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Phytate in seeds of wild plants

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ABSTRACT

Phytic acid, the free-acid form of myo-inositolhexakiphosphate, is found widely among eukaryotes. It constitutes the major storage form of phosphate in seeds and fruit in the form of phytate, a mixed cation salt with, e.g., K or Mg. However, the general claim that phytate represents between 60 and 80% of total phosphorus in mature seeds is based almost entirely on work with crop plants. A few reports with wild plants with contrasting findings raised doubts about the generality of the current view. To put this notion to the test, we collected mature dry seeds and dry fruits from 55 species of wild plants from a number of habitats and determined concentrations of both total P and phytate. In the majority of species the contribution of phytate-P to total P was either higher than the "typical" range or considerably lower, with minimum values of just 12%. The proportion of P in phytate was a function of total P: in high-P seeds c. 80% of P was found in phytate, while this proportion decreased gradually with decreasing total P. We conclude that it was indeed premature to generalize the quantitative role of phytate in seeds based on a highly biased data set.

1. Introduction

Phytic acid, the free-acid form of myo-inositolhexakiphosphate (InsP6), is found widely among eukaryotes. A considerable number of functions of phytic acid have been identified, which are related to the storage of mineral elements, RNA transport, DNA metabolism, or to herbivore defense (Green et al., 2001; Marschner and Marschner, 2012; Raboy, 2003). Most attention, however, is associated with its function as major storage form of phosphate in seeds and fruit in the form of phytate, a mixed cation salt with K, Mg and other cations. Raboy et al. (2007) and others (e.g. Gupta et al., 2015) state that phytate represents "between 60–80% of mature seed total phosphorus". Indeed, on average, phytin-P accounts for 74% of total P in a compilation of data from dry seeds and fruit of 38 species by Lott et al. (2000). The scientific interest in phytate has not ceased since these authors compiled their large data set, mostly motivated by the fact that phytate impacts the nutritional quality of seeds and fruit for both livestock and humans. Limited phytase activity of non-ruminant animals does not allow them to utilise phytate, which reduces the amount of usable P in fodder, contributes to water pollution from animal manure and even leads to mineral deficiencies because phytic acid is a powerful chelator for Ca, Mg, or Fe. Numerous studies that were published after Lott et al.'s (2000) compilation just enlarged the data base, but did not put into question the general quantitative and qualitative view of the role of phytate in seeds.

However, our current understanding of the role of phytate in the plant kingdom is still based almost entirely on data from crops such as wheat, corn or rice and other crop plants such as soybean, peanuts or canola (Lott et al., 2000; White and Veneklaas, 2012). The few reports on the relative importance of phytate in seeds of wild plants give a mixed picture: Ravindran et al. (1994) report 69% for *Ceiba pentandra* and 59% for *Mucuna deeringiana*, Mitchell and Allsopp (1984) report 64% for *Hakea sericea*, which is in line with the account given above, but other values deviate substantially, e.g. seeds of the climbing *Kedrostis africana* with 28% (Unuofin et al., 2017), the tree *Artocarpus altilis* with 22% (Fagbemi et al., 2005), or the herb *Taraxacum officinale* with 10% (Alkarawi and Zotz, 2014). Such low percentages are not entirely exceptional, since there are a few known cases like *Castanea sativa* fruit, in which phytate only accounts for 17% of total P (Lott et al., 2000). However, it highlights a possible problem with such a biased data set when it comes to generalisations. We conclude that there is an obvious need for data from wild plants to test the generality of Raboy et al.'s (2007) statement for seed plants at large. To this end, we collected mature seeds from 55 species of wild plants from a number of habitats and determined both total P and phytate concentrations.

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Table 1
Total P and phytin-P concentrations in the seeds of 55 herbaceous species from five habitat types. Also given is the proportion of total P in phytin in %. Species names follow the The Plant List (2013).

