THE UPTAKE AND EFFECTS OF CALCIUM AND PHOSPHATE ON MATURITY, LIGNIFICATION, AND PEROXIDASE ACTIVITY OF WHEAT INTERNODES

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Summary

Wheat (cv. Gabo) was grown in a controlled environment and supplied with various levels of calcium and phosphate. Low levels of phosphate accelerated maturity irrespective of calcium concentrations, while high phosphate levels delayed maturity. The converse applied to calcium concentrations, and phosphate effects tended to dominate those of calcium. The maturity effects partly explain the lower lignin content of plants grown with high levels of phosphate observed by other workers.

Plants were harvested at the same level of maturity, viz. when the top internode had completed elongating. The top three internodes were analysed for calcium, phosphorus, lignin, and peroxidase.

The internodes of plants grown with higher levels of phosphate contained greater amounts of phosphorus. Internode calcium, however, did not increase above a certain value irrespective of nutrient concentration. Unlike phosphorus, internode calcium was relatively immobile.

Low-calcium plants contained the most lignin, but only with low phosphate treatments. The lignin content of medium and high calcium treatments was not significantly different. Increasing the phosphate level with medium and high calcium levels reduced lignification. Peroxidase specific activity was lower in low and high calcium and phosphate treatments than in controls, but there was no correlation between this activity and lignin content.

Plants grown with low calcium levels had a significantly higher percentage of total peroxidase attached to the internode cell walls than other treatments. Phosphate levels did not affect the amount of wall-bound peroxidase. The percentage of soluble protein loosely bound to cell walls did not differ significantly between treatments.

Phosphate apparently modifies lignification by affecting maturity, but also by some other means. Increasing the levels of calcium probably reduces lignification by releasing peroxidase from the cell wall. Phosphate effects again dominated those of calcium.

I. Introduction

Miller and Anderson (1963, 1965) have shown that high levels of superphosphate decrease lignification in wheat culms, thus making the straw more susceptible to lodging. In the present work wheat was grown under various levels of calcium and phosphorus and the lignin content of internodes determined.

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It has been found that indoleacetic acid inhibits lignification (Petinov and Urmantsev 1964; Stafford 1965), and the lack of lignin in immature tissue may be due to high levels of the growth regulator. Thus, the effects of calcium and phosphate on maturation of wheat have also been studied in the present work. If maturation is delayed then lignification is inhibited and the plant exposed to the danger of lodging for a longer period.

Peroxidase is involved in the formation of lignin (Freudenberg 1959; Brown 1961). Lipetz (1962) found that high levels of calcium reduced lignification in tissue cultures. Lipetz and Garro (1965) showed that high concentrations of calcium release peroxidase from cell walls, and they reconciled this with the effects of calcium on lignification. In the present work the effects of calcium and phosphate levels on peroxidase activity were measured. High concentrations of calcium were added to cell walls and the amount of peroxidase thus released was determined. The effect of calcium nutrition on the binding of peroxidase to the cell wall could then be estimated.

The uptake of calcium and phosphorus into wheat internodes was also measured.

II. Materials and Methods

Wheat (cv. Gabo) was germinated on damp filter paper in Petri dishes stored at 4°C. Nylex pots (5-in. diam.) were filled with CSIRO-grade perlite, and plastic gauze was placed in the bottom of the pots. The perlite of each pot was soaked with the relevant nutrient solution. Germinating seeds, the radicle having just appeared, were then placed three to a pot and sown 1 in. below the surface. The pots were placed in a growth room, the temperature being kept constant at 70°F, and the plants grown under long-day conditions (16 hr of light). Four pots were used for each of the nine treatments, and pots were randomly scattered in the growth room.

The full nutrient solution used was Hoagland's No. 2. The so-called low concentrations of calcium and phosphate were one third and the so-called high concentrations twice the levels specified by Arnon and Hoagland (1940). Plants grown with normal Hoagland's No. 2 were designated medium-calcium and medium-phosphate plants.

Nutrient solutions (100 ml per pot) were added every second day, while double-distilled water was added on alternate days.

When plants of a particular treatment had completed "heading out", i.e. the top internode had finished elongating, the plants were removed and stored in a deep freeze at −5°C. Five plants per treatment were randomly selected and sampled.

