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Does the Maternal Genome Influence Seedling Protein Biochemistry During Plant Embryogenesis?

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Introduction

Biochemical studies of plant seedlings may provide insights into embryogenesis. The focus of the present paper deals with a discovery in sea urchin embryos that maternal m-RNA templates might be directing development during early embryogenesis (Gross et al. 1964). These investigators found that a transcriptional inhibitor (actinomycin D) did not interfere with protein synthesis during the early phase of development, since the embryo used maternal transcripts which were pre-formed before fertilization. However, after some 10 hours (blastula stage), the embryo began to transcribe embryonic templates *de novo* to translate a new genre of protein. This switch over, known as the maternal to zygotic transition (MZT), has since been demonstrated in other animal models (Baroux *et al.*, 2008).

Less is known about MZT in plant systems. Early studies on rice (Zhu *et al*., 1980) and more recent studies in rapeseed plant (Wu *et al*., 2005) have suggested such maternal mediation during early development. The purpose of this research is to encourage exploration regarding the occurrence of MZT in other plant species. In order to facilitate study, we have undertaken pilot experiments with two representatives of the *Brassicaceae* (cabbage and broccoli) as well as one of the *Fabaceae* (sugar snap pea). The study used electrophoresis to determine whether proteins in germinated seedlings are expressed in the presence of transcriptional (actinomycin D) and translational (cycloheximide) inhibitors.

Materials

Seed Growth and Development

Seeds of pea, cabbage and broccoli were disinfected in 10% Bleach for 10 minutes and triple rinsed in distilled water. To initiate germination, seeds were submersed in a beaker of distilled water overnight. The seeds were then divided into three groups: Group "A" (Experimental- Act D) was exposed to a solution of 10 mg % actinomycin D (Enzo) for one hour; Group "B" (Experimental – Hex) was exposed to a solution of 10 mg % cycloheximide (Enzo) for one hour; and Group "C" (Control) remained in sterile distilled water (one hour) . (Warnings: consult MSDS sheets for these chemicals). After treatment these seeds were spread on filter paper in separate sterile plastic Petri dishes and covered with more sterile filter paper. Paper was soaked in sterile distilled water, covered with lid, and placed in a drawer at 25-30 C. Seeds were checked for moisture at least once every day.

Sample Preparation

Plant embryo seedlings were harvested after one day. Thereafter, the seedlings were harvested each week for a three week period. Testae and cotyledons were removed with scissors and forceps; the remaining whole seedlings were weighed at 0.16 g, minced under icy conditions and placed in microfuge tubes. A pea seedling was selectively cut into root, stem and leaf sections that would be equivalent in weight to 0.16 g and treated as above. Tubes are frozen at -80º C, minimally overnight, to disrupt cell membranes.

Preparation of the Seedling Homogenate

After thawing the frozen specimens, distilled water was added in proportion to the weight of pooled sample (e.g. 160 µl for a 0.16 g sample). *Bio-Rad* mini-pulverizing tool was used to make a paste of samples on ice. One could increase the total volume of water to create a visible slurry. Each sample was then briefly vortexed and centrifuged at 10,000 g (4˚C, 2 X 10 min). Supernatant aliquots were delivered into separate microfuge tubes by decanting and/or suctioning with pipet tip. This point can be considered a "stop point" and the samples placed in a freezer.

Protein Calibration Assay

Protein content of 5 µl supernatant of each sample was determined by comparison to a standard curve developed from different concentrations of bovine serum albumin. Bio-Rad protein assay procedure (http://www.bio-rad.com/webroot/web/pdf/lsr/literature/LIT33.pdf) provides instructions.

Electrophoresis

Samples were vortexed and centrifuged for 5 minutes in a Serofuge centrifuge (3400 rpm). After calculating the volume of the supernatant needed to deliver a final content of 10 µg protein to each well, an equal volume of modified 2X Laemmli buffer (5ul mercaptoethanol per 95ul of Laemmli) was added. Each well, of a precast 4-15% Tris-Glycine polyacrylamide-TGX gel, is loaded with equivalent calculated amounts of sample based on standard curve (usually 10-20ul). At least one lane should contain a marker, with 10 pre-stained known proteins (Bio-rad Precision Plus Protein™ Dual Color Standards). Two PAGE protocols explain details of the process (Racusen and Thompson, 1996; Frame, 2000). Electrophoresis should be run at 150V for 40 minutes.

