

## Variation in Phytate Accumulation in Common Bean (*Phaseolus vulgaris* L.) Fruit Explants

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### ABSTRACT

*The in vitro synthesis of phytate was studied in common bean fruit explants. Different concentrations of sucrose; phosphorus (P); myo-inositol; abscisic acid (ABA); glutamine and methionine, were tested. Fixed concentrations of these compounds were tested at different periods (0, 3, 6 and 9 days). Variation in phytate coincided with different concentrations of sucrose, myo-inositol, P and ABA for the duration tested. These compounds caused an accumulation of phytate and were more effective in the presence of myo-inositol and P. The accumulation of P varied less than phytate for the different treatments tested in vitro. In conclusion, P, sucrose, ABA, and myo-inositol caused an increase in the phytate of bean seed, showing that it could be possible to alter its content by culturing bean fruit explants in vitro.*

**Key words:** Abscisic acid; glutamine; phosphorus; phytic acid; sucrose

### INTRODUCTION

The stored phosphorus (P) in the form of phytate (myo-inositol hexakisphosphate - InsP<sub>6</sub>, phytic acid) occurs during the development of common bean (*Phaseolus vulgaris*) fruit and depends on the supply of organic P from other parts of the plant and on the assimilation of myo-inositol or glucose-6P. Phytate is deposited in the bean seeds within single-membrane storage organelles, referred to as protein bodies. These are mostly complexed with minerals (K, Mg, Ca, Fe and Zn) and are known as phytin (Reddy et al., 1989). Phytate or phytic acid represents from 65 to 85% of the total seed P (Reddy et al., 1989). As a polyanion, phytate is an

effective chelator of positively charged molecules and has the potential to form stable insoluble complexes with minerals and proteins. These complexes confer to phytate its notorious antinutritional properties, which are particularly important for the humans and nonruminants, such as poultry, swine and fish that lack the hydrolytic enzyme phytase in their digestive tract and excrete a large fraction of these phytin salts (Cheryan, 1980). Moreover, the amount of phytate that breaks down during the storage of common beans appears to be the most important trait in the development of the hard to cook (HTC) phenomenon (Coelho et al., 2007a).

On the other hand, phytate is generally considered

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an important source of nutrients during bean seed germination, which provides inorganic phosphate, minerals and myo-inositol to the growing seedling, although it is not apparently required at the typical levels (Raboy et al., 2001). Furthermore, a diet rich in phytic acid may also have a beneficial role in human health, as an antioxidant and anticarcinogen (Zhou and Erdman, 1995).

Studies have been conducted with phytate to reduce the levels of InsP6 in the seeds to improve iron and zinc availability in food (Lucca et al., 2001). For instance, studies were carried out with maize (*Zea mays*) mutants, where loss of function mutations at two loci, *low phytic acid 1-1* (*lpa1-1*) and *low phytic acid 2-1* (*lpa2-1*), caused a reduction of InsP6 by about two-third and an increase in inorganic phosphorus by a molar-equivalent when compared with the wild type. In *lpa2-1* maize mutant, InsP6 was reduced by around 50%, while inorganic phosphorus and inositol phosphate contents increased when compared to that of the wild type (Larson et al., 1998; Raboy et al., 2000; Raboy et al., 2001).

Another approach to study phytate would be the identification and regulation of the physiological and biochemical mechanisms that are involved or that control phytic acid accumulation and their relationships with other inositol and phosphorus pools. Essentially, two types of studies could be performed: the elucidation of the exact metabolic pathway of phytic acid, and the understanding of the mechanisms that regulate phytic acid accumulation. However, when the pathway is considered, several points of this cascade are still not well defined due to multiple, simultaneous and non-linear pathways that lead to phytic acid synthesis (Coelho and Vitorello, 2002; Shi et al., 2003; Stevenson-Paulik et al., 2002).

Some reports have provided important evidences to suggest that D-myo-inositol-3-phosphate synthase (MIPS, E.C. 5.5.1.4) plays a regulatory role in phytate synthesis in developing common bean seeds (Coelho et al., 2005b; Hegeman et al., 2001; Yoshida et al., 1999; Coelho et al., 2007b). Moreover, recent studies showed a possible control point in the conversion of inositol pentakisphosphate to inositol hexakisphosphate (Coelho et al., 2005b). Several other studies have shown that the accumulation of phytic acid oscillates in response to the variation of P supplied during seed development (Coelho et al., 2002; Raboy and Dickinson, 1984; Raboy and Dickinson, 1993). The pathway of phytate

synthesis begin with myo-inositol-monophosphate, which is produced if both enzymes, MIPS and myo-inositol-kinase, act upon glucose-6-P and myo-inositol, respectively. Moreover, myo-inositol-monophosphate is a precursor of phytate synthesis since it is a non-reversible reaction (Loewus and Murthy, 2000).

