Changes in *myo*-inositol-1-phosphate synthase gene expression and phytic acid accumulation in oat plants during seed maturation.

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Abstract

Phytic acid (*myo*-inositol 1,2,3,4,5,6-hexakisphospate) is the main storage form of phosphorus in many plant seeds. *Myo*-inositol-1-phosphate (Ins(3)Pi) synthase (MIPS EC 5.5.1.4) catalyzes the first step in phytic acid biosynthestic pathway. An oat (*Avena sativa*) MIPS cDNA was isolated by RACE-PCR using consensus primers designed from highly conserved regions in other plant MIPS sequence. The oat MIPS clone sequence analysis shows an 1936 bp encoding a polypeptide of 510 amino acids with highly homologous to other plants. Northern blot analysis indicated that MIPS gene is high expressed during early stage of seed maturity, while it was decreased at late maturity stage. Phytic acid concentration also gradually increased with seed maturity.

Key words

Avena sativa, *myo*-inositol-1-phosphate synthase, oat, phosphorus, phytic acid

Introduction

Maize, sorghum, soybean and oat plants are widely used in the raw materials of livestock fodder. However, these grains in particularly containing phytic acid (*myo*-inositol 1,2,3,4,5,6-hexakisphosphate), which is the major phosphorus storage compound in these grains and accounts for up to 60% of the total grain phosphorus (Lott et al., 2000). Phytic acid is almost indigestible in monogastric animals such as pigs, chickens and humans, so phytic acid passes through the digestive system. Therefore, poultry and chicken feces contain large amounts of P. As a result, P is lost to the environmental in cereal-consuming regions, where it may contribute to excessive P concentration in soil and water. One effective method to increase the availability of P in grain is by increasing the non-phytic acid compounds such as inorganic P and/or organic P compounds instead of phytic acid. Phytic acid is synthesized form *myo*-inositol-3-phosphate (Ins(3)Pi)(Loewus and Murthy,2000). During Ins(3)Pi biosynthesis the *myo*-inositol-1-phosphate synthase (MIPS; EC 5.5.1.4) catalyze the conversion of D-glucose-6-phosphate into Ins(3)Pi, that represents the first steps in inositol metabolism and phytic acid biosynthesis. In the present study we isolate MIPS cDNA clone from oat plants, and we discussed the role of MIPS gene during development of oat seeds.

Methods

Oat (*Avena sativa* L. cv. Haeibuki) plants were grown in pot of greenhouse under natural light conditions. An oat MIPS cDNA was isolated from immature seed by a RACE-PCR approach (SART RACE cDNA Amplication kit, Clontech, USA). The primers used for RACE cDNA Amplication kit, Clontech), that was based on conservation of MIPS coding sequence among plants. cDNA sequence was carried with a DNA sequencer (Applied Biosystem, model 373A) using an DYEnamic ET Dye Terminator Cycle Sequencing Kit (Amersham, England). Total RNA from developing seed was prepared using RNA isolation kit. Twenty micrograms of RNA was resolved on 1% agarose/formaldehyde/MOPS gel and transferred to nylon membrane. Preparation of MIPS probe, hybridization, and washing were carried out according to instruction provided by the manufacture (Gene Image Kit, RPN3540, Amersham, England). Total P, Ca and Mg concentration in seeds were measured by ICP-AMS after digestion with sulfuric acid. Phytic acid and inorganic P were extracted by HCl/Na_2SO_4 solution as described by Raboy et al.(1984). The iron-

phytic acid precipitate was obtained with $FeCl_3$/HCl/Na_2SO_4 solution, and digested by sulfuric acid. Phytic acid-P and inorganic P concentration were determined using the molybdenum blue method.