(i) *Peroxidase Extraction and Assay*

The top three internodes from plants were separated from leaf sheaths and each internode cut up with a razor-blade. The internode segments were then homogenized in a Servall Omnimixer with 6·7 mM phosphate buffer (pH 7·0) for 6 min at 220 V. The homogenate was centrifuged at 1500 *g* for 10 min. The supernatant was removed and the precipitate washed twice with buffer. The final volume of supernatant was 8·0 ml and this solution was used for peroxidase and protein determination. Extractions were carried out at 0°C.

Two ml of 0·05M Tris–maleate buffer (pH 7·0) containing 10^{-2}M $Ca(NO_3)_2$, was then added to the precipitate. The precipitate was resuspended and stored overnight at 0°C. The supernatant was removed after centrifugation and assayed for protein and peroxidase. Peroxidase activity was determined by the method of Lück (1963) using *p*-phenylenediamine as substrate. Protein was estimated according to Lowry *et al.* (1951).

(ii) *Lignin Extraction*

After peroxidase extraction the internode debris was dried and the dry weight determined. Lignin soluble in cold alcoholic alkali (designated CAA-lignin) was then extracted and estimated by the method of Phillips (1927) as modified by Miller and Anderson (1965).

(iii) *Determination of Calcium and Phosphorus Uptake*

Phosphorus levels of internodes were measured by the method of Strickland, Thompson, and Webster (1956). Calcium was determined by atomic absorption spectroscopy (David 1959).

III. Results

(*a*) *Effects of Calcium and Phosphate Levels on Maturity*

The results show that phosphate effects dominated those of calcium (Table 1). All plants grown with low phosphate were first to complete heading out irrespective of calcium concentration. Plants grown with medium phosphate matured next, except where there was low calcium, in which case maturity was delayed. High phosphate plants were slowest to mature, except where high calcium levels were present. In general, as phosphate levels increased or calcium levels decreased maturity was delayed. The calcium effect was not apparent in plants grown with low phosphate. High levels of calcium considerably reduced the maturity-delaying effect of high phosphate. Low calcium levels considerably delayed maturation when normal phosphate concentrations were present.

Table 1

EFFECTS OF CALCIUM AND PHOSPHATE ON MATURITY OF WHEAT

Number of days between germination and heading out under conditions of low, medium, and high phosphate and calcium levels are recorded

Treatment Level		Days until Heading Out Completed	Treatment Level		Days until Heading Out Completed
Phosphate	Calcium		Phosphate	Calcium	
Low	Low	53	High	High	73
Low	Medium	53	High	Low	93
Low	High	53	High	Medium	93
Medium	Medium	65	Medium	Low	95
Medium	High	65			

(*b*) *Calcium and Phosphorus Uptake by Internodes*

Plants grown under low calcium levels had a lower calcium content than other plants (Table 2). However, no significant differences in calcium content occurred between plants grown with medium and high calcium concentrations. Under conditions of low calcium, increasing phosphate concentrations slightly increased calcium uptake. At medium calcium concentrations phosphate had little effect, while at high calcium concentrations uptake was slightly reduced by increasing phosphate concentrations.

The higher level of phosphate with which the plants were grown, the greater the amount of phosphorus taken up (Table 3). Low calcium levels decreased the phosphorus taken up when phosphate was not limited. High calcium levels tended to decrease phosphorus uptake in plants grown on medium phosphate, but high levels of phosphate overcame this reduction.

The phosphate levels, expressed as a percentage of oven dry weight (Table 4) and total phosphorus (Table 3), were highest in the top internodes, successively decreasing in the second and third internodes. Calcium showed a similar distribution

pattern when expressed as total calcium (Table 2). However, when expressed as a percentage of oven dry weight (Table 4), this pattern was inverted in plants grown with low calcium. At other concentrations no clear pattern emerged. The differences between internodes were much greater in the case of phosphorus than of calcium, suggesting phosphorus may be more readily transferred from maturing to growing tissue.