Considering that sucrose and abscisic acid (ABA) may affect the synthesis of storage compounds in seeds and MIPS expression, a possible alteration in the phytate synthesis may occur since the content of myo-inositol-monophosphate will also be changed (Yoshida et al., 2002; Yoshida et al., 1999).

Other possible regulatory steps of phytate synthesis might involve protein synthesis due to negative correlations between the content of proteins and phytate in grains (Raboy, 1990) and most of the phytic acid is deposited as a mixture and packaged into discrete, membrane bound inclusions referred as globoids (Lott et al., 1985).

Due to many factors involved in phytate accumulation, an *in vitro* system would be an appropriate way to study phytic acid synthesis. Soybean fruit explants cultured *in vitro* showed alterations in their protein patterns and free amino acids of the cotyledons when methionine and different sources of nitrogen were provided (Mosquim and Sodek, 1991; Mosquim and Sodek, 1992). Therefore, based upon this work, an *in vitro* test of cultured common bean fruit explants was conducted with the objective to alter phytate and protein synthesis. This system allowed the elimination of the complex interaction between the fruit and the plant and altered the availability of components involved in the synthesis of both phytate and protein.

## MATERIAL AND METHODS

### Growth of plants

Seeds of a commercial line of common bean (*Phaseolus vulgaris* L.), Una variety, were supplied by the Experimental Research Station of the Instituto Agronômico de Campinas - IAC, in Capão Bonito, SP, Brazil. Plants were grown in a greenhouse in 10-L pots containing 8 L of a 4:1 (v/v) mixture of soil and commercial substrate. A dystrophic soil was collected and amended with lime at 3.8 g.dm<sup>-3</sup>, according to chemical analysis to reach a base saturation of about 70%. Prior to planting, phosphorus, nitrogen and potassium were

applied to the mixture at a rate of 37, 17 and 70.4 mg.dm<sup>-3</sup> of P, N and K, respectively. At 25 and 35 days after planting (DAP), each pot received 27.5 mg N.dm<sup>-3</sup>. The experimental design was a complete randomized block with one variety, four replicates and one harvest time.

All the flowers were labeled with their date of anthesis and pods were collected at one age from independent plants. Each individual plant provided fruit explants (bean pods with immature seeds and a short piece of stem) and was maintained under greenhouse conditions for developmental *in situ* analysis.

To evaluate the synthesis of phytate, two independent experiments were conducted: 1) altering the different concentrations of compounds (*sucrose*; *phosphorus (P)*; *myo-inositol*; *abscisic acid (ABA)*; *glutamine and methionine*) that were directly and indirectly involved in phytate synthesis, and 2) testing various periods of growth on phytate synthesis at a fixed concentrations of the mentioned compounds.

### Experiment 1

*Growth of common bean fruit explant with different compounds at varied concentrations:* The culture system used was according to Mosquin and Sodek (1991) including the basal culture medium (BCM), which was used to cultivate the bean fruit explants. The BCM contained the macronutrients (Chandler et al., 1983) together with Fe-EDTA and micronutrients (Murashige and Skoog 1962). Immature fruits containing seeds (approximately 150 mg of fresh weight) obtained from the greenhouse were cultured for 12 days in BCM amended with one of the following treatments, i.e., compounds at different mM concentrations: Sucrose at 0, 40, 80, 160 and 320; Phosphorus at 0, 3, 6, 12 and 24; Myo-inositol at 0, 1, 5, 10 and 20; Abscisic acid (ABA) at 0, 0.05, 0.10, 0.125 and 0, 150; Glutamine at 0, 10, 20, 40 and 80, and Methionine at 0, 5, 10, 15 and 25. Each concentration tested had three repetitions.

### Experiment 2

*Growth culture of bean fruit explants at different periods:* Culture of fruit explants obtained from the greenhouse was conducted separately with BCM amended with one of the following compounds at a fixed concentration of sucrose (160 mM); 10 mM myo-inositol; 12 mM phosphorus and 0.125 mM ABA for 0, 3, 6 and 9 days. Each period tested had three replicates.

These concentrations were chosen based on the highest accumulation of phytate observed in preliminary tests and on results reported by Coelho et al. (2002b).

