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IMBIBED PHOSPHORUS IN SEED OF BARLEY(*Hordeum vulgare* L.) AND ITS INFLUENCE ON SEEDLINGS

BY

MINGCHU ZHANG

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled IMBIBED PHOSPHORUS IN SEED OF BARLEY (*Hordeum vulgare*) AND ITS INFLUENCE ON SEEDLINGS submitted by MINGCHU ZHANG in partial fulfillment of the requirements for the degree of Master of Science in Soil Fertility.

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Supervisor

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Date: ....,1989.

A thesis dedicated to my wife, for her valuable support.

Abstract

Seed size and seed nitrogen concentration are known to affect seedling growth, but the effect of phosphorus (P) concentration in barley seed on seedling growth is not clear. The objectives of this study were to find: the influence of seed P concentration on seedling growth; ability of seed to imbibe solution P; the location of imbibed P among seed structures; and the time when seedlings begin to assimilate imbibed P. Experiments were conducted in the greenhouse to determine the effect of P concentration in barley seed (*Hordeum vulgare* L., cv. Empress) on the rate of shoot elongation and on seedling dry matter of shoots and roots. Seeds were grown in a greenhouse in sand-filled boxes supplied with Crone's incomplete culture solution which contained KNO_3, $MgSO_4$, $CaSO_4$, $CaCl_2$ and $FeSO_4$. In the first sand experiment, three batches of seed were selected to have similar mass but the batches had different P concentrations: high-P (113.0 mmol P kg^{-1}), medium-P (80.7 mmol P kg^{-1}), and low-P (54.9 mmol P kg^{-1}). Plants were harvested 21 days after sowing. In the second sand culture experiment, high-P and low-P seeds were wetted with distilled water or with a solution of NaH_2PO_4 for 24 h, and then grown for 31 days. Growth and vigor of barley seedlings were reduced at seed P concentrations less than 80.7 mmol kg^{-1}. Seed P concentration was increased without harm to germination by treating seeds with solutions of NaH_2PO_4 for 24 h provided the concentration of P in the solution did not exceed 25.8 cmol L^{-1}. Although solution P was imbibed by barley seeds whether high or low in native P, only seed with native P concentrations less than 80.7 mmol kg^{-1} showed an increased dry matter and shoot elongation from imbibed P. The lack of beneficial effect of imbibed P on seedlings grown in sand from high-P barley seed was associated with low recovery of imbibed P in shoots and roots of those plants.

The location of imbibed P within seed structures was determined by using seeds either high in P concentration (113.0 mmol P kg^{-1}) or low in P concentration (67.8 mmol P kg^{-1}). The two seed batches were both separately treated with distilled water or a solution containing P at 25.8 cmol L^{-1} . The location of the imbibed P in seeds was investigated by analysing SEM-EDXRA spectra from four different areas (hull, aleurone, endosperm and embryo) of P-treated and non P-treated seeds. The relative P concentration was increased by the treatment for each of hull, aleurone and embryo ($P=0.05$). In addition, the four batches of seeds were germinated in the dark and shoots were sampled at 3, 6, 9 and 12 days. The P uptake was greater($P=0.05$) for seedlings grown from P-treated seeds compared to non-treated seeds after nine days of germination. We concluded that part of imbibed P entered into the seeds during the 24 h treatment and that seedlings began assimilation of imbibed P within nine days of the start of germination.

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Chapter 1. Introduction

Seed quality has long been recognized as an important factor affecting seedling establishment. One of the earliest experiments showed that large seeds of Manchuria barley produced taller seedlings than small seeds (Carleton, 1920). Later, Kiesselbach (1924) showed similar results with spring and winter wheat(*Triticum avestivum*) and oats(*Avena sativa*). In the 1960's, much work was done at the Lacombe Research Station, Alberta, which revealed the same phenomenon that large seeds generated more vigorous seedlings, more tillers and greater yield than small seeds (Demirlacakmak *et al.*, 1963; Kaufmann *et al.*,1960,1967, MacFadden, 1963).

Ever since, research has been focussed on the chemical compositions of seeds and the effects on seedling developement. Schweizer *et al.* (1969) showed that high protein content in oats resulted in more growth of seedlings. Lowe *et al.* (1972), Lowe and Ries(1972), Lopez *et al.* (1971) and Ries (1971) obtained similar results in wheat, barley and beans. The majority of N compounds stored in seeds is protein. The remainder is RNA and soluble amino acid. The proportion of the stored N is found greater in the endosperm than in aleurone layer in cereal grains, eventhough the latter is richer in protein than bulk of the endosperm (Murray, 1984). A decrease in the amount of endosperm nitrogen and a concomitant increase in axis nitrogen can be observed within 48 h of the beginning of imbibition (Ingle *et al.*,1964).

Most recently, research interests have emphasized phosphorus concentration in seeds and its effect on seedling growth. Berezkin *et al* (1984) found a positive effect of seed P in wheat on seedling growth. The P stored in seeds can be primarily classified into four groups: phytin acid, nucleic acid, lipid and protein (Murray,1984). Of those phytic acid is most abundant compound in seeds. In cereals, such as barley, 66% to 70% of P appears in the form of phytin

(Lolas *et al.* ,1976). During germination, phytase hydrolyses phytin to release orthophosphate and myo-inositol which are essential for systhesis of the cell wall (Roberts *et al.*,1968, and Loewus,1982).

The objectives of this study were to determine: 1) the effect of barley seed P concentration on seedling growth and seedling dry matter production; 2) if seed imbibed P(seeds treated with P solution) increased seedling growth; 3) the location of imbibed P; and 4) the time at which seedlings began to use imbibed P.

References

Berezkin, A.N., V.N. Guida , N.A. Klochko, L.L. Derezkina, and V.V. Dakeev. 1984. Sowing qualities of barley and winter wheat seeds depend on their phosphorus content. Izvestiya Timiryazevskoi Sel'skokhozyaistvennoi Akademii, 2:51-56.

Carleton, M. A. 1920. The Small Grains. The MacMillan Co., New York. P184-186.

Demirlicakmak, A., A.L. Kaufmann, and L.P.V. Johnson. 1963. The influence of seed size and seed rate on yield and yield component of barley. Can. J. Plant Sci. 43: 330-337.

Ingle, J., L. Beevers and R.H. Hageman.1964 Metabolic changes associated with the germination of corn I. changes in weight and metabolites and their redistribution in the embryo axis, scutellum, and endosperm. Plant Physiol 39:735-740.

Kaufmann,M.L., and A.A. Guitard. 1967. The effect of seed size on early plant development in barley. Can. J. Plant Sci. 47: 73-78.

Kaufmann, M.L. and A.D. McFadden. 1960. The competitive interaction between barley plants grown from large and small seeds. Can. J. Plant Sci. 40:623-629.

Kiesselbach, T.A. 1924. Relation of seed size to yield in small grain crop. J. Am. Soc. Agron. 16:670-682

Loewus, M.W., and Loewus, F. A.,1982. Myo-inositol-1-phosphate from the pollen of *Lilium longiflorun* Thunb. Plant Physiol. 70:765-770.

Lolas,G.M., N. Palamidis and P. Markakis 1976. The phytin acid-total phosphorus relationship in barley, oats, and soybeans and wheat. Cereal Chem. 53:867-871.