TABLE 2

CALCIUM CONTENT OF INTERNODES OF PLANTS UNDER VARIOUS LEVELS OF PHOSPHATE AND CALCIUM NUTRITION

Treatment Level		Calcium Content (mg) of Internodes:			
Calcium	Phosphate	Top	Second	Third	Total
Low	Low	0·191	0·121	0·139	0·451
Low	Medium	0·257	0·156	0·130	0·543
Low	High	0·173	0·165	0·130	0·468
Medium	Low	0·478	0·229	0·195	0·902
Medium	Medium	0·285	0·200	0·173	0·658
Medium	High	0·457	0·219	0·087	0·763
High	Low	0·266	0·135	0·121	0·522
High	Medium	0·321	0·165	0·303	0·789
High	High	0·468	0·169	0·117	0·754

TABLE 3

PHOSPHORUS CONTENT OF INTERNODES OF PLANTS UNDER VARIOUS LEVELS OF CALCIUM AND PHOSPHATE NUTRITION

Treatment Level		Phosphorus Content (mg) of Internodes:			
Calcium	Phosphate	Top	Second	Third	Total
Low	Low	0·225	0·043	0·010	0·278
Medium	Low	0·160	0·032	0·005	0·197
High	Low	0·100	0·037	0·010	0·147
Low	Medium	0·389	0·083	0·024	0·496
Medium	Medium	0·424	0·269	0·094	0·786
High	Medium	0·340	0·100	0·046	0·486
Low	High	0·438	0·295	0·204	0·937
Medium	High	0·707	0·269	0·233	1·209
High	High	0·578	0·373	0·205	1·156

(c) *Effects of Calcium and Phosphate Levels on CAA-lignin Content of Internodes*

Plants grown with low calcium and low phosphate concentrations contained a higher percentage of lignin than other plants (Table 5). However, increasing the level of phosphate resulted in less lignin under low calcium levels. No significant differ-

ences in lignin content existed between high and medium calcium treatments. Medium phosphate-treated plants grown with medium and high calcium concentrations contained higher lignin levels than low phosphate plants; increasing the phosphate concentration further resulted in a decreased lignin content. The effects of treatments on lignin content of top internodes were less marked or even contradictory to the effects observed in second and third internodes. No consistent pattern in the lignin percentage of internodes of each plant became apparent. When expressed as milligram of lignin per centimetre, however, the figures generally increased from top to third internodes, presumably because the latter had completed maturing (Table 5). This suggests that the rate of lignin deposition may differ before and after heading out in different treatments.

TABLE 4

CALCIUM AND PHOSPHORUS CONTENT (AS PERCENTAGE OF OVEN DRY WEIGHT) OF INTERNODES OF PLANTS UNDER VARIOUS LEVELS OF CALCIUM AND PHOSPHATE NUTRITION

Treatment Level		Calcium Content (%) of Internodes:			Phosphorus Content (%) of Internodes:		
Calcium	Phosphate	Top	Second	Third	Top	Second	Third
Low	Low	0·173	0·187	0·250	0·204	0·066	0·018
Low	Medium	0·173	0·195	0·219	0·263	0·105	0·041
Low	High	0·250	0·307	0·344	0·633	0·550	0·540
Medium	Low	0·388	0·272	0·304	0·130	0·038	0·008
Medium	Medium	0·368	0·304	0·302	0·547	0·409	0·164
Medium	High	0·275	0·244	0·118	0·426	0·300	0·316
High	Low	0·505	0·285	0·431	0·190	0·079	0·036
High	Medium	0·237	0·168	0·394	0·251	0·102	0·059
High	High	0·331	0·212	0·178	0·409	0·469	0·311

(*d*) *Effects of Calcium and Phosphate Levels on Specific Activity of Peroxidase in Internodes*

Low and high levels of calcium depressed peroxidase activity, the former considerably more than the latter (Table 6). At all levels of calcium, low and high phosphate decreased the peroxidase activity. High phosphate decreased the activity more than low phosphate under low calcium levels, but less than low phosphate at medium and high calcium levels. Comparison between internodes of various treatments showed similar trends as the combined internode activities of each plant. The top internode, except with low phosphate and low calcium, had a lower peroxidase activity than the other internodes. Internodes of plants grown with low and high levels of phosphate had lower peroxidase activity than controls. Activity was lowest under low levels of phosphate. Low and high levels of calcium decreased peroxidase activity at all levels of phosphate. Changes in the peroxidase activities of second and third internodes were similar to changes in the combined internode activities of each plant.