Conclusion

In the present study MIPS cDNA clone was isolated from immature seeds of oat plants. Total length of the cDNA obtained was 1936 bp (accession no. AB059557), and encodes 510 amino acid. The putative oat MIPS protein has predicted molecular mass of 56.13 kD. The reduced amino-acid sequence of oat MIPS showed regions of strong homology with MIPS from 77 to 88 % among MIPS sequence from different plant species (Yoshida et al. 1999, Hageman et al. 2001). Northern blot analysis indicated that the level of MIPS mRNA was accumulated at higher level in immature seeds, while signals were not detected in stem and leaves (Fig.1 A). MIPS transcript was slightly observed in the flower, and it was increased with seed maturity (Fig.1 B). Maximal levels of MIPS RNA were observed in immature seed, and as development progressed MIPS transcripts levels gradually decreased.

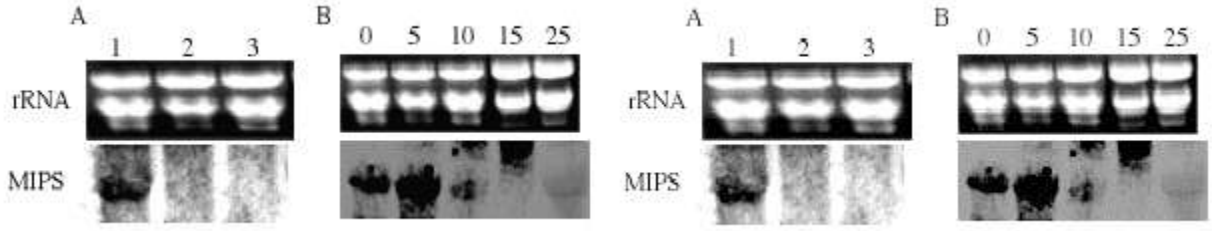

Fig. 1. Northern-blot analysis of oat plants. A : expression of MIPS in immature seed(1), leaves(2) and stem (3) at 5 days after flowering. B: expression of MIPS in developing seeds at 0, 5, 10, 15 and 25 days after flowering.

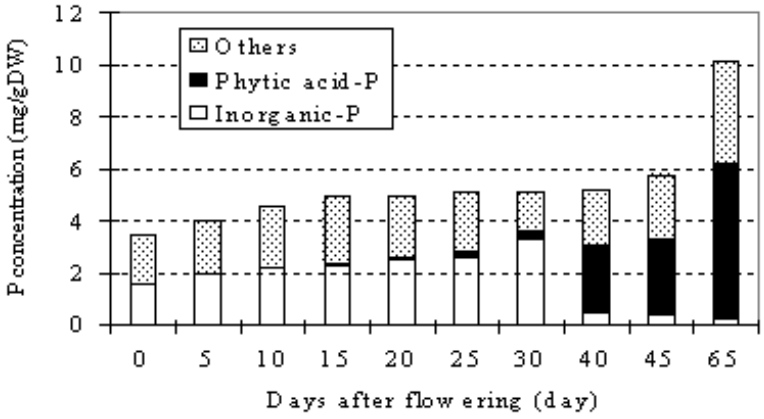

Fig. 2. Changes in total P, phytic acid-P and inorganic-P concentration after flowering.

The total P concentration in flowers was 3.51 mg/g dry weight (DW), and was gradually increase with seed maturation (Fig.2). The total P concentration in mature seeds was reached 10.15 mg/ g DW at 65 days after flowering (full maturity stage). The phytic-acid P was began to accumulate at early stage and increased rapidly with seed maturity, and its concentration was 5.97 mg/g DW. The inorganic P concentration was 1.58 mg/gDW in flower, and was gradually increase up to 3.30 mg/g DW at 30 days after flowering, after that rapidly decreased with accumulation of phytic acid-P. Other P compound except for phytic acid and inorganic acid was high as same inorganic-P at early stage of seed development. Other P compound contained cellular P defined as the cell membrane and low molecular weight sugar P compounds. These compounds are the precursors of inositol phosphate and phytic acid biosynthesis. P

absorbed by plants is translocated to the seed and plays an important role in organic-P and cellular compound synthesis at the early maturity stage. And also, the P from these P compounds was may be used for phytic acid synthesis from middle to the end of the maturity.

These results suggested that MIPS are expressed strongly in seeds and play a role in phytic acid biosynthesis during seed development.

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