Data Analysis

After the run, gels were rinsed for 10 minutes (3X) in distilled water on a rocking platform, stained with Bio-Rad Coomassie Blue Safe stain for one hour and then destained in distilled water for 2 days. Upon scanning (Bio-Rad ChemiDoc™ XRS scanner), the image revealed the position and intensity of the protein bands in each of the lanes. Other scanners will perform similarly but regardless all images must be exported to the .jpg format before presentation. Band intensity was quantified using software from the scanner and this data was exported to Microsoft Excel. Once exported, this data is analyzed and plotted with Excel's software.

Notes for the Instructor

Results

(1) Though actinomycin D (transcriptional inhibitor) does not prevent germination of pea and broccoli seeds (Fig. 1), it is evident that after two weeks, there is retardation of their physical development (Fig. 2). Similar changes occur in broccoli seedlings (figure not shown). Cycloheximide (translational inhibitor) seems to prevent germination of all seeds (Fig. 3). The seeds do not appear to sprout and show signs of infection.

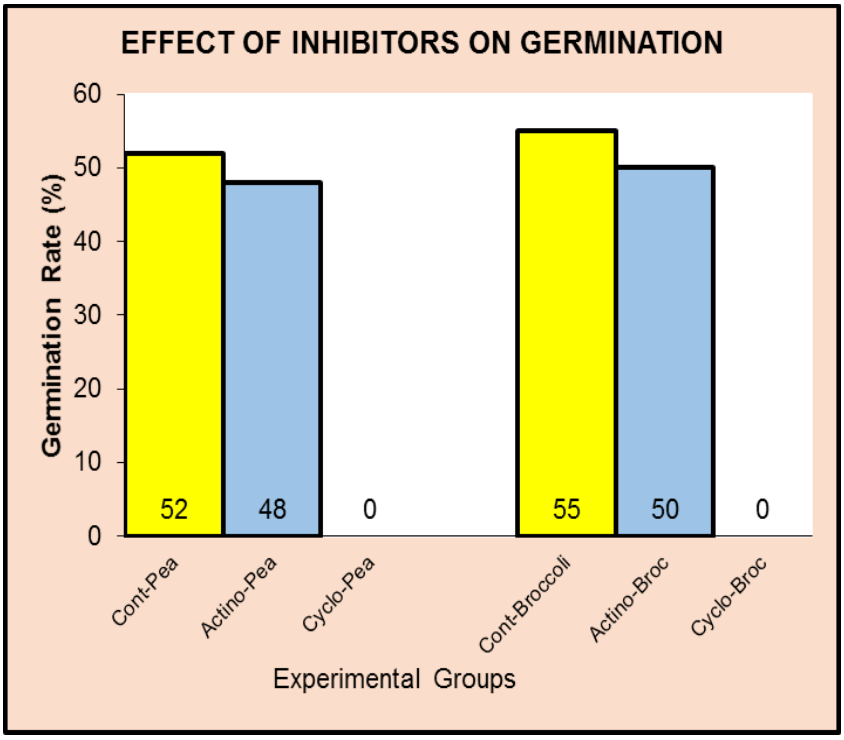

Figure 1. Germination rates of pea and broccoli seeds at two weeks, after exposure to actinomycin D and cycloheximide.

Figure 2. Germinating sugar snap pea seedlings (*Fabaceae* family) after 2 weeks. "Exp" seed was exposed to actinomycin D. Note bleaching of the treated seedlings. Millimeter scale on left.