### Controls

The following controls were conducted for both the experiments. CT<sub>0</sub>: fruit explants were harvested before initiating *in vitro* culture (at zero time). The following treatments were explants maintained in the greenhouse for a determined period followed by *in situ* analysis: CT<sub>3</sub>: 3 days; CT<sub>6</sub>: 6 days; CT<sub>9</sub>: 9 days, and CT<sub>12</sub>: 12 days.

### Seed analysis

Seeds obtained from the Experiments 1 and 2 were separated from the bean pod and lyophilized. The weight and the number of the seeds were documented and finely ground in the presence of liquid nitrogen. The following parameters were analyzed: total phosphorus (P) and the content of total protein and phytate.

### Determination of total phosphorus (P)

The content of P was determined colorimetrically following nitric/perchloric acid digestion, using the metavanadate method, which was based on the formation of a yellow vanadomolybdophosphoric complex (Bernhart and Wreath, 1955).

### Determination of phytic acid

The method of Latta and Eskin (1980) was employed to determine the phytic acid. A 500 mg sample was extracted with 20 mL of 2.4% HCl (0.65 N) for 2 h at room temperature on a rotatory shaker (200 rpm). The extract was centrifuged (10,000 g, 15 min) and the supernatant was decanted and filtered through Whatman number 1 filter paper. A 3-mL aliquot of the filtrate was diluted to 18 mL with distilled water and the diluted sample was passed through a 200-400 mesh AG1-X8 chloride anion exchange resin, taking care that no more than 3 mg phytate per 0.5 g of resin was applied. Inorganic phosphorus was eluted with 0.07 M NaCl followed by elution of phytate with 0.7 M NaCl. Phytate was determined colorimetrically based on the pink color of the Wade reagent, which was formed upon the reaction of ferric ion and sulfosalicylic acid, and had an absorbance maximum at 500 nm.

### Total nitrogen determination (total protein)

Total protein content was determined by total N

content of the sample, where % Protein = N content (%) x 6.25. Samples (200 mg) were placed in 75-mL digestion tubes and 6 mL of digestion mixture, which consisted of concentrated sulfuric acid, 30% hydrogen peroxide, 14% lithium sulfate and 42% selenium, was added to each sample. The samples were placed in a digestion block at 250 °C until samples became clear. Samples were diluted with water to a final volume of 75 mL. The N content of the samples was quantified according to Parkinson and Allen (1975).

## RESULTS

### Accumulation of the seed dry matter

The growth of seeds from the common bean fruit explants cultured for 12 days was determined by the accumulation of dry matter. Data for the dry matter of the seeds before and after the culture at different concentrations of sucrose, myo-inositol, phosphorus (P), abscisic acid (ABA), glutamine and methionine are shown in Table 1. The accumulation of dry matter from *in vitro* culture of fruit explants was different when compared to that for seeds from *in situ* culture (CT<sub>12</sub>, 0.183 g.seed<sup>-1</sup>). A considerable augmentation in the dry matter of the seeds was observed due to the increase in concentrations of sucrose, phosphorus and myo-inositol. Increases of 42, 127, 49, 50 mg/seed were observed for the highest concentrations of sucrose, phosphorus, myo-inositol and glutamine, respectively, when compared with their corresponding 0 mM levels (Table 1).

The accumulation of dry matter of the seeds at 24 mM P (0.203 g/seed) was higher than that for *in situ* culture at 12 days (CT<sub>12</sub> 0.183 g/seed) and triggered seeds to accumulate 13 times more dry matter over a 12 day period (Table 1). At 320 mM sucrose, the accumulation of dry matter (42 mg/seed) was higher than reported by Mosquim and Sodek (1991), who observed a 26 mg/seed increase for soybean fruit explants cultured at 46 to 292 mM of sucrose. These results indicated that the common bean seeds underwent considerable development, which was higher than other explant cultures reported in the literature, such as soybean and pea (Barratt, 1986; Mosquim and Sodek, 1991; Mosquim and Sodek, 1992).

The accumulation of dry matter observed at the end of the 9 days of *in vitro* culture (Table 2) and in the presence of sucrose at the initial time (CT<sub>0</sub>) was around a three-fold increase. This was higher

than that obtained when the concentration of sucrose varied (from 0 mM to 320 mM) for the 12-day culture (Table 1), which caused a 1.47-fold increase in the dry matter. This indicated that the culture period caused a greater effect on the development of the seed rather than the concentration of sucrose tested.

Regarding the growth period (Table 2), no considerable effect was observed between the treatments for the seeds cultured *in vitro* for up to 9 days. However, the dry matter obtained for the *in vitro* sucrose treatment was approximately 1.4-fold lower when compared to that of *in situ* up to 9 days.