Species	Family	Habitat type	country	Total P (mg g^{-1})	Phytin-P (mg g^{-1})	Phytin-P (% total P)
Ammophila arenaria (L.) Link	Poaceae	Coastal	Germany	0.87	0.11	12.3
Armeria maritima (Mill.) Willd.	Plumbaginaceae	Coastal	Germany	1.25	0.40	33.4
Artemisia maritima L.	Asteraceae	Coastal	Germany	3.26	2.32	71.1
Bolboschoenus maritimus (L.) Palla	Cyperaceae	Coastal	Germany	0.84	0.31	37.0
Cakile maritima Scop.	Brassicaceae	Coastal	Germany	2.01	0.99	49.4
Elymus athericus (Link) Kerguélen	Poaceae	Coastal	Germany	2.64	2.08	78.7
Eryngium maritimum L.	Apiaceae	Coastal	Germany	2.35	1.90	80.7
Phragmites australis (Cav.) Trin. ex Steud.	Poaceae	Coastal	Germany	1.76	0.90	51.4
Plantago maritima L.	Plantaginaceae	Coastal	Germany	6.44	5.07	78.8
Triglochin maritimum var. *debile* M.E. Jones	Juncaginaceae	Coastal	Germany	4.41	3.57	80.9
Astragalus hauarensis Boiss.	Leguminosae	Desert	Iraq	1.71	0.50	29.4
Atriplex leucoclada Boiss.	Amaranthaceae	Desert	Iraq	1.36	0.39	28.6
Eragrostis barrelieri Daveau	Poaceae	Desert	Iraq	2.09	0.69	32.9
Farsetia heliophila Bunge ex Coss.	Brassicaceae	Desert	Iraq	2.24	0.99	44.5
Gymnarrhena micrantha Desf.	Asteraceae	Desert	Iraq	1.26	0.23	17.8
Horwoodia dicksoniae Turrill	Brassicaceae	Desert	Iraq	1.60	0.33	20.5
Launaea mucronata (Forssk.) Muschl.	Asteraceae	Desert	Iraq	1.64	0.33	20.1
Malcolmia grandiflora Kuntze	Brassicaceae	Desert	Iraq	1.39	0.42	30.1
Monsonia nivea (Decne.) Webb	Geraniaceae	Desert	Iraq	2.04	0.45	22.0
Neurada procumbens L.	Neuradaceae	Desert	Iraq	1.38	0.45	33.1
Peganum harmala L.	Nitrariaceae	Desert	Iraq	1.24	0.30	24.7
Picris cyanocarpa Boiss.	Asteraceae	Desert	Iraq	2.07	0.72	34.5
Plantago boissieri Hausskn. & Bornm.	Plantaginaceae	Desert	Iraq	2.23	1.17	52.3
Rumex vesicarius L.	Polygonaceae	Desert	Iraq	2.28	0.47	20.7
Savignya parviflora (Delile) Webb	Brassicaceae	Desert	Iraq	1.83	0.53	28.8
Anemone nemorosa L.	Ranunculaceae	Forest	Germany	5.14	4.44	86.5
Circaea lutetiana L.	Onagraceae	Forest	Germany	6.72	5.60	83.3
Digitalis purpurea L.	Plantaginaceae	Forest	Germany	3.46	2.84	82.0
Hieracium umbellatum L.	Asteraceae	Forest	Germany	5.22	4.55	87.0
Sanicula europaea L.	Apiaceae	Forest	Germany	3.62	1.81	50.0
Stachys sylvatica L.	Lamiaceae	Forest	Germany	4.61	4.09	88.6
Stellaria holostea L.	Caryophyllaceae	Forest	Germany	6.01	5.07	84.4
Anthriscus sylvestris (L.) Hoffm.	Apiaceae	Ruderal	Germany	10.44	7.40	70.8
Capsella bursa-pastoris (L.) Medik.	Brassicaceae	Ruderal	Germany	4.53	3.63	80.2
Cirsium vulgare (Savi) Ten.	Asteraceae	Ruderal	Germany	4.20	3.90	92.9
Daucus carota L.	Apiaceae	Ruderal	Germany	4.97	4.22	84.9
Geum urbanum L.	Rosaceae	Ruderal	Germany	4.57	3.73	81.7
Heracleum sphondylium L.	Apiaceae	Ruderal	Germany	5.87	4.58	78.0
Lapsana communis L.	Asteraceae	Ruderal	Germany	4.72	4.25	90.0
Medicago lupulina L.	Fabaceae	Ruderal	Germany	1.76	0.90	51.1
Papaver rhoeas L.	Papaveraceae	Ruderal	Germany	8.67	7.10	81.9
Saponaria officinalis L.	Caryophyllaceae	Ruderal	Germany	3.26	2.32	71.2
Sisymbrium officinale (L.) Scop.	Brassicaceae	Ruderal	Germany	8.03	6.84	85.4
Trifolium repens L.	Fabaceae	Ruderal	Germany	3.00	2.55	84.8
Tripleurospermum maritimum (L.) W.D.J.Koch	Asteraceae	Ruderal	Germany	3.10	2.20	62.9
Cirsium palustre (L.) Coss. ex Scop.	Asteraceae	Wetland	Germany	8.53	7.27	85.3
Eriophorum angustifolium Honck.	Cyperaceae	Wetland	Germany	6.32	5.43	85.8
Filipendula ulmaria (L.) Maxim.	Rosaceae	Wetland	Germany	1.66	0.75	45.5
Hypericum perforatum L.	Clusiaceae	Wetland	Germany	6.72	5.60	83.4
Juncus maritimus Lam.	Juncaceae	Wetland	Germany	4.41	3.58	81.2
Molinia caerulea (L.) Moench	Poaceae	Wetland	Germany	4.97	4.22	84.9
Narthecium ossifragum (L.) Huds.	Nartheciaceae	Wetland	Germany	0.69	0.56	80.4
Phalaris arundinacea L.	Poaceae	Wetland	Germany	4.66	4.08	87.5
Rhynchospora alba (L.) Vahl	Cyperaceae	Wetland	Germany	0.86	0.11	12.5
Stellaria aquatica (L.) Scop.	Caryophyllaceae	Wetland	Germany	2.35	1.90	80.6