(2) Protein banding pattern in actinomycin-treated seedlings is quite similar to the banding in control seedlings during the first two weeks of development in pea (Figs. 4 and 6) and cabbage.(Figs. 5 and 7)

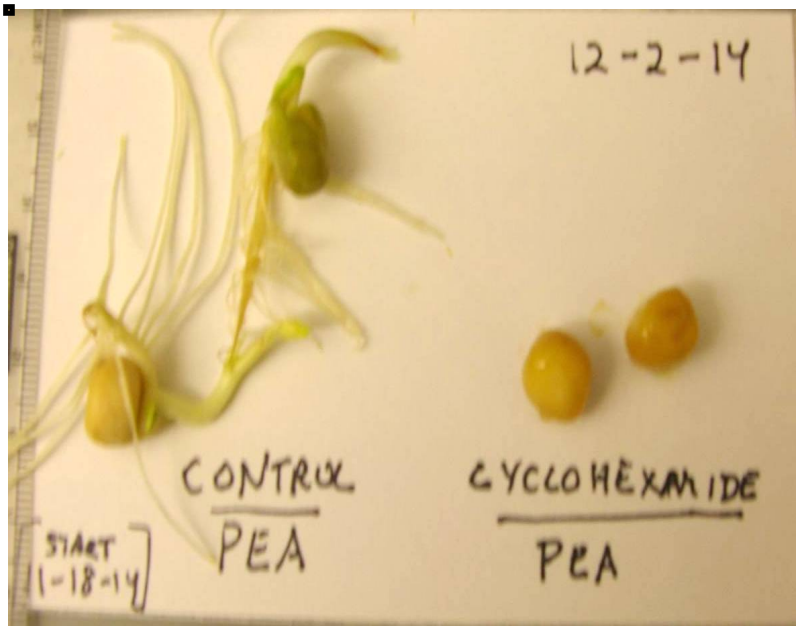

Figure 3. Germinating sugar snap pea seedlings (*Fabaceae* family) after 2 weeks. "Exp" seed was exposed to cycloheximide. Note the lack of germination in the cycloheximide-exposed seeds.

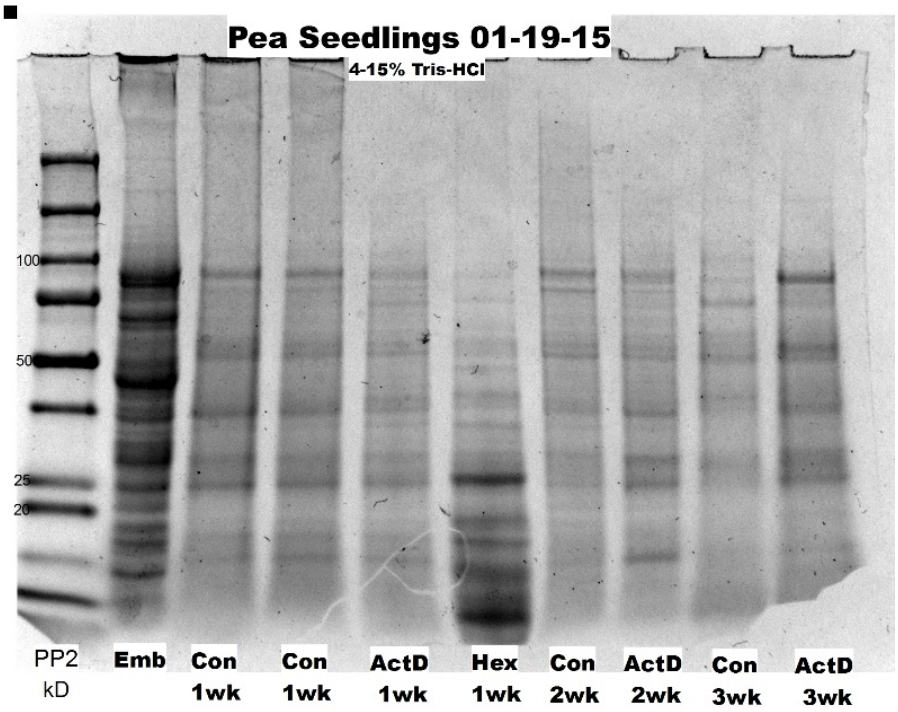

Figure 4. Protein banding profiles of germinating pea (*Fabaceae*) seedlings. The twenty-four hour pea embryo banding pattern (lane 2) contrasts with 1 week controls (lanes 3, 4). After one-week (lane 5) and two-week (lane 8), the actinomycin-D (ActD) -treated seedlings (lane 5) exhibit similar banding to controls (lanes 4 and 7 resp.). The ActD-treated three week seedlings (lane 10) exhibit different banding than controls

(lane 9). Cycloheximide-treated (lane 6) specimens also exhibit different banding than controls (lane 4) after one week.