### Phytate, phosphorus and protein seed accumulation

The accumulation of phytate increased based on the given concentration of the compounds, and occurred mainly for the treatment 4 (T<sub>4</sub>) with myo-inositol, phosphorus, sucrose and ABA at 10, 12, 160 and 0.125 mM, respectively (Fig. 1A, B). However, a decrease in the phytate accumulation was found above these concentrations, with the exception of phosphorus, in which the accumulation was not altered (Fig. 1A). Overall, myo-inositol caused the greatest effect on the accumulation of phytate (Fig. 1A). On the other hand, the different concentrations of methionine caused an oscillation in the accumulation of phytate, while glutamine above 10 mM caused a decrease in phytate accumulation (Fig. 1B).

An increase of 0.35 mg/seed of P was found after adding different concentrations of P in the culture medium (T<sub>1</sub> to T<sub>5</sub>) (Fig. 1C). For other treatments, only myo-inositol and glutamine, both at 10 mM, caused a considerable accumulation of P (Fig. 1C, 1D).

The increase in the accumulation of seed proteins with P, myo-inositol and sucrose treatments was very similar, i.e., approximately 10 mg/seed when compared to the highest concentrations of the respective treatments (Fig. 1E). On the other hand, no considerable alteration in the accumulation of seed proteins was found for other treatments (glutamine, methionine and ABA) (Fig. 1F). When the values for the treatments of the *in vitro* culture were compared with those of the control CT<sub>0</sub> (0.095 mg/seed) and CT<sub>12</sub> (1.062 mg/seed) from the *in situ* culture, only phytate accumulated at a large proportion in the presence of myo-inositol and P, reaching 1.65 mg/seed and 1.1 mg/seed, respectively (Fig. 1A, B). The accumulation of P

in seeds from *in vitro* culture was similar to that found for the control CT<sub>0</sub> (0.165 mg/seed) and CT<sub>12</sub> (0.781 mg/seed) for the *in situ* culture. In the presence of myo-inositol and P, phytate

accumulated to 0.75 mg/seed and 0.8 mg/seed, respectively. These values were similar to that found for CT<sub>12</sub> (Fig. 1C, D).

**Table 1** - Dry matter of common bean (*Phaseolus vulgaris* L.) of seeds (Una variety) cultured *in vitro* and *in situ* for 12 days. Seeds were cultured *in vitro* in basal medium amended with one of the following compounds at different concentrations: phosphorus, myo-inositol, abscisic acid, glutamine and methionine. Seeds cultured at zero time (CT<sub>0</sub>) had a dry matter seed weight of 0.014 ± 0.007. Seeds from *in situ* culture, after 12 days (CT<sub>12</sub>), had dry matter seed weights of 0.183 ± 0.014. (\*standard error of mean, n=3).

Sucrose		Phosphorus		Myo-inositol	
(mM)	mass (g/seed)	(mM)	mass (g/seed)	(mM)	mass (g/seed)
0	0.089 ± 0.019*	0	0.076 ± 0.022	0	0.082 ± 0.006
40	0.086 ± 0.008	3	0.108 ± 0.016	1	0.111 ± 0.013
80	0.093 ± 0.018	6	0.114 ± 0.018	5	0.105 ± 0.015
160	0.101 ± 0.011	12	0.098 ± 0.015	10	0.114 ± 0.029
320	0.131 ± 0.016	24	0.203 ± 0.014	20	0.131 ± 0.023
Abscisic acid		Glutamine		Methionine	
(mM)	mass (g/seed)	(mM)	mass (g/seed)	(mM)	mass (g/seed)
0	0.103 ± 0.011	0	0.085 ± 0.015	0	0.085 ± 0.018
0.050	0.119 ± 0.028	10	0.146 ± 0.016	5	0.131 ± 0.017
0.100	0.118 ± 0.031	20	0.139 ± 0.014	10	0.130 ± 0.013
0.125	0.116 ± 0.042	40	0.128 ± 0.013	15	0.128 ± 0.014
0.150	0.106 ± 0.022	80	0.135 ± 0.026	25	0.104 ± 0.018

**Table 2** - Dry matter of common bean (*Phaseolus vulgaris* L.) seeds (Una variety) cultured *in vitro* and *in situ* (control) for 0, 3, 6 and 9 days. Seeds were cultured *in vitro* in basal medium amended with fixed concentration of compounds: phosphorus (12 mM), myo-inositol (10 mM), abscisic acid (0.125 mM), glutamine (80 mM) and methionine (22 mM). (\*standard error of mean, n=3).

