De novo synthesis of enzymes participating in phytate breakdown during germination of lentils (*Lens culinaris* var. Magda)

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The phytate-degrading activity of grains, seeds and pollen has been shown to be responsible for phytate degradation during germination to make phosphate, minerals and *myo*-inositol available for the purpose of plant growth and development (Reddy *et al*., 1989). Usually there is a good correlation between the rapidity of phytate breakdown and phytate-degrading activity of the seed or seedling. Although large increase in phytate-degrading activities have been extensively reported in germinating seeds as well as pollen, the biochemical mechanism leading to this rise in phytate-degrading activity is not well understood. In pollen, phytate-degrading enzymes induced during germination may be synthesised from long-lived, pre-existing mRNA (Jacksoon and Linskens, 1982). In cereals and legumes, some studies suggest d*e novo* synthesis (Bianchetti and Sartirana, 1967), while others suggest activation of pre-existing enzymes (Eastwood and Laidman, 1971). In addition, several molecular forms of phytate-degrading enzymes, which may be regulated in different ways, have been identified in a certain plants (Greiner, 2002).
With 16.91 mU g^{-1} dry basis, non-germinated lentils (*Lens culinaris* var. Magda) showed only a very low phytate-degrading activity. During germination for 6 days at 20°C in the dark a 17.5fold increase in extractable and a 2.5fold increase in total phytate-degrading activity determined at pH 5.0 with a concomitant reduction in phytate content (66%) was observed. The increase in phytate-degrading activity was inversely correlated with the disappearance of phytate. No accumulation of *myo*-inositol penta*kis*-, tetra*kis*-, and trisphosphates occurred during germination. *myo*-Inositol bis- and monophosphates were not be detected by the HPLC method used. Lentils exhibited no phytate-degrading activity at pH 8.0 as reported for other legumes (Scott, 1991), so alkaline phytase activity does not seem to play a role in phytate degradation during germination. Since the non-extractable phytate-degrading activity (pH 5.0) was not significantly affected by germination, only the extractable phytate-degrading activity was suggested to be responsible for phytate breakdown during germination of lentils.

Table 1. Effect of cylcoheximide and actinomycin D on phytate-degrading activity and phytate reduction after 6 days of germination when applied after 24 h (first column) and 72 h (second column) of germination.

	Phytate-degrading activity [mU g^{-1} d.m.] extractable	total	Phytate content [µmol g^{-1} d.m.]
control	296.3 ± 1.0	548.7 ± 4.6	1.12 ± 0.1
cycloheximide			
1 µM	114,9 ± 0.6 / 162.5 ± 0.8	362.2 ± 3.3 / 401.2 ± 3.4	2.27 ± 0.6 / 1.95 ± 0.8
5 µM	61.9 ± 0.3 / 75.1 ± 0.6	303.1 ± 2.9 / 322.6 ± 2.8	2.53 ± 0.3 / 2.53 ± 0.6
10 µM	22.9 ± 0.3 / 50.5 ± 0.5	273.7 ± 1.8 / 300.9 ± 3.8	2.80 ± 0.3 / 2.65 ± 0.6
actinomycin D			
50 µM	100.6 ± 0.8 / 225.9 ± 1.4	345.9 ± 4.3 / 480.5 ± 4.1	2.36 ± 0.1 / 1.61 ± 0.4
100 µM	39.6 ± 0.2 / 177.5 ± 0.9	281.7 ± 2.4 / 417.3 ± 2.7	2.71 ± 0.3 / 1.92 ± 0.7

Cycloheximide, a competent inhibitor of translation in eukaryotes, and actinomycin D, which blocks transcription in eukaryotes, were applied to germinating lentil seeds after 24 h and 72 h of germination, respectively. A significant reduction in the rise of extractable as well as total phytate-degrading activity, and a reduced drop in phytate content, was observed (Table 1). The observed effects were more pronounced at higher concentration of cycloheximide and actinomycin D and by administration of these compounds earlier in germination. Application of cycloheximide or actinomycin D after 96 h of germination had no significant effect on phytate-degrading activity or phytate hydrolysis compared to the control.

During germination phytate-degrading activity may be controlled by the action of gibberellic acid and phosphate, respectively. The action of gibberellic acid on the phytate-degrading enzymes of the germinating seed is not well understood. It is claimed that gibberellic acid merely increase the secretion of phytate-degrading enzymes but does not stimulate their synthesis (Gabard and Jones, 1986), thus giving phytate-degrading enzymes access to phytate, and that gibberellic acid stimulates phytate-degrading activity (Srivastava, 1964). In lentils, administration of gibberellic acid during germination remarkably enhanced extractable phytate-degrading activity (1.4-fold at 1 µM, 1.6-fold at 5 µM, 1.8-fold at 10 µM) and stimulated phytate degradation (1.2-fold at 1 µM, 1.3-fold at 5 µM, 3-fold at 10 µM) in a dose dependent manner. These effects were reduced by 79–84% (phytate-degrading activity) and 52–66% (phytate degradation) with 5 µM cycloheximide or 50 µM actinomycin D.

Two main mechanisms appear to be involved in the regulation of phytate-degrading activity by phosphate. Acid phytate-degrading enzymes are strongly inhibited by phosphate, thus the enzyme activity itself may be controlled by phosphate. It was concluded that phosphate also acts at the transcription level, since phosphate added early enough in the germination sequence can repress the increase in phytate-degrading activity (Sartirana and Bianchetti, 1967). The addition of up to 10 mM phosphate during germination of lentils did not affect significantly phytate-degrading activity and phytate degradation as compared to the control.

In light of the data presented, the rise in phytate-degrading activity during germination of lentils is due to *de novo* synthesis of the corresponding enzyme(s). Inhibition of transcription as well as inhibition of translation strongly reduced the increase in phytate-degrading activity and the disappearance of phytate during germination. Gibberellic acid, but not phosphate, seems to control phytate breakdown within the lentil seedling. Since the stimulating effect of gibberellic acid on phytate-degrading activity and phytate breakdown was inhibited by cycloheximide and actinomycin D, we conclude that this effect of gibberellic acid is at least partly due to a stimulation of *de novo* synthesis of enzymes participating in phytate degradation. Neither addition of cycloheximide and actinomycin D nor administration of gibberellic acid significantly affects non-extractable phytate-degrading activity, which further suggests that non-extractable phytate-degrading activity does not contribute to phytate degradation during germination.

References

Bianchetti, R., Sartirana, M.L., 1967. *Biochim. Biophys. Acta* 145, 485-590.
Eastwood, D., Laidman, D., 1971. *Phytochemistry* 10, 1275-1284.
Gabard, K.A., Jones, R.L., 1986. *Plant Physiol.* 67, 182-192.
Greiner, R., 2002. *J. Agric. Food Chem.* 50, 6858-6864.
Jackson, J.F., Linskens, H.F., 1982. *Acta Bot. Neerl.* 315, 441-447.
Reddy, N.R., *et al.*, 1989. *Phytates in Cereals and Legumes.* CRC Press, Boca Raton, Florida, USA.
Sartirana, M.L., Bianchetti, R., 1967. *Plant Physiol.* 20, 1066-1075.
Scott, J.J., 1991. *Plant Physiol.* 95, 1298-1301.
Srivastava, B.I.S., 1964. *Can. J. Bot.* 42, 1303-1305.