(3) After one week of development in cycloheximide treated seedlings, protein banding at high molecular weights (range >25kD), is reduced in intensity and number in both peas (Fig. 6) and cabbage (Fig. 7). In contrast, there are increases of banding intensity in the range of MW less than 25kD. (Fig. 4, lane 6 and Fig. 5, lane 6).

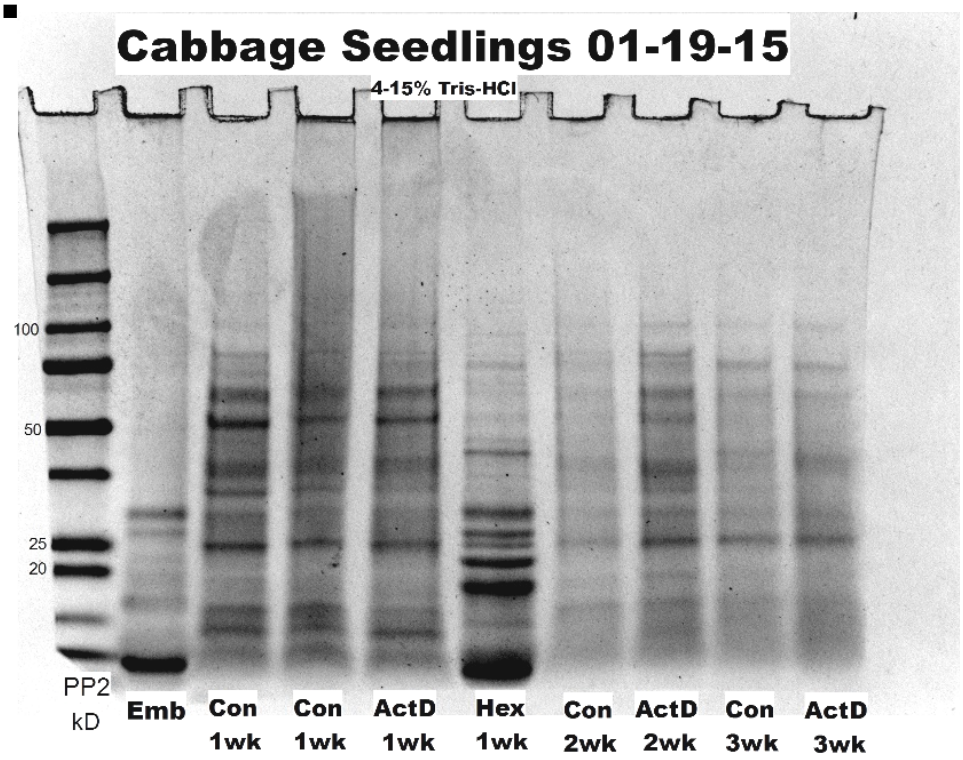

Figure 5. Protein banding profiles from cabbage (*Brassicaceae*) seedlings. The twenty-four hour embryonic band pattern (lane 2) contrasts with 1 week controls (lanes 3, 4).After one-week (lane 5), two-week (lane 8) and three-weeks (lane 10), the actinomycin-D (ActD)-treated exhibit similar banding to controls (lanes 4 , 7 and 9 resp.). Cycloheximide-treated (lane 6) specimens exhibit different banding than controls (lane 4) after one week.