2. Material and methods

2.1. Species

We studied a total of 55 plant species from 23 families from four habitat types in Northern Germany (coastal marshes and beaches, deciduous forest, ruderal sites, and wetlands) and from one desert habitat in Iraq. Species details are given in Table 1. Plant names follow The Plant List (2013).

2.2. Sampling and measurement

Field collections were done in 2015 and 2016. Samples were obtained from a number of sites in the vicinity of the city of Oldenburg, Lower Saxony (53°08′N, 8°13′E), along the coast of the North Sea (53°42′N, 8°50′E) and from a nutrient-poor desert ("Razzaza") that is located close to the city of Karbala, Iraq (32°35′N, 43°52′E). There, all harvested plants were growing in sandy soil.

We collected seeds or fruits from at least three individuals per species. Plants were collected in Germany between early June and September in 2015 and 2016, and in Iraq between early March and middle April 2016. Samples were oven dried at 80 °C for approximately 48 h and ground using a ball mill (MM200, Retsch, Haan, Germany). For each species, seeds/fruit of all individuals were joined and subsamples used for the analysis of total phosphorus and phytic acid. The former was determined colorimetrically using ammonium heptamolybdate (Chapman and Pratt, 1961). Phosphorus in phytic acid was also assayed colorimetrically with a phytic acid / total P assay kit (K-PHYT;

Megazyme International, Wicklow, Ireland). This kit determines the P that is released from a ground sample after treatment with phytase and alkaline phosphatase. Parallel samples that are not treated with phytase allow the quantification of monophosphates not associated with phytic acid. This allows for the necessary correction to avoid an overestimation of P in phytic acid. Since P comprises 28.2% of phytic acid (molar mass 660.04), multiplying phytic acid-P (atomic mass 30.97) with the factor of 3.55 yields the amount of phytic acid. Analogously, dividing phytic acid data by 3.55 allows the quantification of phytate-P from phytate values: this was used to calculate the ratios of phytate-P to total P from the data provided by Lott et al. (2000). As suggested by the manufacturer, we routinely used oat samples supplied with the kit as control.