Lopez, A. and D.F. Grabe. 1971. Effect of seed Protein content on plant growth of barley and wheat. Agron. Abstr. P44.

Lowe, L.B., G.S. Ayers, and S.K. Ries. 1972. The relationship of seed protein and amino acid composition to seedling vigor and yield of wheat. Agron. J. 64:608-611.

Lowe, L.B. and S.K. Ries. 1972. Effects of environment on the relation between seed protein and seedling vigor in wheat. Can. J. Plant Sci. 52:157-164.

McFadden, A.K. 1963. Effect of seed source on comparative test results in barley. Can. J. Plant Sci. 43:295-300.

Murray, D. R. 1984. Seed Physiology. Academic Press Astralia. vol. 2 p163-196.

Ries,S.K. 1971. The relationship of size and protein content of bean seed with growth and yield. J. Amer. Soc. Hort. Sci. 96:557-560.

Roberts, R. M., J. Deshusses and F. Loewus 1968. Inositol metabolism in plants V. conversion of myo-inositol to uronic acid and pentose units of acidic polysaccarides in root tips of zea mays. Plant Physiol. 43:979-989.

Schweizer, C. J. and S.K. Ries. 1969. Protein content of seed; increase improves growth and yield. Science 165:73-75.

Chapter 2. Phosphorus concentration in barley(*Hordeum vulgare* L.) seed: influence on seedling growth and dry matter production[1]

Introduction

Seed quality components, such as seed size and seed nitrogen concentration, influence seedling establishment and grain yield of cereals (Kaufmann, 1967 and Ries, 1973). Large seeds usually generate vigorous seedlings, more tillers per plant and higher yield at harvest (Austenson,1970, Demirlicakmak,*et al.* 1963 and Kaufmann,1967). McFadden (1963) suggested that the chemical composition of barley seed could be associated with yield performance, although quantitive data were not given. Subsequent research has focussed on seed protein or N content and its relationship to seedling vigor and yield. Lowe (1972) and Ries (1971 and 1972) found seed protein content related positively to seedling vigor and yield.

Research into the influence on plant performance of P concentration in seeds has developed more recently. Read (1983) suggested that high P concentration in seed may compensate for stress conditions. Berezkin *et al.* (1984) found that higher P concentration in winter wheat seeds increased seedling growth, but seed P of barley had less effect.

We have observed from field studies that P content of barley seeds is influenced both by P status of the soil and by fertilizer P added during growth of the parent crop (Nyborg, 1986 unp[illegible]l. data). Technologies also exist for direct preplanting imbibition of P into seeds. The form of the P in the former case would be primarily inositol compounds, in the latter, orthophosphate. The

1. A version of this chapter has been submitted for publication.Zhang, M.,Nyborg, M., and McGill,W.B. 1989. Plant and Soil.

influence on seedling performance of P content of barley seeds and of imbibed P is unclear.

The objectives of this research were to test the hypothesis that native and imbibed P in barley seed directly increase seedling growth and seedling dry matter accumulation; and to discover: i) the concentration of P that can be imbibed before germination is affected; and ii) the recovery in seedlings of seed P during barley growth in sand culture under greenhouse conditions.

Materials and methods

Three batches of barley seed (*Hordeum vulgare* L, cv. Empress) were used to provide a range in native P content. One was a commercial seed source with P content of 113.0 mmol kg^{-1}, the other two were from field research plots treated with either NPKS or NKS and having P contents of 80.7 and 54.9 mmol kg^{-1}, respectively. We designated those three batches of seeds as high-P, medium-P and low-P seed, respectively.

All experimental designs were randomized complete blocks, replicated three times. Barley seedlings were grown in a greenhouse at Edmonton (Lat. 54^{o} N, Long. 114^{o} W) without artificial lighting in June and July, 1987. The greenhouse glass was partially screened with whitewash to reduce bench temperature. Night and day temperatures varied between 15 and 28 ^{o}C, respectively.

Shoot height of each plant was measured to the tip of the tallest leaf at two-day intervals during the period of plant growth. Seedlings were harvested by cutting the shoots at the sand surface and by washing the roots from sand. The shoots and roots were dried at 65 °C and weighed.

The seeds, shoots or roots were ground to <1.3-mm prior to analysis. N and P contents of plant materials was determined by digestion in H_2SO_4 and H_2O_2 according to Technicon (1977), followed by a colorimetric measurement of N and P concentration (Technicon, 1975).

Effect of native seed P

Seeds of similar size were visually selected from each of the three sources. The seeds were soaked in distilled water for 24 h , and then 20 seeds from each group were grown in sand held in a wooden box (20 cm long, 15 cm wide and 30 cm deep). The bottom of the box was perforated to allow drainage. Crone's incomplete culture solution (Hewitt, 1966) was supplied to each box during the growth period to eliminate factors other than seed P concentration, which could affect seedling growth. The solution contained 9.9, 4.2, 3.7, 3.2 and 0.3 mmol L^{-1} of KNO_3, $MgSO_4$, $CaSO_4$, $CaCl_2$ and $FeSO_4$, respectively. The experiment had three treatments (high-P, medium-P and low-P seeds) and was terminated 21 days after planting.

Tolerance to imbibed P

To determine tolerance of seed germination to NaH_2PO_4 solution, seeds were soaked for 24 h with P concentrations ranging from 0 to 45.2 cmol L^{-1}. Percentage germination was determined at 23°C by placing 60 soaked seeds in a petri dish between moist filter papers, or by placing 100 soaked seeds on a moist paper towel in a foil pan. Germinated seeds were counted at 24 h intervals for three days.

Effect of imbibed P and fate of seed P

Seeds were selected by sieving the seeds through a 2.5 by 19.1 mm sieve and retaining them on a 2.0 by 19.1 mm sieve. The P concentration of low-P seeds after sieving was slightly increased to 67.8 mmol kg^{-1}. Low-P and high-P seeds were soaked in distilled water or in 25.8 cmol L^{-1} P solution (NaH_2PO_4) for 24 h, and then 20 seeds were planted in each box and maintained as above. There were four treatments: low-P seed soaked in water; low-P seed soaked in P solution; high-P seed soaked in water and high-P seed soaked in P solution. This experiment was terminated 31 days after planting.

Results and discussion

Effect of native seed P

Average mass per 100 seeds and percentage germination were only moderately different among the three seed batches, but the P concentration differed greatly (Table II.1). By holding seed size constant and providing plants with adequate nutrients, other than P, differences in shoot elongation from the various batches of seed were thus attributed to differences in P contents(Fig.II. 1). The plants grown from high-P seed were consistantly taller than from medium-P seed, and much better than from low-P seed. The shoot height of high-P seed and medium-P seed at harvest (21 days) was significantly different (P= 0.05) from that of low-P seed (Table II. 2).

Seedling vigor and seedling relative growth rates can also be inferred from short-term seedling dry matter production(Lowe, *et al.* 1972). Dry matter accumulation was not significantly different between seedlings grown from high-P and medium-P seeds, but was significantly greater (P= 0.05) than that by

Table II.1. Characteristics of barley seed selected to provide a range in P concentration.