TABLE 5

CAA-LIGNIN CONTENT OF INTERNODES OF PLANTS UNDER VARIOUS LEVELS OF CALCIUM AND PHOSPHATE NUTRITION

Treatment Level		CAA-lignin Content of Internodes (% dry weight):				CAA-lignin Content of Internodes (mg/cm):			
Calcium	Phosphate	Top	Second	Third	Mean	Top	Second	Third	Mean
Low	Low	11·21	7·95	19·71	12·9	2·37	3·60	6·78	3·91
Low	Medium	2·23	1·37	2·23	1·9	1·49	0·92	2·22	1·54
Low	High	1·12	3·72	6·20	3·7	0·53	2·73	4·80	2·69
Medium	Low	3·34	2·71	1·90	2·6	1·35	1·85	1·54	1·58
Medium	Medium	3·22	1·90	5·63	3·6	1·51	1·57	3·86	2·31
Medium	High	2·93	2·02	2·06	2·6	1·32	1·48	2·22	1·67
High	Low	2·01	1·71	2·73	2·1	1·09	1·29	2·78	1·72
High	Medium	2·42	5·40	6·62	4·8	1·12	3·90	4·55	3·19
High	High	3·31	3·72	3·91	3·6	1·69	2·80	3·66	2·72
Least significant difference ($P = 0·05$)		1·72	1·63	1·03		0·83	0·76	0·63	

(e) *Effects of Calcium and Phosphate Levels on Wall-bound Protein and Peroxidase*

The percentage of the total soluble protein released from internode cell walls by calcium ions was similar in all treatments (Table 7). The percentage of total peroxidase activity released, however, differed between treatments (Table 7). A

TABLE 6

SPECIFIC ACTIVITY OF PEROXIDASE IN INTERNODES OF PLANTS UNDER VARIOUS LEVELS OF CALCIUM AND PHOSPHATE NUTRITION

Activity is expressed as $10^3 \times$ units per milligram soluble protein

Treatment Level		Specific Activity of Peroxidase in Internode:			Total Peroxidase Activity	Mean Peroxidase Activity
Calcium	Phosphate	Top	Second	Third		
Low	Low	0·856	0·786	0·820	2·462	0·820
Low	Medium	0·576	0·800	1·237	2·613	0·871
Low	High	0·265	0·346	0·340	0·951	0·317
Medium	Low	0·482	1·192	1·440	3·114	1·038
Medium	Medium	1·130	7·550	7·100	15·780	5·260
Medium	High	0·617	2·322	4·660	7·599	2·533
High	Low	0·320	0·986	0·371	1·677	0·559
High	Medium	0·598	4·350	2·320	7·268	2·422
High	High	0·828	1·463	0·902	3·193	1·064
L.S.D. ($P = 0·05$)		0·328	0·407	0·372		

TABLE 7

PERCENTAGE OF TOTAL PEROXIDASE ACTIVITY AND SOLUBLE PROTEIN (VALUES IN PARENTHESES) RELEASED FROM CELL WALLS OF INTERNODES BY CALCIUM IONS

Treatment Level		Peroxidase Activity (%) and Soluble Protein (%) from:			Mean Values
Calcium	Phosphate	Top Internode	Second Internode	Third Internode	
Low	Low	15·3 (19·0)	26·8 (13·0)	23·1 (12·3)	21·7 (14·8)
Low	Medium	22·5 (19·1)	34·9 (16·6)	20·4 (18·1)	25·9 (17·9)
Low	High	20·9 (16·6)	38·5 (16·3)	18·4 (19·7)	25·9 (17·5)
Medium	Low	32·3 (15·2)	27·0 (17·8)	23·1 (17·4)	27·1 (16·8)
Medium	Medium	15·6 (15·4)	12·0 (16·5)	10·1 (15·9)	12·6 (15·7)
Medium	High	11·8 (14·4)	12·1 (12·9)	3·78 (13·5)	9·22 (13·6)
High	Low	14·3 (15·7)	15·0 (15·8)	11·9 (16·0)	13·7 (15·8)
High	Medium	9·70 (16·3)	11·1 (16·4)	23·8 (15·7)	16·1 (16·1)
High	High	9·50 (14·1)	27·5 (13·4)	11·4 (14·6)	14·0 (14·0)
L.S.D. ($P = 0·05$)		4·61 (5·31)	6·27 (4·92)	6·12 (7·10)	

significantly higher percentage of peroxidase was released from the internode cell walls of plants grown with low calcium, irrespective of phosphate level, than with

other treatments. Normal calcium and low phosphate was the only other treatment with significantly higher amounts of wall-bound peroxidase. Phosphate levels, except in the latter case, did not significantly affect wall-bound peroxidase levels.

