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PHYTIC ACID IN POLLEN

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Key Word Index—Gymnosperms; angiosperms; *Petunia hybrida*; Solanaceae; NMR spectroscopy; paper electrophoresis; style length; cell-wall synthesis; myoinositol.

Abstract—Phytic acid has been identified by paper electrophoresis and NMR spectroscopy in extracts of pollen from *Petunia hybrida*. In this species pollen was found to contain an extraordinarily high amount of phytic acid (2.1% by weight), which makes up 37% of the total phosphorus of the pollen grain. Examination of the phytic acid content of pollen from a wide range of plant species showed that all species tested with a style length of 5 mm or more had a significant (0.05–2.1% by weight) amount of pollen phytic acid. On the other hand, the composites and grasses with very short styles had no detectable phytic acid in pollen, with the exception of *Zea mays*, which significantly has a very long style (250 mm) and a substantial amount of pollen phytic acid. It is suggested that phytic acid plays a role in providing precursors for cell-wall synthesis necessary for rapid pollen tube elongation down the style of pollinated plants.

INTRODUCTION

Phytic acid is known to occur in certain seeds, where it appears to act as a storage substance [1]. During seed germination it is rapidly broken down to myoinositol or its derivatives [1, 2]. In many plant cells, the latter may serve as a precursor for the synthesis of cell-wall components [3–5]. There has been no report of phytic acid occurring in pollen, even though rapid pollen tube elongation following germination implies a high demand for cell-wall precursors [4]. We have therefore examined pollen from this point of view and report herein on the identification of phytic acid in pollen and draw some conclusions as to its function.

RESULTS

Identification of phytic acid in Petunia hybrida *pollen*

Paper electrophoresis of extracts from *Petunia hybrida* pollen showed two main phosphate-containing spots, one migrating with the same mobility as phytic acid and the other moving in the same position as inorganic phosphate. Phytate was identified in the pollen extract from the fully proton decoupled ^{31}P NMR spectrum. Four peaks in the spectrum at δ 3.87, 4.04, 4.38 and 5.22 were identical to those of an authentic sample of dodecasodium phytate at the same pH (pH measured as 10.40) and may be compared with the ^{31}P NMR spectrum observed for dodecasodium salt of cereal grain phytic acid [6]. A broad peak, essentially at the same frequency as the reference standard of 85% phosphoric acid, was assigned to an inorganic phosphate component in the extract, thus confirming observations made from paper electrophoresis. It was estimated from paper electrophoresis, NMR spectroscopy and phosphate analyses that pollen from *Petunia hybrida* has 2.1% by weight phytic acid, and that phytic acid makes up 37% of the total phosphate of *P. hybrida* pollen grain.

Phytic acid content of pollen from a wide range of plant species

The extraordinarily high concentration of phytic acid that we have found in the pollen grain (e.g. 2.1% by weight in *Petunia hybrida* pollen) and the singularly high electrophoretic mobility of phytic acid [7, 8] means that crude extracts from pollen can be subjected to paper electrophoresis without further processing in order to survey them for phytic acid. The results of such a survey are presented in Table 1, together with our estimation of the proportion of the total phosphorus of the pollen grain locked up in phytic acid and the average style length of the plant species involved. Although phytic acid content was not found to be directly correlated with style length, it was found that all species tested with styles of 5 mm in length or greater had detectable phytic acid in the pollen grain (i.e. more that 0.05% phytic acid by weight). The grasses and Compositae in general had no detectable phytic acid, with the notable exception of *Zea mays* pollen, which develops an extremely long pollen tube in order to penetrate a style at least 250 mm long. Other species with shorter styles were found to be variable in phytic acid content depending on the species.

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Table 1. Phytic acid content of pollen

	Phytic acid (wt %)	Phytic acid P (% total P)	μmol total P/g	Average style length (mm)
GYMNOSPERMS				
Pinaceae				
Abies nordmanniana (Steven) Spach	0.13	7	180	n
Pinus flexilis James	0.10	7	130	n
Araucariaceae				
Araucaria bidwillii Hook.	0.59	34	160	n
ANGIOSPERMS				
Magnolidae (Dicots)				
Fagaceae				
Quercus robur L.	0.66	33	180	s
Quercus lusitanica Lam.	0.53	27	180	s
Juglandaceae				
Juglans regia L.	0.80	49	150	5
Malvaceae				
Hibiscus huegelii Endl.	0.26	13	180	40
Anacardiaceae				
Pistacia chinensis Bang.	0.26	13	180	5
Mimosaceae				
Acacia saligna Wendl.	0	0	70	4
Onagraceae				
Fuchsia spec. hort.	0.13	6	190	75
Oenothera striata Ledeb ex Link	0.40	28	130	25
Myrtaceae				
Eucalyptus youngiana F. Muell.	0.20	10	180	40
Solanaceae				
Solanum mauritianum Scop.	1.1	74	130	7
Petunia hybrida hort.	2.1	37	520	35
Scrophulariaceae				
Antirrhinum majus L.	2.1	38	500	25
Asteraceae				
Arctotheca calendula Wendl.	0	0	170	2
Helianthus annuus L.	0	0	290	2
Senecio cruentus DC.	0	0	190	2
Liliatae (Monocots)				
Liliaceae				
Aloe excelsa Berger	0.1	9	100	40
Lilium henryi Baker	1.1	6	270	70
Araceae				
Zantedeschia aethiopica (L.) Spreng	0.26	12	200	s
Poaceae				
Chloris gayana Kunth	0	0	120	1
Hordeum bulbosum L.	0	0	130	1
Secale cereale L.	0	0	200	1
Triticum aestivum L.	0	0	140	1
Zea mays L.	0.26	14	170	250
Musaceae				
Strelitzia reginae (Banks) Ait.	0.53	20	240	95
Orchidaceae				
Dendrobium lowii Lindl.	0.13	7	180	10

0—Phytic acid not detected. Assay sensitive to 0.05% by wt phytic acid.
s—Sessile stigma. n—No style.