Seed batch	Mass/100 seed	Germination	Concentration N	Concentration P
	g	%	mmol kg^{-1}	
Low-P	3.43	98	1,270	54.9
Medium-P	3.95	81	1,340	80.7
High-P	3.81	98	1,330	113.0

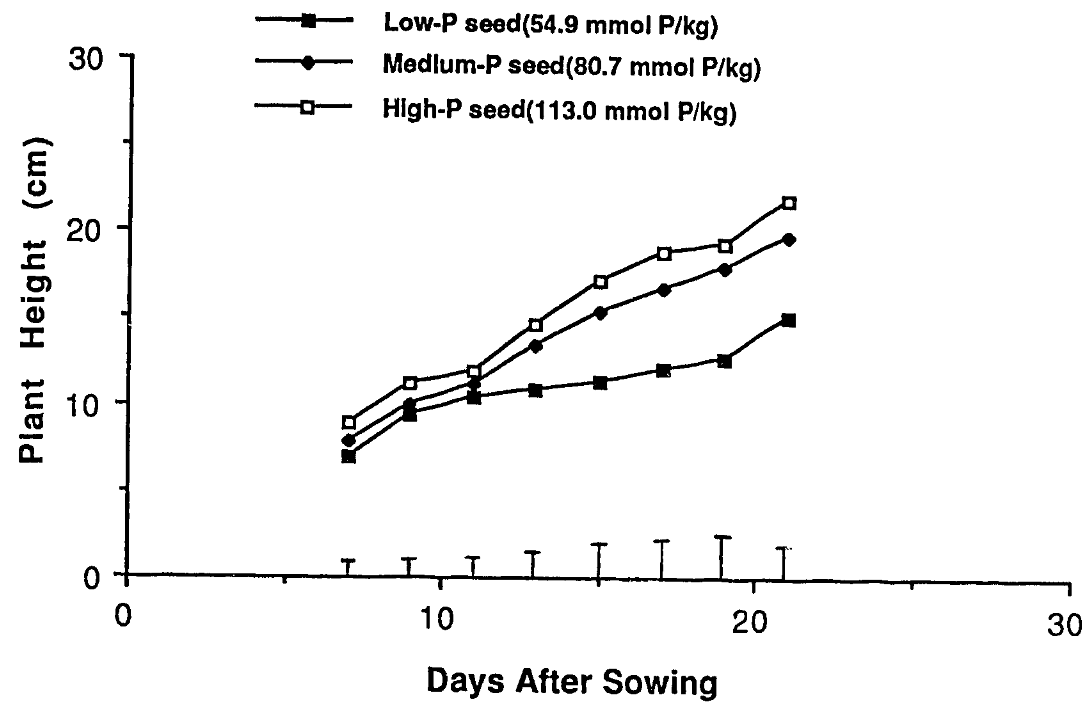

Fig.II.1. Shoot elongation by seedlings grown from seeds with diffferent P concentrations. Each value represents mean (n=3). Vertical bars indicate LSD (P=0.05).

Table II.2.. Plant height and dry matter produced during 21 days from seeds having different P concentrations.

Seed batch	Plant height	Dry matter Shoot	Dry matter Root	Dry matter Total
	cm	g		
Low-P	15.1	0.77	0.78	1.55
Medium-P	19.7	1.23	1.07	2.30
High-P	21.8	1.25	1.07	2.32
LSD (0.05)	2.0	0.16	0.16	0.31

seedlings grown from low-P seed (Table II. 2). Berezkin *et al.* (1984) found little increase of dry matter production of spring barley seedlings with increasing P concentration of seed batches, but the P concentration in the seed batches tended to be high and similar (range 102 to 145 mmol kg^{-1}). They obtained substantial positive results with winter wheat, and those seeds were low in P concentration and had marked differences in P contents among seed batches. In present work, the ratio of concentration of N:P in the seedlings was much greater than healthy plants (about 10:1) (TableII.3). That is, the high N:P ratio for the seedlings indicated the seed P was depleted by harvest.

Table II.3 N and P concentrations of plant tissue in the first sand culture experiment

	N		P		N:P	
Treatments	Shoot	Root	Shoot	Root	Shoot	Root
	%					
Low-P	3.82	2.15	0.078	0.059	49	36
Medium-P	3.91	2.19	0.082	0.061	47	36
High-P	3.69	2.24	0.083	0.059	44	38

In summary, barley seed with less than 80.7 mmol kg^{-1} native P yielded less vigorous seedlings than did seeds having 80.7 mmol P kg^{-1} or greater.

Effect of imbibed P

Having demonstrated that P concentration in seed affected barley seedling growth, the question remained: would increasing seed P content by seed treatment also benefit seedling growth? When adding P to seeds through imbibition, it is desirable to achieve sufficient imbibition of P to benefit developing seedlings while minimizing osmotic damage to the seed embryo. An optimum concentration was

Table II.4. Cumulative germination of barley seed treated with NaH_2PO_4 solution for 24 h

Concentration of P in solution	Cumulative germination		
	2D[a]	3D	4D
cmol L^{-1}	%		
0	98	98	98
6.5	98	98	98
12.9	72	81	83
19.4	62	75	84
25.8	58	78	86
32.3	47	63	75
38.7	35	48	59
45.2	38	54	70
LSD (0.05)	9	13	14

[a] Days after completion of soaking with P solution.

obtained by treating healthy medium-P seeds with P solution (NaH_2PO_4) for 24 h at concentrations ranging from 0 to 45.2 cmol L^{-1}. Germination was delayed following treatment with NaH_2PO_4 at 32.3 cmol L^{-1} or more (Table II. 4). There were no differences, however, among treatments from 12.9 to 25.8 cmol L^{-1} after 4 days. A P concentration in solution of 25.8 cmol L^{-1} was accepted as the 'optimum' for seed treatment. The P content of high-P and low-P seed was increased by soaking for 24 h at this solution concentration (Table II.5). Shoot elongation rates of seedlings from these

Table II.5. Mass and P concentration of barley seed with and without P treatment

Seed batch	Mass/100 seeds	P concentration in seed
	g	mmol kg^{-1}
Low-P seed	4.29	67.8
Low-P seed +P treat.[a]	4.33	126.0
High-P seed	4.30	113.0
High-P seed +P treat.[a]	4.27	154.8

[a] Soaked for 24 hours in water solution of NaH_2PO_4 containing 25.81 cmol L^{-1} of P (as NaH_2PO_4).