Culture (days)	Sucrose Mass (g/seed)	Phosphorus Mass (g/seed)	Myo inositol Mass (g/seed)	Abscisic acid Mass (g/seed)
0	0.043 ± 0.0015*	0.042 ± 0.0016	0.042 ± 0.0014	0.045 ± 0.0081
3	0.063 ± 0.0059	0.066 ± 0.0058	0.064 ± 0.0022	0.078 ± 0.0064
6	0.081 ± 0.0071	0.088 ± 0.0011	0.087 ± 0.0042	0.086 ± 0.0068
9	0.123 ± 0.0092	0.091 ± 0.0085	0.093 ± 0.0072	0.111 ± 0.0099

Culture (days)	Glutamine Mass (g/seed)	Methionine Mass (g/seed)	<i>in situ</i> (Control) Mass (g/seed)
0	0.044 ± 0.0016	0.046 ± 0.0011	0.047 ± 0.0031
3	0.065 ± 0.0026	0.062 ± 0.0064	0.087 ± 0.0056
6	0.089 ± 0.0081	0.093 ± 0.0069	0.166 ± 0.0082
9	0.091 ± 0.0078	0.099 ± 0.0065	0.172 ± 0.0089

The accumulation of protein seed in the *in vitro* culture experiment (average of 25 mg/seed) (Fig. 1E, F) was lower in all treatments when compared to that of the control CT<sub>0</sub> (14.22 mg/seed) and CT<sub>12</sub> (41.22 mg/seed) from the *in situ* culture (data not shown). This finding was probably due to the amount of the accumulated dry matter observed for the seeds cultured *in vitro*, which was 60.6%

lower than that found for those cultured *in situ* for the majority of the treatments (Table 1). If the amount of phytate accumulated was compared to that of P, the data showed that overall, P was mainly altered in the phytate form rather than in the total P.

By altering the period of the *in vitro* culture (0, 3, 6 and 9 days), the increased amount of phytate was

similar, at 9 days, to that of the *in situ* culture in the presence of sucrose, phosphorus, myo-inositol and ABA. On the other hand, glutamine and methionine had the least effect on phytate accumulation in the seeds since they caused an increase only up to 6 days followed by a constant level up to nine days in culture (Fig. 2A, B). Phosphorus accumulated in the seeds as the period of the *in vitro* culture increased for the first 6 days of all the treatments, followed by a constant level after this period, with the exception of sucrose and ABA, which caused an increase in P levels up to 9 days (Fig. 2C, D).

When compared to the *in situ* culture, the accumulation of P observed in the seeds from *in vitro* culture was lower (50%), starting at 3 days and remained lower for all the treatments. This low P accumulation was due to the reduced accumulation of dry matter found for *in vitro* culture (Table 2). The accumulation of phytate in the seed was greater in both experiments 1 and 2, for P, myo-inositol, sucrose and ABA. These results suggested an effect of these compounds on the synthesis of phytate.

Seed proteins were accumulated in a similar manner to that observed for P during the *in vitro* culture (Fig. 2E, F). This mainly occurred after the addition of glutamine, methionine and ABA, which caused an increase in the protein levels up to the sixth day and remained constant till the end of the experiment (9 days) (Fig. 2E, F). After 6 days, a slight increase in the accumulation of seed proteins, approximately 7.5 mg/seed, was observed in the presence of sucrose, P and myo-inositol at 9 days of *in vitro* culture. When compared to *in situ* conditions, the accumulation of seed proteins from *in vitro* culture was lower from the third to the ninth day. This value was approximately 18 mg/seed and corresponded to around 50% of the value found for the *in situ* conditions. This percentage was due to the dry matter accumulated, which was 50% lower than that observed for *in situ* conditions (Table 2).