IV. Discussion

Miller and Anderson (1965) suggested that the decreased lignification they observed in the presence of high superphosphate levels was a result of delayed maturation. Since in the present work plants were harvested at the same stage of maturity, viz. when heading out was complete, the extent to which calcium and phosphate affected lignification via maturity or some other means could be established. Low phosphate, irrespective of calcium level, and high calcium accelerated maturity. Wheat grown under either of these conditions would, therefore, be expected to contain more lignin early in the season than wheat grown with medium levels of phosphate or calcium. The converse would apply to plants grown with high phosphate since these conditions delayed maturity. High calcium considerably reduced the maturity-delaying effect of high phosphate. However, the observations of Miller and Anderson (1965) could be at least partially explained by the effects of phosphate on maturity. The reported advance in the date of harvest caused by high phosphate levels in field experiments (Russell 1961) conflicts with observations made during the present work.

As phosphate levels increased, increasing amounts of phosphorus were detected in the wheat internodes. The uptake was not significantly affected by changes in calcium concentration. Calcium uptake into the internode was greater in medium than in low calcium treatments, but no significant difference existed between medium and high calcium treatments. Therefore, high calcium levels must exert their effect on maturity indirectly, perhaps by modifying the uptake of other ions (Hooymans 1964; Hirata and Mitsui 1965; Van Steveninck 1965).

The level of CAA-lignin was high in plants grown with low calcium provided phosphate levels were also low. High and medium calcium treatments with low phosphate contained similar amounts of lignin, suggesting that the effect of calcium on lignification is a direct one. If phosphate levels simply modify the rate of lignification by accelerating or delaying maturity, one would expect plants of similar maturity, no matter what the phosphate level, to contain similar amounts of lignin. However, in the second and third internodes of plants grown with high levels of calcium and in the third internodes of plants grown with medium calcium, both high and low phosphate levels significantly decreased the CAA-lignin content (expressed either as percentage dry weight or as milligrams per centimetre) when compared with medium phosphate levels. This suggests that phosphate may be affecting lignification by other means in addition to controlling maturity.

Peroxidase specific activity is decreased by both low and high levels of calcium and phosphate. Since calcium uptake is limited, the effect of high levels must again be indirect. Peroxidase is required for lignification, but lignification may also be limited by the availability of lignin precursors (Brown 1961; Higuchi 1966) or the level of indoleacetic acid (Petinov and Urmantsev 1964; Stafford 1965). No consistent correlation existed between lignin content and peroxidase activity. This may be explained if the activity of peroxidase decreases after a certain degree of

lignification has occurred. However, other work (Galston and Dalberg 1954; Parish 1968*a*, 1968*b*) suggests that peroxidase activity increases continually during maturation and senescence. Thus it is doubtful whether peroxidase synthesis is the determining factor in the control of lignification by calcium and phosphate levels.

It has been suggested that high calcium levels reduce lignification in tissue cultures by releasing peroxidase from the cell wall (Lipetz 1962; Lipetz and Garro 1965). In all low calcium treatments in the present work there was a significantly higher percentage of total peroxidase activity loosely bound to the internode cell wall than in other treatments. Medium and high calcium treatments did not differ significantly, which was to be expected, as the internodes contained similar levels of calcium. Medium and high levels of phosphate did not affect the amount of wall-bound peroxidase in the presence of low calcium levels, but did reduce lignification. This further supports the postulate that phosphate effects dominate those of calcium and do not act via peroxidase.

The amount of protein loosely bound to cell walls did not differ significantly between treatments. This suggests that the release of peroxidase by increasing concentrations of calcium is not a non-specific effect.

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