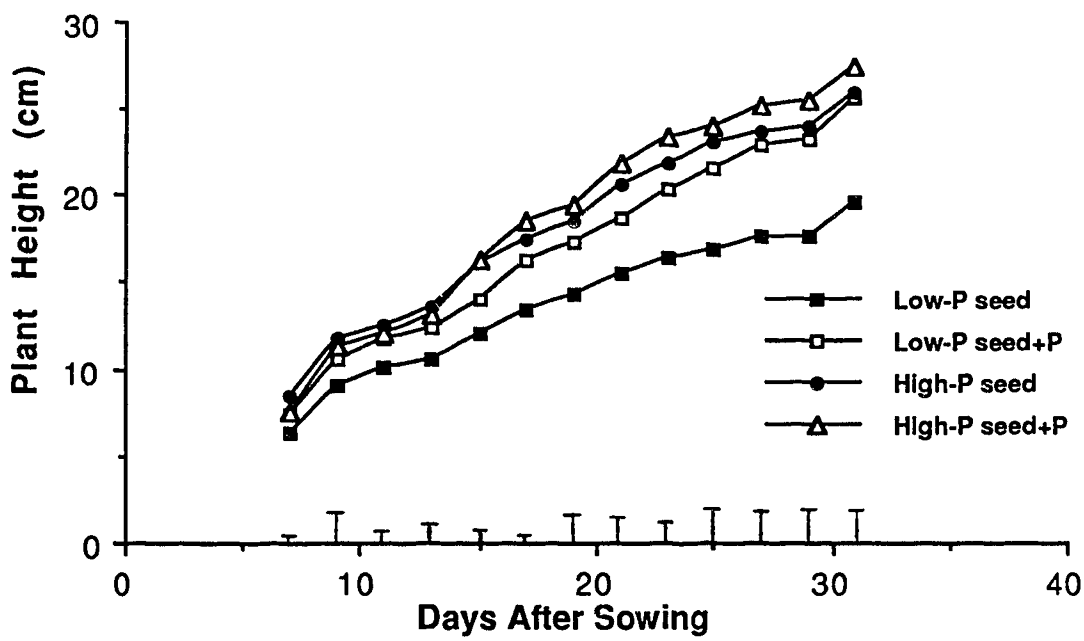

Fig.II.1.Shoot elongation by seedlings grown from seeds with different concentrations of native and imbibed P. Each value represents mean (n=3). Vertical bars indicate LSD (P=0.05).

seeds related directly to P concentration in the seed(Fig.II. 2). Shoots from high-P seed treated with 25.8 cmol L^{-1} P solution tended to be shorter than high-P seed without treatment during the first 15 days, after which the trend reversed. In contrast, growth from low-P seed was less than from low-P seed treated with P solution.

The dry matter of plant tissues (31 days) was not different among high-P seed, high-P seed + P treatment, and low-P seed + P treatment, but dry matter in these three treatments were all significantly greater(P=0.05) than from low-P seed (Table II. 6). The shoot height from different seed batches exhibited the same behaviour as the dry matter (Table II. 6). The benefit from imbibed P appeared, therefore, to be a function of initial P content of the seed and of seedling age. In addition, N:P ratio of those seedlings was also very high (Table II.7).

Table II.6. Plant height and dry matter produced during 31 days from seeds containing a range of native and imbibed P.

Seed batch	Plant height	Dry matter		
		Shoot	Root	Total
	cm	g		
Low-P	19.6	1.26	1.14	2.40
Low-P+P treat	25.7	1.73	1.52	3.25
High-P	26.0	1.87	1.57	3.44
High-P+P treat	7.4	2.02	1.65	3.67
LSD (0.05)	1.8	0.17	0.21	0.38

Table II.7 N and P concentrations of plant tissue in the second sand culture experiment

	N		P		N:P	
Treatments	Shoot	Root	Shoot	Root	Shoot	Root
	%					
Low-P	3.40	2.32	0.075	0.053	45	44
Low-P+P treat.	3.39	2.23	0.083	0.063	41	35
High-P	3.48	2.24	0.073	0.057	48	39
High-P+P treat.	3.34	2.22	0.078	0.055	43	40

The recovery of seed P, or of P added to the seed, was greater from low-P seed (Table II. 8). Recovery of P in plants grown from the low-P containing seed was nearly complete (89%), while recovery in plants grown from the high-P seed was somewhat less (75%). If recovery of native seed P was independent of imbibed P, then apparent recovery of imbibed P was less than for native seed P. Only 60% of imbibed P was recovered from low-P seed; this dropped even further (to 21%) when high-P seed was treated. A possible explanation may be leaching of imbibed P from germinating and emerging seeds during watering. Initially we added 0.40 L culture solution to the sand surface of each box (0.03 m^2) each day, which resulted in water drainage from holes in the bottoms of the boxes. If native P was used first by the plants, then the added P would be vulnerable to leaching, especially for the high-P seed. Work is underway to determine the location of imbibed P in seeds, and to compare the effect of imbibed P when seeds are grown in soil instead of sand.

Table II.8. Recovery in shoots and roots at 31 days of native seed P and imbibed P

Seed batch	P recovery[a]	Recovery of seed P	Apparent Recovery of added P
	mg	%	
Low-P seed	1.59	89	
Low-P seed + P treat	2.49		60
High-P seed	2.28	75	
High-P seed + P treat.	2.47		21
LSD (0.05)	0.47	17	37

[a] recovery of P per box (shoot+root).

Conclusions

Reduced growth and vigor of barley seedling (cv. Empress) can be expected at seed P concentrations less than 80.7 mmol kg^{-1} when grown in sand under greenhouse conditions. Seed P concentration can be increased by treating seeds with solutions of NaH_2PO_4 for 24 h without harm to the embryo provided the concentration of P in solution did not exceed 25.8 cmol L^{-1}. Although solution P can be imbibed by barley seeds which are either high or low in native P, only seeds with a native P concentration less than 80.7 mmol kg^{-1} can be expected to benefit in terms of dry matter accumulation and shoot elongation from imbibed P. The lack of beneficial effect of imbibed P on seedlings grown in sand from high-P barley seed was associated with low recovery of imbibed P in shoots and roots of those plants.

References

Austenson, H.M., and P.D. Walton.1970. Relationship between initial seed weight and mature plant characters in spring wheat. Can. J. Plant Sci.50:53-58.

Berezkin, A.N., V.N. Guida , N.A. Klochko, L.L. Derezkina, and V.V. Dakeev. 1984. Sowing qualities of barley and winter wheat seeds depend on their phosphorus content. Izvestiya Timiryazevskoi Sel'skokhozyaistvennoi Akademii, 2:51-56.

Demirlicakmak, A., M.L. Kaufmann, and L.P.V. Johnson. 1963. The influence of seed size and seed rate on yield and yield component of barley. Can. J. Plant Sci. 43: 330-337.

Hewitt, E.J. 1966. Sand and Water Culture Method Used in the Study of Plant Nutrition p 187-198. Commonwealth Agriculture Bureaux Farnham Royal. Bucks . England.

Kaufmann,M.L., and A.A. Guitard. 1967. The effect of seed size on early plant development in barley. Can. J. Plant Sci. 47: 73-78.

Lowe, L.B., G.S. Ayers, and S.K. Ries. 1972. Relationship of seed protein and amino acid composition to seedling vigor and yield of wheat. Agron. J. 64:608-611.

McFadden, A.D. 1963. Effect of seed source on comparative test results in barley. Can. J. Plant Sci. 43:295-300.

Read, D.W.L.,1983. Residual phosphorus increase the P content of wheat. Better Crops, Spring 67:26-27.

Ries, S.K. 1971. The relationship of size and protein content of bean seed with growth and yield. J. Amer.Soc. Hort. Sci. 96:557-560.

Ries, S. K., and E.H. Everson. 1973. Protein content and seed size relationship with seedling vigor of wheat cultivars. Agron. J. 65:884-886.

Technicon, 1975. Industrial Method No. 369-75A/A+ Technicon Instrument Corp, Tarrytown, N.Y.

Technicon, 1977. Industrial Method No. 334-74W/ B+ Technicon Instrument Corp, Tarrytown, N.Y.