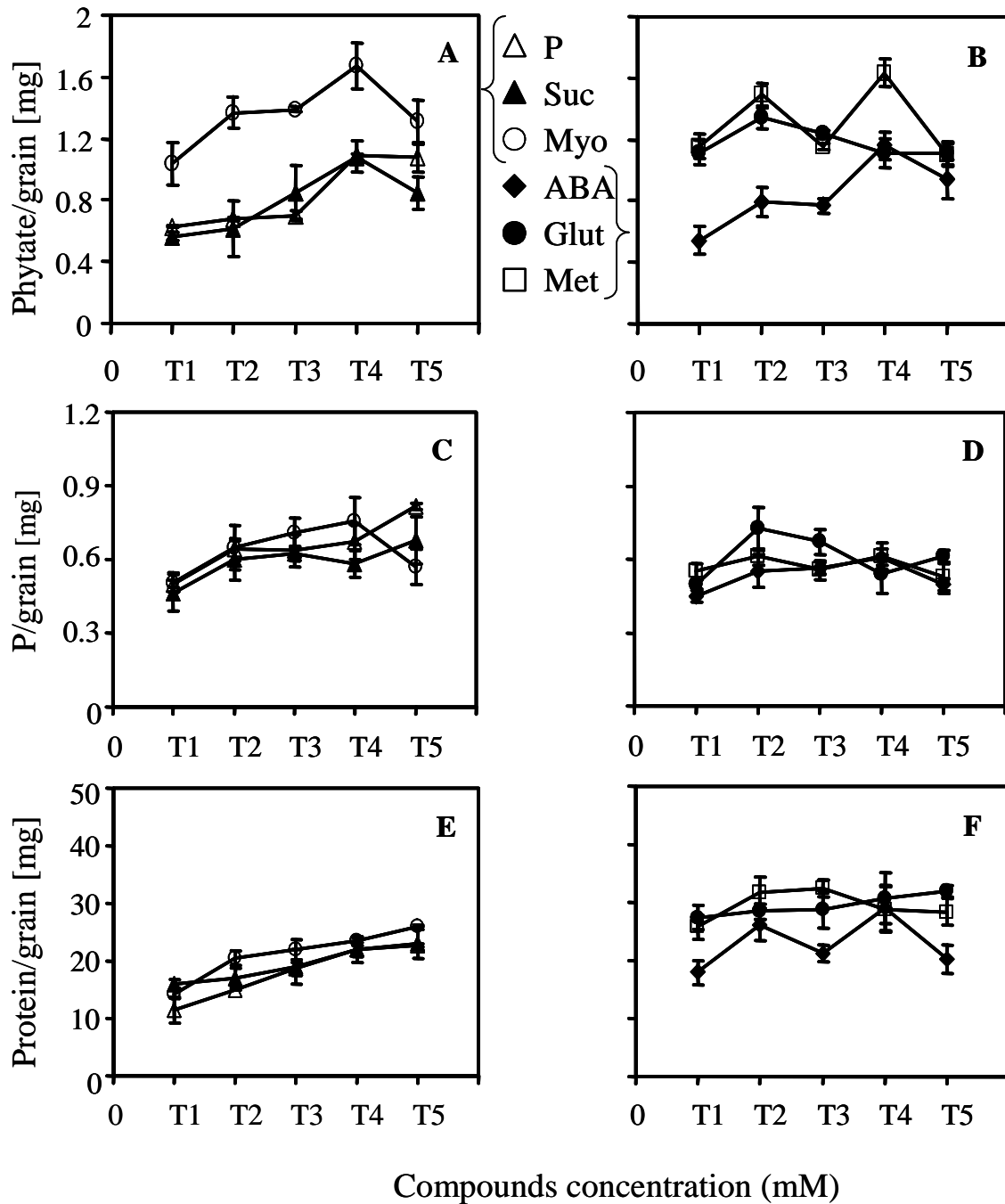
## DISCUSSION

The *in vitro* system used for common bean fruit explants showed a considerable alteration in the accumulation of the parameters tested, total phosphorus (P), total protein content and phytate in seeds after the addition of P, sucrose, myo-