2.3. Data analysis

Apart from our own data, we used those compiled by Lott et al. (2000) for 38 species of crop plants with dry seeds or fruit for comparison. We analyzed differences among sites in seed P concentrations and the ratio of phytase-P to total P with two one-way ANOVAs. Although the second data set is comprised of percentages, visual inspection of the residuals suggested that no transformation was necessary to fulfil ANOVA assumptions. A level of $p < 0.05$ was accepted as significant, and a Tukey HSD test was used to detect among-group differences, again with $p < 0.05$ indicating significance. All statistical tests were performed with the program R 3.3.2 (R Development Core Team, 2014).

3. Results and discussion

The proportion of total P found in phytate varied almost 8-fold among the sampled species, from 12% in *Ammophila arenaria* to 93% in *Cirsium vulgare* (Table 1, Fig. 1b). The significant differences at the habitat level (ANOVA, $p < 0.05$) were entirely due to the low values of desert plants (Tukey HSD test, $p < 0.05$). In these plants, < 30% of total P, on average, was stored as phytate. In all other groups, the contribution of phytate to total P was statistically indistinguishable from that previously reported for crop plants (Tukey, $p > 0.05$).

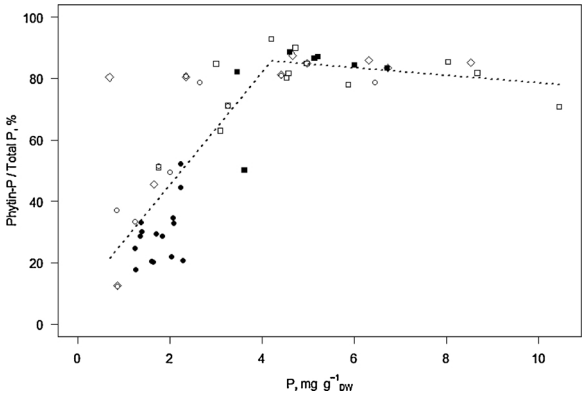

Fig. 2. The relationship between seed P levels and the ratio of phytin-P to total P. The different symbols indicate different habitat types (coastal marshes = open circles; wetlands = diamonds, desert = closed circles, ruderal = open squares, forest = closed squares). The dotted lines show the result of a segmented regression. The complete data set is given in Table 1.

The desert plants from Iraq as a group had also the lowest levels of total seed P (Table 1, Fig. 1a), but seed P levels of some species of other groups, i.e. plants from coastal habitats or wetlands, were also low – the Tukey test did not single out desert plants as it had done for proportional phytate-P. The relationship of seed P to phytate-P/total P was analysed with a segmented regression. Fig. 2 shows the significant linear increase of phytate-P/total P with increasing seed P up to a concentration of c. 4 mg P g^{-1} (breakpoint: c. 4.2 mg P g^{-1}), with no further increase at higher seed P levels. There is a strikingly similar increase in the relative importance of phytin-P during the early stages of seed and grain development (Marschner and Marschner, 2012). Initially low, phytin concentrations increase sharply during seed development, both absolutely and relatively (Greenwood et al., 1984; Michael et al., 1980). These trends may reflect a common mechanism governing phytin synthesis as a function of total P in tissues.