Chapter 3. Phosphorus imbibed by barley seeds: location within seed and assimilation by seedlings[1]

Introduction

The influence of P concentration in seeds on the establishment of seedlings has been recently studied. Berezkin *et al.* (1984) found an increase in seed P in wheat resulted in more seedling growth. Sand culture experiments indicated that high P concentration in barley seed (*Hordeum vulgare* L.) increased seedling growth (Chapter 2). We also found that seed imbibed P (treating seeds with P solution) resulted in higher dry matter accumulation in seedlings. Seed P concentration was increased when seeds were treated with P solution, but location of the imbibed P within the seed was not determined. Separating different structures of seeds (hull, aleurone, endosperm and embryo) would be difficult and subject to contamination. Scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDXRA), however, provided a useful tool to determine the relative abundance of elements in a small volume (Norton *et al.*,1983, 1984).

The objective of this study was to examine the changes of relative P abundance in hull, aleurone,endosperm and embryo of seeds before and after treatment with P solution; and to determine when seedlings begin to use imbibed P.

Materials and Methods

Two sources of barley seed were selected in the experiments with P concentration of 113.0 and 67.8 mmol kg^{-1}. They were designated as high-P

1. A version of this chapter will be submitted for publication Zhang, M.,Nyborg, M., and McGill, W.B. 1989. Plant and Soil.

and low-P seed, respectively.

To determine location of imbibed P in seeds, high and low P seeds were treated with 25.8 cmol P L^{-1} (NaH_2PO_4) or distilled water for 24 h, respectively, in four replicates. After treatments total P concentration was increased to 154.8 and 126.0 mmol P kg^{-1} for high-P and low-P seeds, respectively. Seeds were cut asymmetrically so that the embryo was evenly divided between the two portions. The cut seeds were attached to Cambridge style SEM stubs and then air dried. They were photographed and analysed on a Cambridge 250 SEM with a Tracor Northern 5500 computerized X-ray spectrometer. The background of EDXRA spectra generated from hull, aleurone, endosperm or embryo was kept at the same level. The P peak height from P-treated and non-treated seeds was measured, and the differences were statistically analysed.

The experiment was in a randomized factorial design. Because there was no statistically difference between the high-P and low-P seeds in terms of P peak height in all four areas, the two sources of seeds were combined for statistics. The EDXRA data were finally analysed by mean differences with eight replicates.

To determine the time of uptake of imbibed P, low and high P seeds were soaked with 25.8 cmol P L^{-1} or distilled water for 24 h. The seeds were placed on moist paper towels in foil pans and were kept in the dark with a temperature of 23 ^{o}C. The shoots were harvested at 3, 6, 9 and 12 days after initiation of germination by cutting them near the seeds. The experiment design was a randomized complete block, replicated three times.

The shoots were dried at 65 ^{o}C and P concentration of shoot was determined by digestion in H_2SO_4 and H_2O_2 in accordance with Technicon (1977) followed by a colorimetric measurement of P concentration (Technicon,1975).

Results and Discussion

The EDXRA spectra generated from the four areas within the seed (hull, aleurone, endosperm and embryo)(Fig.III.1) revealed marked differences in P distribution. The P peak of non treated seeds was higher in the aleurone and embryo than in the hull and endosperm (Fig.III.2 a,b,c,d). However, in P-treated seeds, the height of P peak was increased in all four areas, especially in the hull and aleurone (Fig.III.2 e,f,g,h). The stored P in seeds was mainly in the form of phytin (Murray,1984). O'Dell *et al.* (1972) found 85% of the phytin in wheat seeds was contained in the aleurone layer, with 13% in the embryo and 2% in the endosperm. Using peak height as an indicator of relative abundance of P, the differences of peak height between P-treated and non P-treated seeds were found greater in hull, aleurone and embryo (P=0.05)(Table III.1).

Table III.1. Comparison of P peak height between P-treated and non-treated seeds

Treatment	Part of seed			
	Hull	Aleurone	Endosperm	Embryo
Seeds	1.9	20.9	2.6	15.6
Seeds + P treat[a]	43.7	39.8	5.8	21.6
t value	19.9	2.7	1.5	2.3
t (0.05)	2.1	2.1	2.1	2.1

a Seeds were treated with 25.8 cmol P L^{-1}.

P uptake in young seedlings varied with P concentration in seeds whether that P was original or imbibed. The P uptake in seedlings grown from P-treated

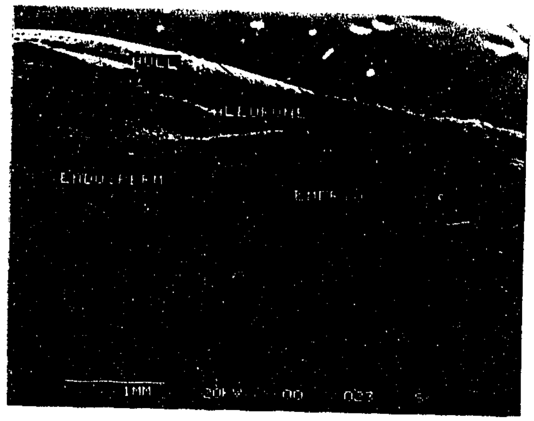

Fig. III.1. SEM image of a sliced seed showing the locations of EDXRA spectra at four different areas.