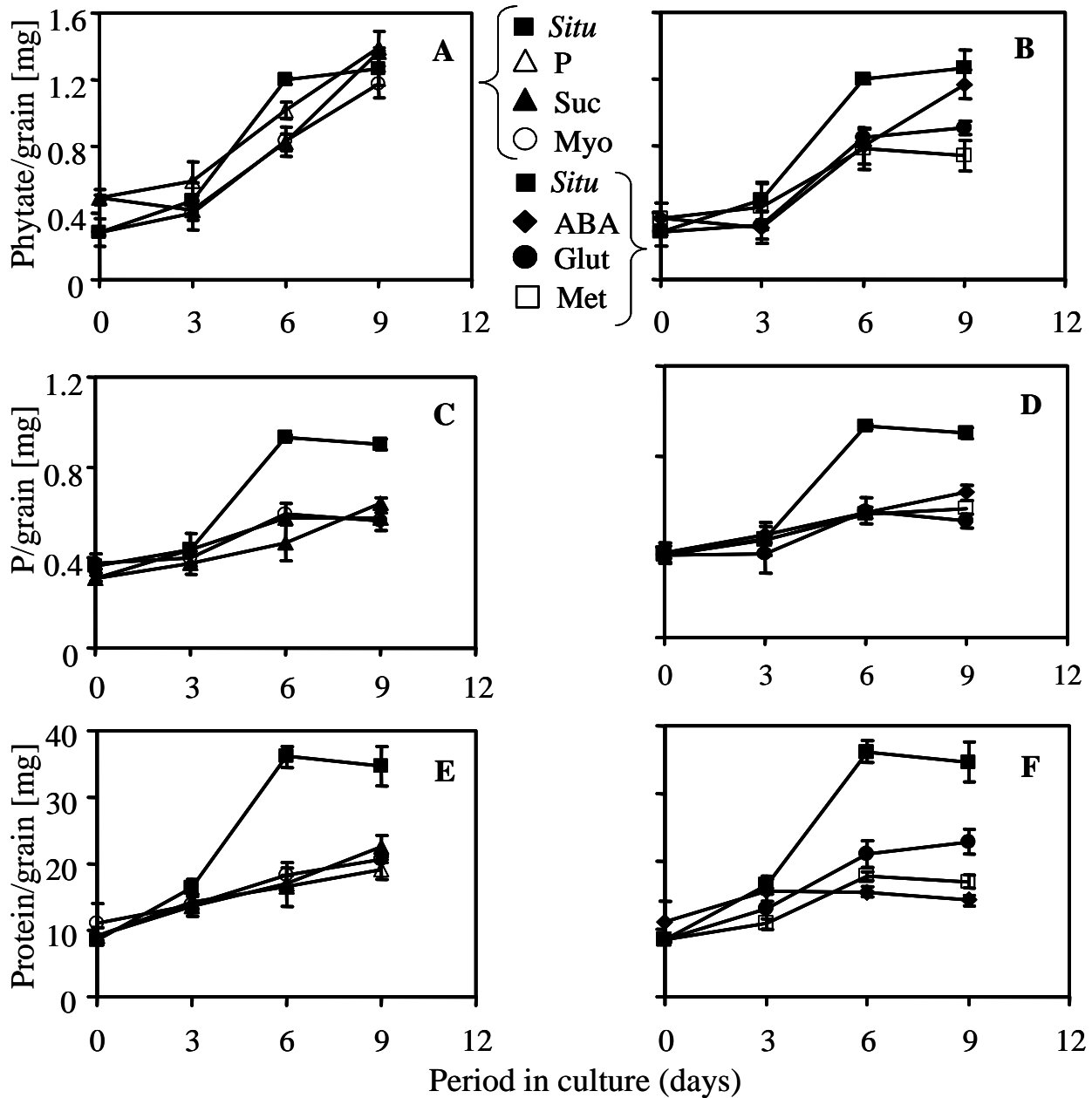
inositol and ABA to the culture medium. No effect on the seeds was observed with glutamine and methionine for the accumulation of these parameters. Overall, the variation found for the accumulation of phytate concurred in the presence of sucrose, myo-inositol, P and ABA after altering their concentrations as well as with the period of *in vitro* culture (Fig. 1A, B and 2A, B). Moreover, the accumulation of phytate was higher mainly after adding myo-inositol and P to the medium when compared to that for the *in situ* conditions (Fig. 2A). Studies conducted *in situ* have shown an increase in the accumulation of seed phytate in response to a higher availability of P (Coelho et al., 2002; Raboy and Dickinson, 1993). The addition of myo-inositol to the medium can provide monophosphate myo-inositol through the activity of myo-inositol kinase, or it can be supplied by *de novo* synthesis from glucose-6-phosphate via MIPS activity. It can also be recycled from the stored or translocated forms, since myo-inositol-1P is produced by MIPS and/or myo-inositol-kinase enzymes for which myo-inositol is a substrate (Loewus and Murthy, 2000). In the present study, this substrate might have caused an increase in the expression of these enzymes and consequently an increase of the final product, phytate, in the seeds since the reaction forming myo-inositol-1P was irreversible.

The positive response of the seed to accumulate phytate in the presence of sucrose and ABA might be due to the fact that both could affect the synthesis of the stored compounds in the seeds (Coelho et al., 2005b) and the MIPS expression. Moreover, a possible alteration of the synthesis of phytate might occur since the content of myo-inositol-1P would also be changed (Yoshida et al., 2002; Yoshida et al., 1999).

Values observed for phytate and P exhibited an interesting relationship since the accumulation of P varied less than the increase of phytate in the different treatments tested *in vitro*. Seeds accumulated P mainly due to the increase of the concentration of P in the medium (Fig. 1C).