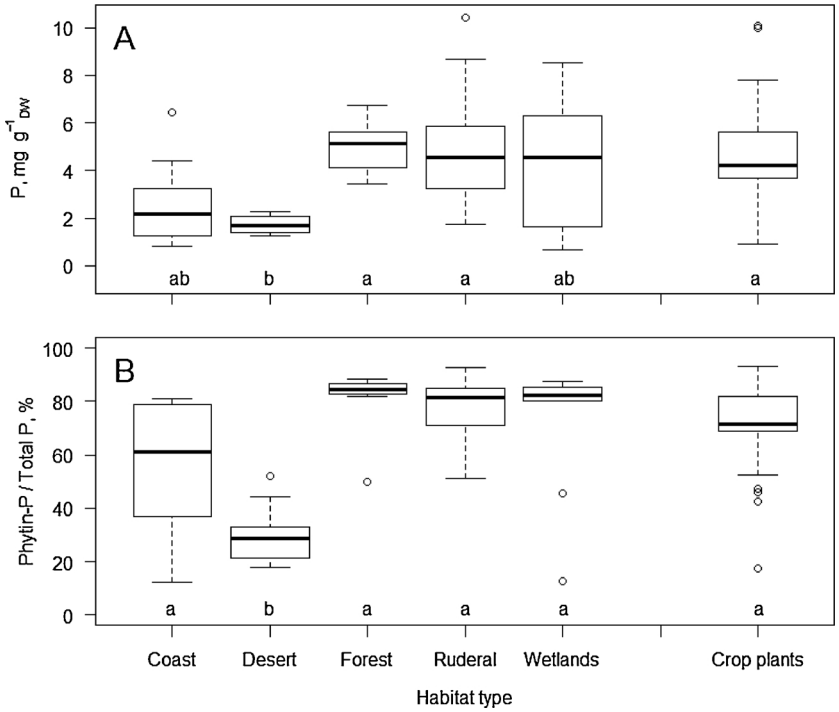

Fig. 1. Seed P levels (A) and the ratio of phytin-P to total P (B) as a function of habitat type. Also shown are data from Lott et al. (2000), who compiled data from 38 species of crop plants with dry seeds or fruit, for comparison. Different lower case letters indicate significant differences between groups (Tukey HSD, $p < 0.05$).

The documented variation in the concentrations of P and phytin in the seeds of 55 wild plant species (Table 1) suggests that it was indeed premature to make generalizing statements on the relative proportions of phytate in seeds and fruits. Although we acknowledge that phytate probably plays a similarly important role in most wild plants as in the abundantly studied crop plants, the data emphasize a much more pronounced variability as the typical figure of "60–80%" (Raboy et al., 2007) implies. In our data set the ratio of phytate-P to total P in almost 90% of all species deviated from this standard value. The ratio was either below 60% (24 species) or above 80% (24 species).

The low concentrations of both total P and phytate in the studied desert plants are not necessarily indicative of P limitation. Quite the contrary, species in regions known for severe P-limitation such as South-West Australia or the Cape province typically show very high P concentrations in their seeds: values as high as 36 mg P g^{-1} dry mass have been reported (*Hakea pycnoneura* (Proteaceae); Groom and Lamont, 2010). Seeds of Proteaceae rich in P did not show extraordinary concentrations of phytate: 63% of the 11.6 mg P g^{-1} in seeds of *Hakea sericea* were stored in the form of phytate (Mitchell and Allsopp, 1984). We did not directly study nutrient availability in the desert habitat and the few available studies on the regional soils (Muhaimeed et al., 2013; Yahia, 1971) do not supply the necessary information either. Thus, we cannot relate the concentrations in seeds to environmental conditions. This must be left to future studies. Given the results of the present study it seems highly promising to investigate the local edaphic conditions and to manipulate P supply to fruiting plants under controlled conditions. Such a study should shed light on the P economy of these desert plants and lead to a better understanding of the regulation of phytic acid synthesis in seeds in general.

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