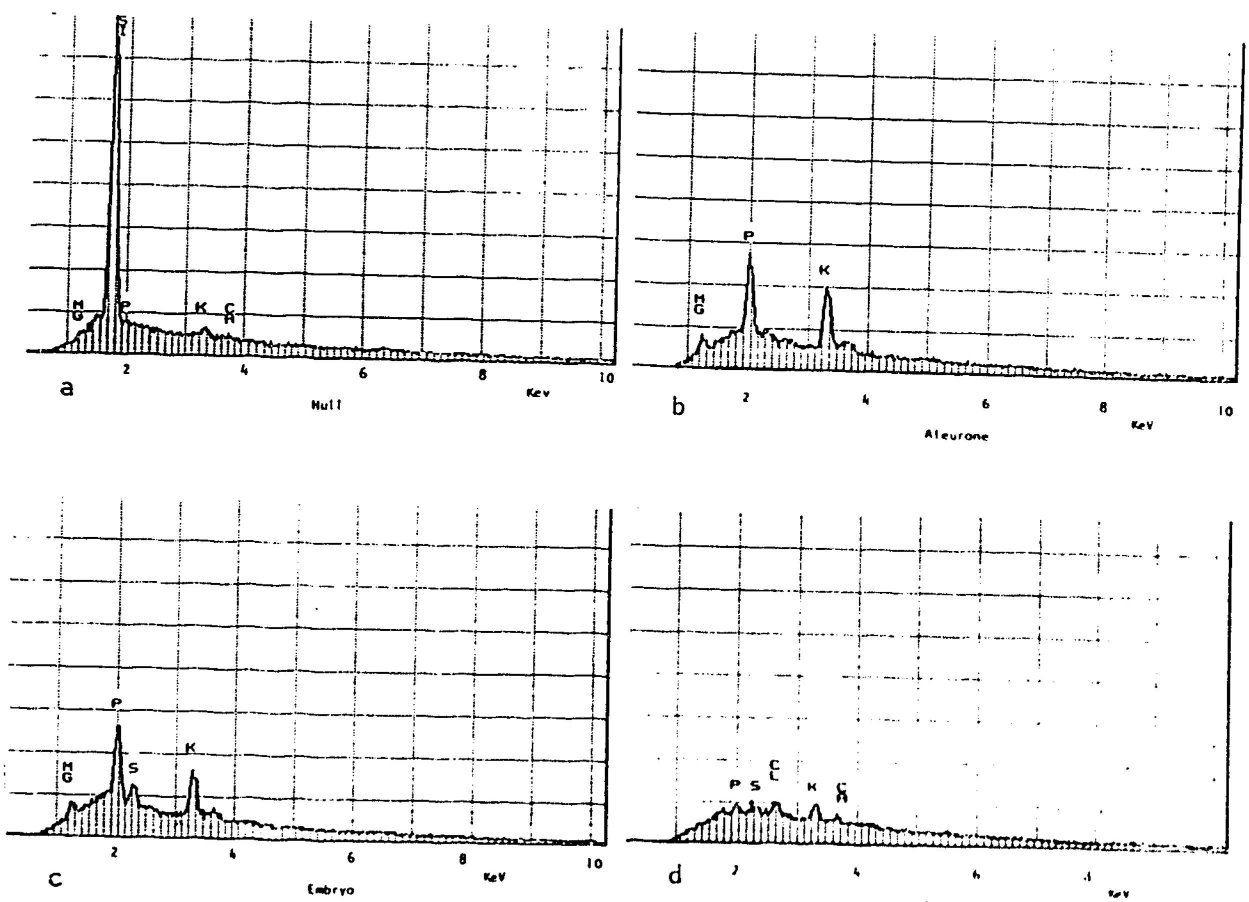

Fig. III.2. EDXRA spectra from the four corresponding areas, a,b,c,d were non P treated seed, e, f, g, h from seed treated with P solution.

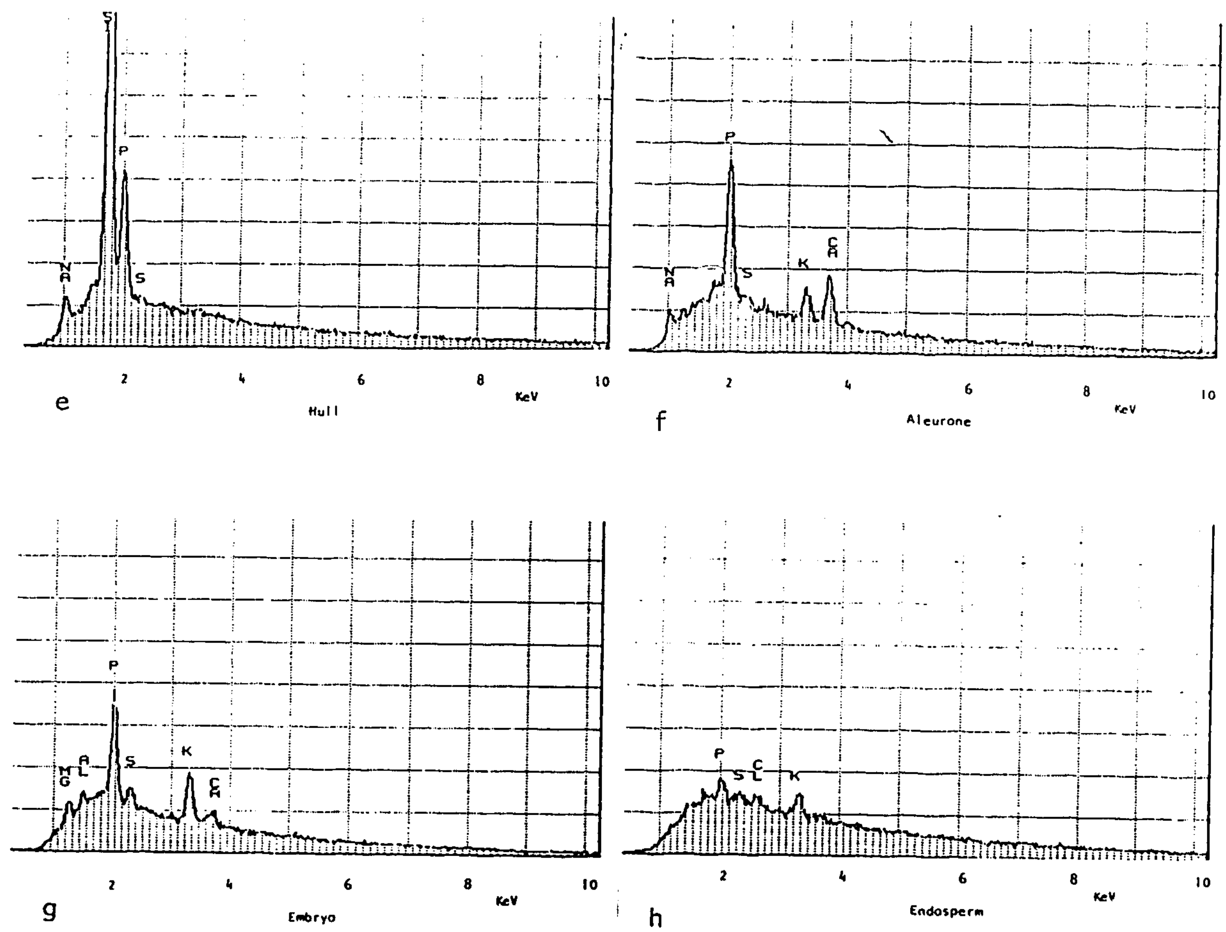

Fig. III.2. (continue)

seeds was not different from that of non-treated seeds at 3 days after germination, but P uptake in seedlings from treated seeds and high-P seed were higher (P=0.05) than that of low P seeds at 6 days. After that, the differences of P uptake in seedlings between P-treated and non treated seeds became greater(P=0.05).