DISCUSSION

The utilization of myoinositol for pollen tube wall polysaccharides has been demonstrated in *Lilium longiflorum* by Loewus *et al.* [4]. It is thought that myoinositol is converted by an oxidation pathway to UDP-glucuronic acid and a portion of it thence into tube wall polysaccharides. Another portion was found to be converted to starch by gluconogenesis during pollen germination [9], a surprising result considering that while much energy is needed for rapid tube wall synthesis, the pollen grain should be building up a storage material such as starch. The solution to this apparent paradox may lie in the large amount of phytic acid found in the pollen of many species during the present study. Over the first few hours of pollen germination the phytic acid may be broken down to myoinositol and related compounds, perhaps leading to an excess for the needs of tube wall synthesis, resulting in a synthesis of starch while it is in excess. It is noteworthy that a phytase has already been described in pollen from *Petunia hybrida* [10].

Although style length may be the most important factor in determining whether or not pollen from a particular species has significant quantities of phytic acid due to a demand for this compound for rapid tube wall polysaccharide synthesis, other factors may also be involved. Thus, both species examined for the family Solanaceae had very large quantities (1–2.1%) of phytic acid, as did the related *Antirrhinum majus* with 2.1% phytic acid, and yet none of these species has styles as long as *Fuchsia* or *Hibiscus huegelii*, which have pollen with a much lower (but still significant) phytic acid content. Again, the gymnosperms listed in Table 1 have no style at all, and yet pollen from the gymnosperms has a significant phytic acid content. This could be related to the requirements of myoinositol for the synthesis of such compounds as pinitol, so common in cells of conifers [11]. Similarly, *Acadia saligna* was the only tree to yield pollen with no significant phytic acid content. However, this pollen has a low total phosphate content and additionally has composite-like flowers with very short styles.

While it is evident that the presence of high concentrations of phytic acid in pollen can be readily explained through it being a storage compound for precursors of rapid tube wall polysaccharide synthesis, it should be noted that the male reproductive tracts of animals have high concentrations of the closely related compound, myoinositol [12]. The function of myoinositol in this case is not known. Pollen in many situations has to withstand extremes of desiccation. The report that myoinositol protects bacteria from damage due to both UV radiation and desiccation [13] therefore deserves further investigation with respect to the high phytate content of pollen.

EXPERIMENTAL

Source of pollen. All pollen was obtained from plants grown at the Waite Agricultural Research Institute, except for *Petunia hybrida* and *Lilium henryi* (from Department of Botany, Nijmegen University, The Netherlands), *Pinus flexilis* (from Dr A. Matheson, Division of Forest Research, CSIRO, Canberra), and *Oenothera striata* (collected near Wellington, South Australia).

Preparation of extracts. Dry pollen (200 mg) was ground in a mortar precooled with liquid N_2, then 2 ml 0.02 M Na_4EDTA was added, allowed to freeze over the pollen and the whole ground into a fine powder. The powder was allowed to thaw, with further grinding, poured into a small tube and heated in a boiling water bath for 20 min. After cooling to 0°, centrifugation (Sorvall SSl; 6000 *g* for 20 min) yielded the first supernatant. The first pellet was re-extracted in a boiling water bath, with 2 ml 0.02 M Na_4EDTA, cooled and centrifugation as above yielded a second supernatant.

Paper electrophoresis of extracts and phytic acid estimation. Both the first and second supernatants from each pollen extraction were subjected to paper electrophoresis (0.1 M oxalic acid, pH 1.5; 550 V for 2 hr; on Whatman 3 MM paper 10 × 43 cm). Under these conditions phytic acid migrates well ahead of other cellular phosphate esters [7, 8]. Phosphate-containing areas in the dried electrophoretogram were then stained by dipping in an acid molybdate solution in acetone [14], dried overnight in the dark and exposed briefly to sunlight next day. The intensity of the discrete blue spot from pollen extracts with the same mobility as phytic acid, was compared with that from a series of standard phytic acid spots run under the same conditions. It was found that as little as 1 nmol phytic acid (6 nmol of phosphate) could be detected by this method. Preliminary extractions showed that 95% of the extractable phytic acid was obtained in the first and second supernatants (above). Little or no phytic acid appeared in subsequent extractions. Total phosphate analysis was carried out on aliquots of dry pollen [15].

NMR spectroscopy. The first supernatant fraction (above) from *Petunia hybrida* pollen was titrated to pH 12 with 10 N NaOH and freeze dried. The sample was dissolved in D_2O. ^{31}P NMR spectra were obtained on a JEOL FX-900 Fourier transform spectrometer operating at 36.23 MHz with a probe temp. of 26° and using proton noise decoupling. An external reference of 85% phosphoric acid was used.

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