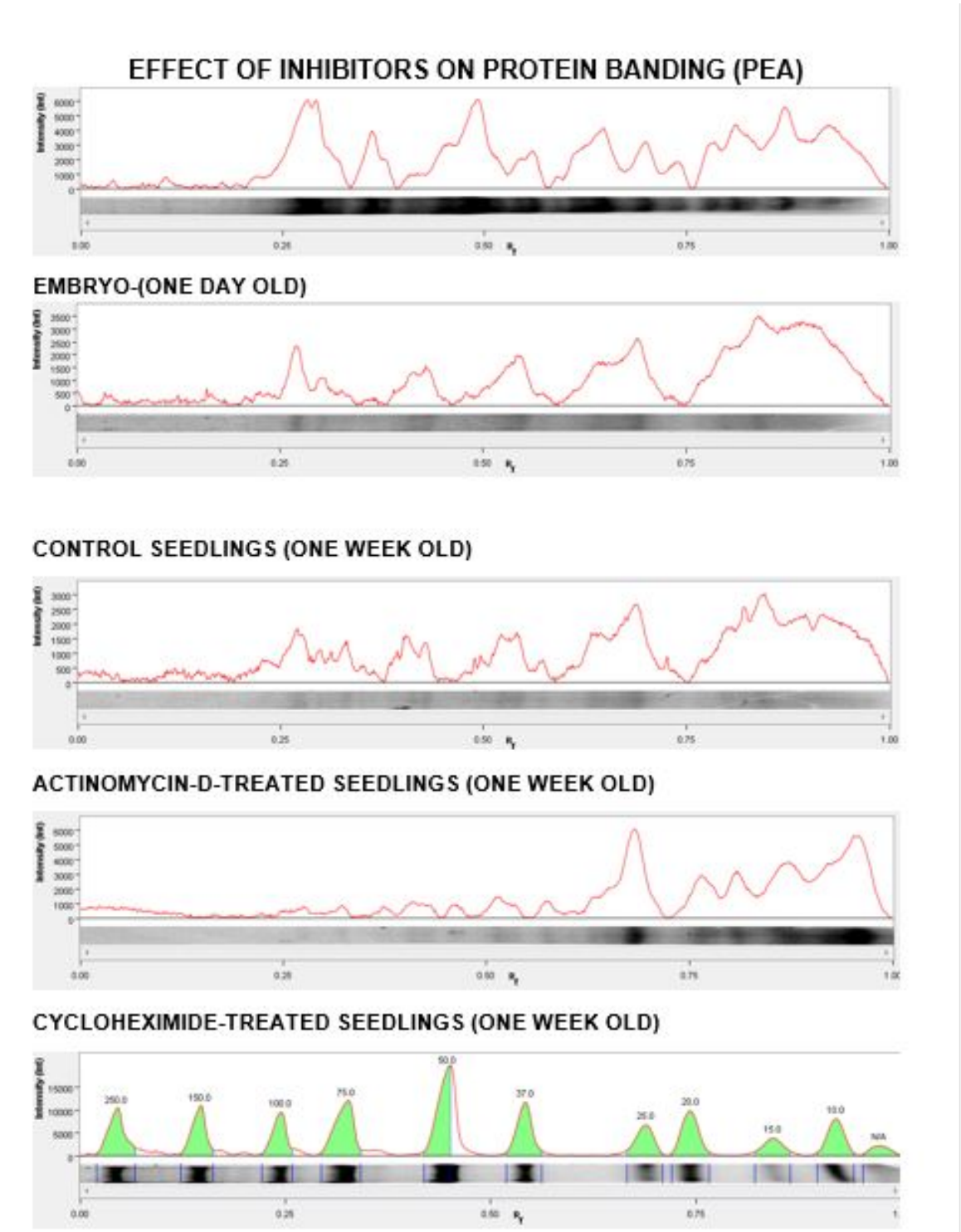

Figure 6. A graphic representation of protein banding in one-week old pea seedlings previously exposed to inhibitors. Note the similarity in pattern between controls and ActD exposed; there is less between controls and cycloheximide-exposed. Molecular weight standard on bottom.