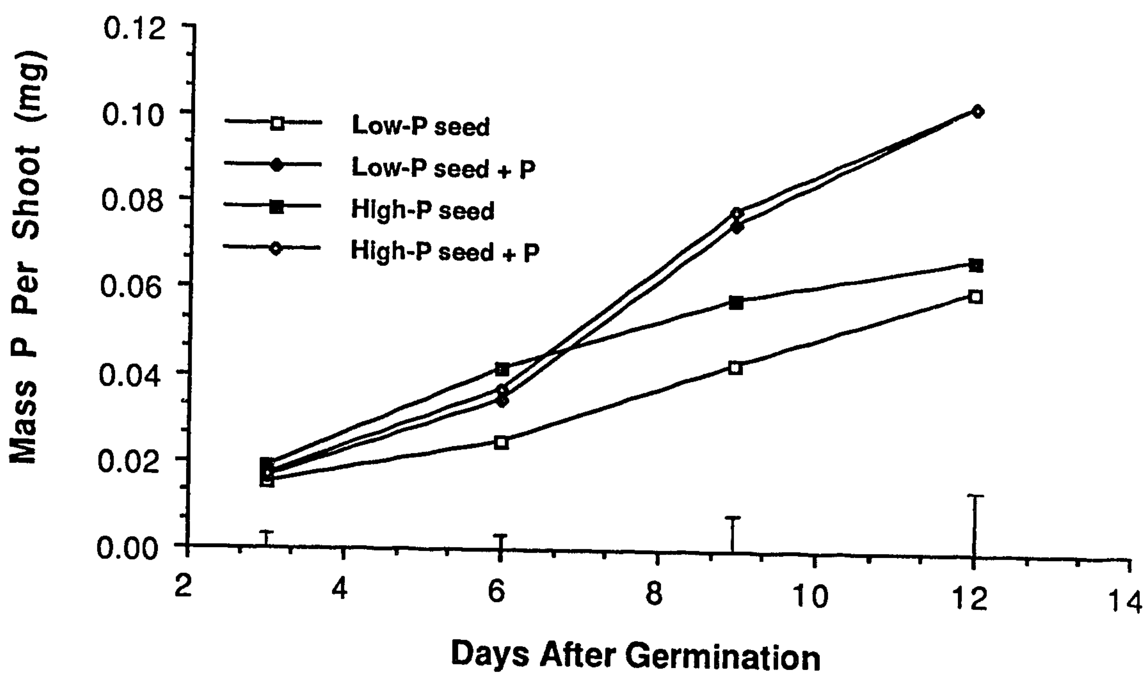

Fig. III.3. P uptake in seedlings from P-treated and non-treated seeds high or low in original P content at 3, 6, 9 and 12 days after germination.The values are mean of 3 replicates and vertical bars indicate LSD (P=0.05) at each date.

Phytin is the most abundant P compound stored in seeds, and is hydrolysed to release orthophosphate during germination (Murray,1984). The form of the imbibed P was orthophosphate in this experiment. As a general rule, increase of the products of a chemical reaction will slow the reaction. In this experiment, increase of the product (orthophosphate) did not inhibit the hydrolysis reaction of phytin during the seed germination. In the first three days, the mass of P in seedlings was not different between treated and non-treated seeds. However, as the depletion of seed-stored P, imbibed P was transferred to seedlings at six days in low P seeds and nine days in high P seeds. Therefore, the translocation of seed-stored P and imbibed P were two independent processes, that is, the seed-stored P was first used by the seedlings and then the imbibed P.

Conclusions

The P abundance in the hull, aleurone and embryo was increased when barley seeds were treated with P solution. As a result, seedlings began to use the imbibed P within nine days after germination.

References

Berezkin, A.N., V.N. Guida , N.A. Klochko, L.L. Derezkina, and V.V. Dakeev. 1984. Sowing qualities of barley and winter wheat seeds depend on their phosphorus content. Izvestiya Timiryazevskoi Sel'skokhozyaistvennoi Akademii, 2:51-56.

Murray, D. R. 1984.Seed Physiology. Academic Press Australia. Vol. 2 p169-196.

Norton, L.D., J.M. Bigham, G.F. Hall and N.E. Smeck. 1983. Etched thin sections for coupled optical and electron microscopy and micro-analysis. Geoderma. 30:55-64.

Norton, L.D., G.F. Hall, N.E. Smeck and J.M. Bigham.1984. Fragipan bonding in a late-Wisconsinan loess-derived soil in east-central Ohio. Soil Sci. Soc. Am. J., Vol 48 p1360-1366.

O'Dell, B. L., A. R. de Boland and S. R. Koirtyohann 1972. Distribution of phytate and nutritionally important elements among the morphological components of cereal grains. J. Agr. Food Chem. 20:718-721.

Technicon, 1975. No. 369-75A/A+ Technicon Instrument Corp, Tarrytown, N.Y.

Technicon, 1977. No. 334-74W/ B+ Technicon Instument Corp, Tarrytown, N.Y.

Chapter 4. Synthesis

Summary

Seedling growth was affected by seed P concentration. When seed P concentration was below 80.7 mmol kg^{-1}, the growth rate of seedling (using shoot elongation as an indicator) was stunted. Total P concentration of seed was enhanced by treating seeds with a 25.8 mmol P L^{-1} solution, but the germination velocity (number of seeds germinated per day) was delayed. The benefit that seedlings got from the 'imbibed P' in terms of dry matter production was observed only when the original seed P concentration was less than 80.7 mmol kg^{-1}. This phenomenon was observed in sand culture and in soil. The SEM-EDXRA analysis indicated that part of the imbibed P entered into the seeds from a 25.8 mmol P L^{-1} solution. As a result, seedlings began to use the imbibed P within nine days after initiation of germination; and the P concentration in seedlings grown from P treated seeds increased substantially. Over all, this research found a positive effect of seed imbibed P on seedling growth, and this may lead to an improved way of P added to crops and promoting P efficiency.

An approach toward the P efficient use.

When P fertilizers are applied to soil, they can react with cations (Ca^{2+} Fe^{3+}, Al^{3+}) and form less soluble compounds like variscite and strengite (Tisdale, *et al.*,1984). Research into the efficiency of P has been in progress for many years. Banding as compared to incorporation is an example of improved efficiency. However, most experiments showed much less than 24% of added P was recovered by a crop (Barber,1978). Our research indicated that high P content in seeds (original or imbibed) resulted in vigorous growth of seedlings. Vigorous seedlings simply mean high potential yield. Therefore, this study could lead two ways of promoting P efficient use. One is selecting high P content of seeds. Those seeds would generate large root systems in the early growth stage so roots would contact relative large soil volumn. As a result, more nutrients including P are absorbed by plants. The other way, however, is treating seeds with P solution or coating them with P compounds. This treating or coating would make P more adjacent to plant roots and in less contact with soil, and thus the efficiency of P fertilizers would result.

A new goal for plant breeding

Phosphorus stored in seeds is not only important for seedling development, but also nutritional important to man and animals(Murray, 1982). For years, high yields and high protein content of grains have been priorities in plant breeding. Such attention has not yet been paid to P content in grains. Dudal (1976) stated " The current approach to soil fertility gives emphasis to changing the soil to fit the plant. However, certain soil conditions cannot economically be corrected with current technology, so that efforts to adapt plants to soil conditions should be given more attention." For example, Andosols and Ferralsols have a remarkable ability to immobilize added P and these soils occupy 9% of world farming land (Dual,1976). High P content seed could supply adequate P in seedling development, and thus compensate P stress when crops are young in Andosols and Ferralsols. One day, if a genotype of high P content seeds of crops become possible, Andosols and Ferralsols will have bright future for crop production.

Future research.

Before the technique of treating seeds with P solution is put into practice in agriculture production, further research is needed:

1) The use of other cereal grains in addition to barley as sources for study of the effect of imbibed P on seedling growth.

2) Find the uptake rate of seed-imbibed P by plants grown in soil.

References

Barber, S.A. 1978. Nutrient flux at the soil-root interface. Proceedings of 11th ISSS. Edmonton, Alberta Canada. Vol. 3, p45-48.

Dudal, R. 1976. Inventory of the major soils of the world with special reference to mineral stress hazards. Proceedings of workshop on plant adaptation to mineral stress in problem soils. Beltsville, Maryland U.S.A. p3-14.

Murray, D.R. 1984. Seed Physiology. Academic Press Australia. Vol. 1 p140-147.

Tisdale, S.L., W.L. Nelson, J.D. Beaton. 1984. Soil Fertility and Fertilizers. 4th ed. Macmillan Company. p197-199.