**Figure 1-** Phytic acid, phosphorus (P) and protein contents of common bean seed from fruit explants cultured for 12 days in basal medium amended with one of the following compounds at different concentrations: P (△) (T1= 0 mM P; T2= 3 mM; T3= 6 mM; T4= 12 mM; T5= 24 mM); glutamine (●) (T1= 0 mM; T2= 10 mM; T3= 20 mM; T4= 40 mM; T5= 80 mM); myo-inositol (○) (T1= 0 mM; T2= 1 mM; T3= 5 mM; T4= 10 mM; T5= 20 mM); abscisic acid (◆) (T1= 0 mM; T2= 0.05 mM; T3= 0.10 mM; T4= 0.125 mM; T5= 0.150 mM); methionine (□) (T1= 0 mM; T2= 5 mM; T3= 10 mM; T4= 15 mM; T5= 25 mM), and sucrose (▲) (T1= 0 mM; T2= 40 mM; T3= 80 mM; T4= 160 mM; T5= 320 mM).



**Figure 2** - Phytic acid, phosphorus (P) and protein contents of common bean seed from fruit explants cultured in medium basal amended with fixed concentration of compounds at: 12 mM phosphorus ( $\Delta$ ); 80 mM glutamine ( $\bullet$ ); 10 mM myo-inositol ( $\circ$ ); 0.125 mM abscisic acid ( $\blacklozenge$ ); 22 mM methionine ( $\square$ ) 160 mM sucrose ( $\blacktriangle$ ) and *in situ* culture ( $\blacksquare$ ) for 0, 3, 6 and 9 days. Bars indicate standard errors (n=3).

Moreover, the relationship between phytate and P at a fixed concentration of P and myo-inositol during the growth period (Fig. 2A, C) revealed considerable alterations in the accumulation of phytate, but not of P. Likewise, at fixed concentrations, sucrose and ABA also caused

alterations in the accumulation of phytate (Fig. 2A, B, C, D). The content of P in common bean seeds was 2-fold higher as the concentration of available P in the culture medium increased (Fig. 1C). These results corroborated with *in situ* studies reported in the literature, which showed that an increase in the



available P caused an increase of P in seeds, mainly in the phytate form (Coelho et al., 2002; Raboy and Dickinson, 1984; Raboy and Dickinson, 1993).

Seed proteins also increased with longer growth culture periods (Fig. 2E). This increase of 10 mg/seed was low if the variations observed for the *in situ* growth period (9 days), which caused an increase of 25 mg/seed, were considered (Fig. 2E). These results were supported by the dry matter value obtained for seeds from *in vitro* culture, which was 60% lower than that from *in situ* (Tables 1 and 2). Future studies should be conducted to measure the alteration of the protein synthesis *in vitro* through the analysis of the accumulation of stored proteins, or the total amino acid content (Mosquim and Sodek, 1991; Mosquim and Sodek, 1992), employing available proteomic and metabolomic techniques (Azevedo et al., 2004a; Azevedo et al., 2004b; Helm et al., 2004), which would allow a more comprehensive evaluation of phytate metabolism.

The results obtained with phytate, P and protein accumulation in grain using *in vitro* the conditions corroborated with the previous studies using *in situ* conditions and *in vitro* condition with radiolabeled precursors (Coelho et al., 2002a e 2002b; Coelho et al., 2005b). In conclusion, P, sucrose, ABA and mio-inositol caused an increase of phytate in common bean seeds (variety Una), thus showing that it could be possible to alter the synthesis of phytate by culturing bean fruit explants *in vitro*. The results obtained from this study would be useful for future research aimed at analyzing the regulation of phytate synthesis in seeds, since contrasting growth culture conditions were conducted should be to evaluate the accumulation of phytate in seeds. To better understand the regulatory mechanisms of phytate synthesis, studies conducted to analyze MIPS expression and seed storage proteins that might be differentially expressed under contrasting conditions, through SDS-PAGE and bi-dimensional gel electrophoresis.

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## RESUMO

O fósforo é armazenado na forma de fitato nas sementes, o qual forma complexos estáveis e insolúveis com minerais e proteínas, conferindo efeito antinutrientes. A síntese de fitato foi estudada em cultivo de explantes de fruto de feijão *in vitro* sob diferentes concentrações de sacarose, fósforo (P), mio-inositol, ácido abscísico (ABA), glutamina e metionina. Fixada a concentração destes compostos, testou-se os diferentes tempos de cultivo (0, 3, 6 e 9 dias). A variação no acúmulo de fitato ocorreu na presença de sacarose, mio-inositol, P e ABA nas diferentes concentrações e tempos testados. O acúmulo mais efetivo de fitato ocorreu na presença de mio-inositol e P. O acúmulo de P variou menos do que fitato em todos os tratamentos. Em conclusão, P, sacarose, ABA e mio-inositol causaram aumento no fitato acumulado nas sementes, mostrando que foi possível alterar a síntese de fitato em cultivo de explantes de fruto de feijão.

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