PHYTIC ACID (INOSITOL HEXAPHOSPHATE): SCREENING FOR LOW PHYTIC ACID CONCENTRATION IN PEA SEEDS FOR INCREASED BIOAVAILABILITY IN HUMANS

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Phytic acid ($InsP_6$), the primary storage form of phosphorus (P) in seeds, is essential for successful growth of young seedlings. It is also a strong chelator of mineral cations such as calcium, iron, and zinc, forming insoluble complexes that are largely excreted by humans and other non-ruminant animals. For this reason, excessive amounts of phytic acid may limit the bioavailability of important nutrients such as iron and zinc. Therefore, scientists are interested in identifying common legumes low in phytic acid.

The objective of this study was to locate accessions of pea seeds (*Pisum sativum*) that are nutrient-rich but low in phytate. Four hundred and eighty-five pea accessions were ground into a fine powder. Each sample (.30 grams) was digested overnight in 2.4% hydrochloric acid (HCl) on an orbital shaker. After centrifuging and micro-centrifuging the samples, supernatant (0.5 mL) was added to 7 mL of water to form the assay sample. The colorimetric assay involved adding 900 µL of WADE reagent and was read by a spectrophotometer. A colorimetric procedure was optimized to measure phytic acid concentration by using accessions of pea seeds. Preliminary results show a two-fold concentration range of approximately 15.0–30.0 mg/g DW with the average phytic acid concentration of pea seeds being 21.7 mg/g. Continuing research will involve the digestion of selected samples with digestive enzymes to measure iron availability.

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INTRODUCTION

Phytic Acid ($InsP_6$) is the first known form of phosphorus (P) in seeds. The phytic acid is essential for the ultimate growth of pollen grains and plant embryos. Yet, this essential acid limits the plants' ability to absorb essential nutrients, such as calcium, iron, and zinc by chelating onto them before they can be properly digested into the body. *Pisum sativum,* the plant whose accessions are used as the primary testing subject, is more commonly known as the typical garden pea or English pea. The plant originates from the legume family and has many predominant characteristics. The *Pisum sativum* has a high-growth rate, is morphologically highly variable in phenotypic and genotypic ways, but most are predominantly self-pollinating.

PURPOSE

The purpose of our research is to locate an accession of *Pisum sativum* that is: 1) high in nutrients, especially iron, zinc, and phosphorus; 2) low in phytate; and 3) has the seed capability to perform digestive and developmental functions normally. We hypothesized that we will find a good number of nutritious pea accessions that have low phytate concentrations.

METHODS

All the pea accessions were first ground to a fine powder using a Wiley Mill (Thomas Scientific, USA). After finalizing our optimized assay protocol, we decided that .3 grams would be ideal

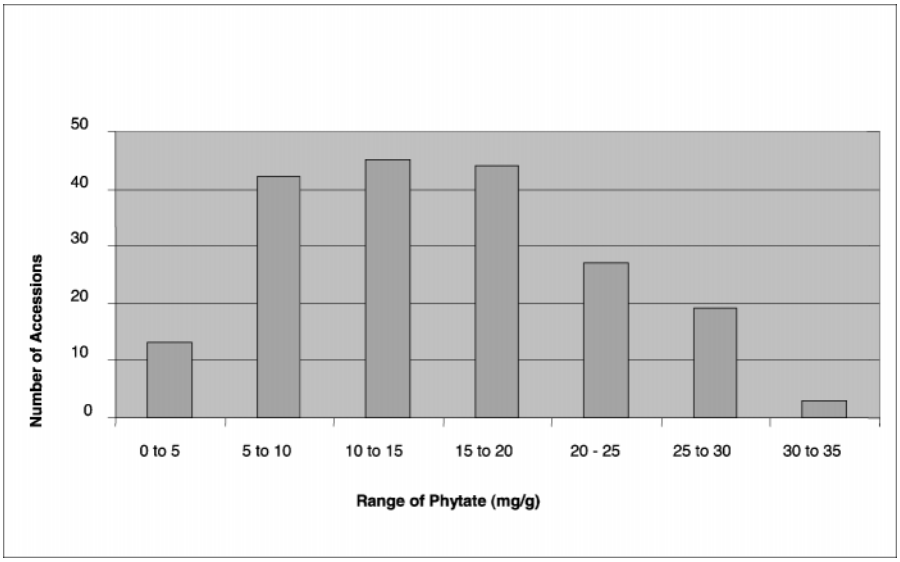

Fig 1. Phytic acid concentration

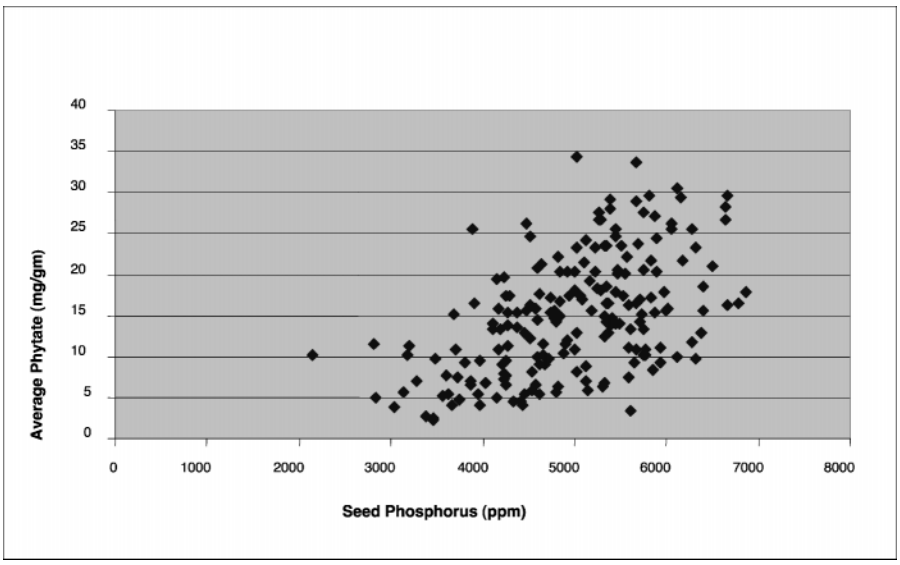

Fig 2. Seed phosphorus vs average phytate

to use to find a correlation between nutrient yield and levels of phytate. Samples (.3 g) were measured in triplicate and then digested overnight in 5 mL of 2.4% hydrochloric acid (HCl). All the samples in the set were centrifuged at 3700 RPM for 10 minutes and then 1 mL of each sample was micro-centrifuged at maximum speed for another 10 minutes. The supernatant (.5 mL) was then added to 7 mL of H_2O. The assay solution was prepared by adding 900 µl of the sample with 300 µl of WADE reagent (0.3% $FeCl_3 \times 6\ H_2O$ containing 0.3% sulfosalicylic acid). The absorbance of each solution was then read by a spectrophotometer at 500 nm.

RESULTS AND DISCUSSION

Two hundred accessions have been screened for phytic acid concentration. We found that all accessions ranged from 2 to 34 µg phytic acid/g DW (Figure 1). Thirteen accessions looked very promising due to their low phytic acid concentration (2–5 mg/g phytic acid). Two hundred eighty-five other accessions are still available for analysis. The optimized assay protocol can now be used to determine phytate concentration in other seeds or vegetables (Figure 2). Information from the project can be used to grow low-phytate pea accesses for further studies.