Appendices

Appendix 1. Dry mass of shoot and root from replications of the first and second sand culture experiments

Table A1.1. Dry matter production of the plant tissue in first sand culture experiment

	Replication					
	1		2		3	
Treatments	Shoot	Root	Shoot	Root	Shoot	Root
	g					
Low-P	0.79	0.84	0.84	0.81	0.68	0.68
Medium-P	1.14	0.97	1.23	1.09	1.31	1.15
High-P	1.18	0.97	1.20	1.10	1.37	1.15

Table A1.2 Dry matter production of the plant tissue in second sand culture experiment

	Replication					
	1		2		3	
Treatments	Shoot	Root	Shoot	Root	Shoot	Root
	g					
Low-P	1.28	1.20	1.17	1.02	1.32	1.19
Low-P+P	1.73	1.55	1.59	1.36	1.87	1.67
High-P	1.92	1.72	1.86	1.61	1.82	1.38
High-P+P	2.26	1.91	1.91	1.52	1.88	1.51

Appendix 2. Dry mass of shoot experiments with potted soil.

To further ascertain the effect of seed P concentration on the seedling growth and the recovery of imbibed P and fertilizer P, high or low P seeds and those seeds treated with P solution were grown in Grey Luvisolic soil low in available P concentration (7 ug/g soil by Bray's extraction). The basic soil characteristics were pH 6.2, C % 1.64, and EC 0.11 mS /cm. The soil texture was silt loam. In the experiment of determining recovery of imbibed P, seeds (high or low) were treated with ^{32}P solution (0.01 mCi) for 24 hours, then grew in soil. Because the recovery data of ^{32}P was variable, only the dry mass were shown (Table A2.1). In Chapter 2, it was shown that seed P concentration had positive effect on seedling growth. This effect was also shown in soil (Table A2.2). Table A2.3 was a summary of Table A2.1 and Table A2.2. The dry matter production was found in the same pattern as in the sand culture experiments. In the experiment of determining recovery of fertilizer P by different batches of seeds, tagged P fertilizer was added to each pot. Dry mass of shoots (48 days) and recovery of ^{32}P of different treatments were quite similar (Table A2.4).

Table A2.1 Shoot mass after 37 days in soil experiment of determining recovery of imbibed P

	Replication		
Treatments	1	2	3
		g pot $^{-1}$	
Low-P	11.52	9.82	10.51
Low-P+P treat.	13.31	10.27	11.34
High-P	9.85	10.91	12.47
High-P+P treat.	12.06	11.77	9.50

Table A2.2 Effect of seed P concentration on seedlings growth in soil after 48 days

Treatments	Replication 1	2	3
	g pot $^{-1}$		
Low-P	11.19	10.44	11.43
Low-P+P treat.	13.14	12.30	13.18
High-P	13.74	13.45	12.83
High-P+P treat.	13.64	12.80	13.00

Table A2.3 Plant dry matter production from seeds containing a range of native and imbibed P

Seed batches	Dry matter production: Exp. No.1[a]	Exp. No.2[b]
	g pot $^{-1}$	
Low-P	10.61	11.02
Low-P + P treat.	11.64	12.87
High-P	11.08	13.34
High-P + P treat.	11.11	13.15
LSD (0.05)	1.96	0.55

a. Exp. No.1 was from Table A2.1.
b. Exp. No.2 was from Table A2.2.

Table A2.4 Shoot mass in the experiment of determining recovery of fertilizer P[a]

Treatment	Replication 1	2	3	Means	P Recovery
	g pot $^{-1}$				%
Low-P+P Fer.	15.22	14.92	16.85	15.33	16.5
Low-P+P treat.+P Fer.	16.34	15.96	15.62	15.97	16.7
High-P+ P Fer.	15.04	16.69	17.40	16.37	15.2
High-P+P treat.+ P Fer.	15.85	15.83	15.98	15.89	16.7

a. 0.0679 g P pot^{-1} (20 kg P ha^{-1}).

Appendix 3. N and P concentrations of the plant tissues in potted soil experiments

Shoot N or P concentration in the table below was from the experiment of determining the recovery of fertilizer P by high or low P seeds or those seeds treated with P solution. Shoot P concentration was higher than that of the same batches of seeds grown in sand.

Table A3.1 N and P concentrations of plant tissue in the experiment of determining the recovery of fertilizer P

	Shoot		
Treatments	N	P	N:P
	%		
Low-P	1.76	0.28	6
Low-P+P treat.	1.61	0.27	6
Low-P+Fer. P	1.83	0.28	7
Low-P+P treat. + Fer. P	1.77	0.27	7
High-P	1.65	0.27	6
High-P+P treat.	1.49	0.27	6
High-P+Fer. P	1.84	0.30	6
High-P+P treat.+ Fer.P	1.79	0.28	6

Appendix 4. Root length of plants grown in sand from different P concentration of seeds

Root length indicates the growth of root. As a widely accepted view, the longer the root length, the greater soil volumn that root can contact.

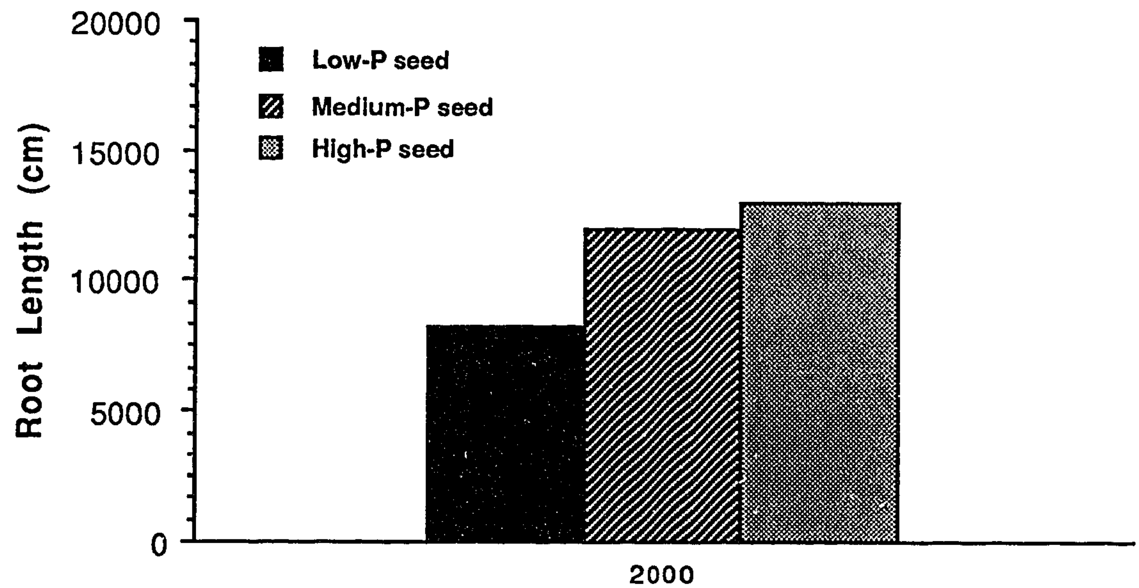

Fig.A4.1 Total Root Length From Different P concentration Seeds

Root length(R)= 11/14 x Number of intersection x Grid unit

Number of intersection: The intersections between roots and regular pattern of lines (grid).
Grid Unit: A length conversion factor. The factors for 1-,2-, and 5-cm grid squares are 0.79, 1.57, and 3.39, respectively.

Reference

Bohm, W. 1979. Method of Studying Root System. Springer-Verlag Berlin Heidelberg. P132-135.