorghum height isogenic pairs and their segregating F_2 plants indicate that the specific height locus (dw) corresponds inversely to peroxidase activity in the internodes. The inverse relationship between internode length and peroxidase further suggests a working hypothesis that peroxidase, acting as an oxidase, may directly inactivate indole-3-acetic acid, which in turn may regulate internode length.

Other enzymes (acid phosphatase and nitrate reductase) assayed showed no correlation with height differences in the same isogenic lines. We had thought nitrate reductase might correlate with height because dwarf varieties have higher seed protein, and nitrate must be reduced to the ammonium form to participate in protein synthesis. However, nitrate reductase did not differ between tall and short isogenic lines, so differences in seed protein of height isogenic pairs appear to be caused by other mechanisms in the assimilation process.

The isozyme banding patterns, which are essentially identical, suggest that differences in peroxidase isozymes among height isogenic types are primarily quantitative; that is, tall and short plants of the same isogenic set have the same kinds of peroxidase isoenzymes, which then differ in activities in some bands. Variation in peroxidase isoenzyme position is also under additional genetic control. Other sorghum lines that carry different height genes show different banding patterns (unpublished). Felder (1976) reported similar dingings in barley. Whether or not height genes affect traits other than length of internodes has been controversial. Our data suggest that the height gene does not affect acid phosphatase or nitrate reductase in the same quantitative manner that it affects peroxidase. Internode length and peroxidase activity are clearly associated and the relative internodal peroxidase concentrations in I.U./gram tissue follow the same simple inheritance pattern as height. We intend to further explore the cause-effect relationship between peroxidase activity and internode length by measuring both IAA oxidase and peroxidase activities. Our working hypothesis is that the height gene controls peroxidase which, by means of its oxidase activity, inversely regulates indole-3-acetic acid levels. Lower endogenous IAA levels should result in shorter internode sections.

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Received February 1, 1977
Communicated by H.F. Linskens

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