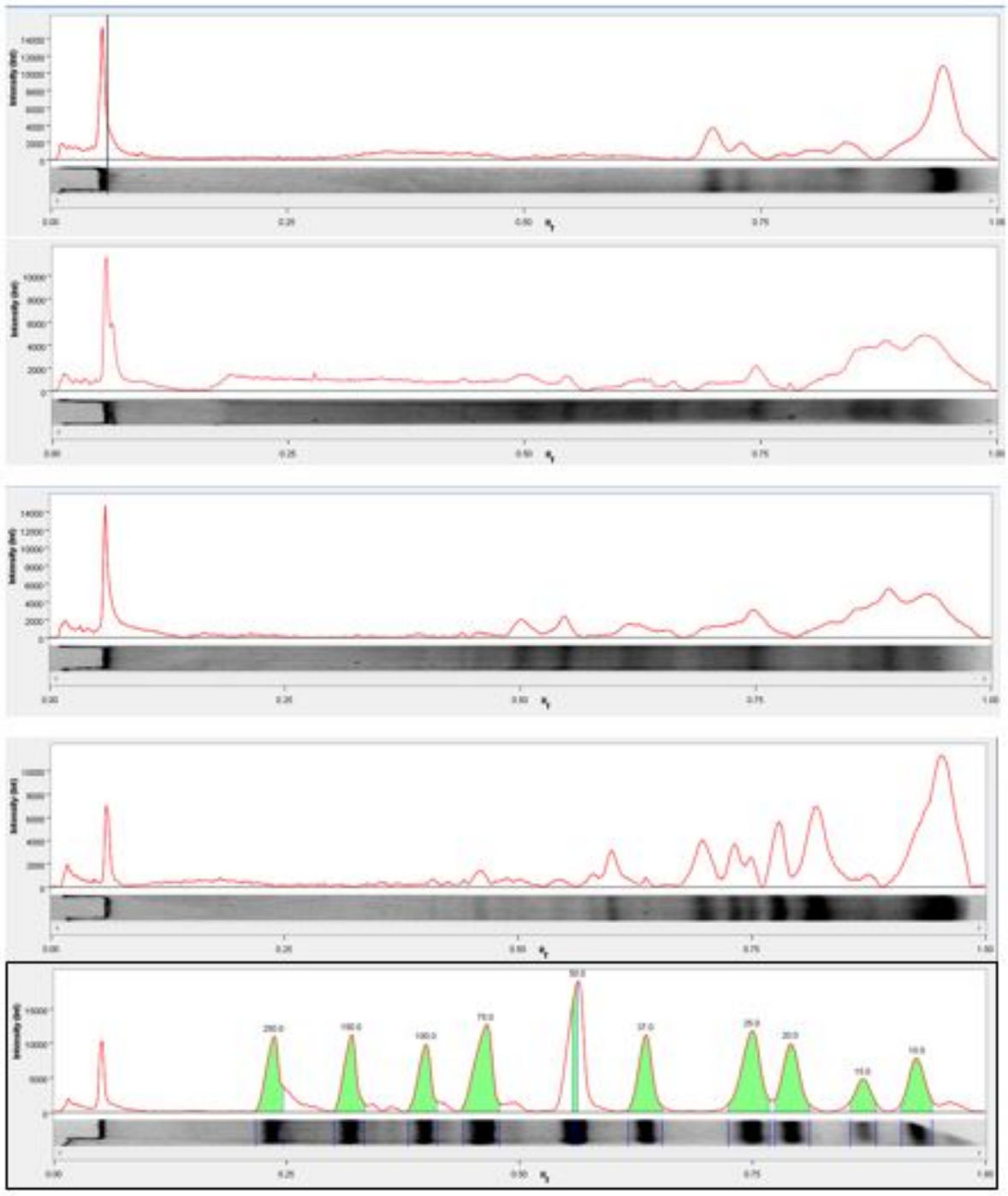

Figure 7. A graphic representation of protein banding in one-week old cabbage seedlings. Note the similarity in pattern between controls and ActD-exposed and the lack thereof between controls and cycloheximide specimens. Top to bottom panels: Embryo (1 d); Control (1 wk); Actinomycin (1 wk); Cycloheximide (1 wk) Molecular weight marker on bottom.

Conclusions

In peas, cabbage and seedlings, the range of expressed protein during the first three weeks of development remains stable for any one plant member (Figure 4 and 5). This may at first suggest that the RNA transcripts show stability.

If one poisons the pea and broccoli seedlings with a transcriptional inhibitor (e.g. actinomycin D), protein band fingerprint during the first two weeks is similar to development of controls, suggesting that the RNA transcripts were preformed maternally. During the third week, there is a loss of protein banding for the pea seedlings, indicating that actinomycin D may be inhibiting both embryonic transcription as well as the production of new proteins *de novo*. At the same time, extant preformed proteins or those made from maternal transcripts stored in the cytoplasm may be degraded. If this is the case, we suggest the existence of maternal zygotic transition (MZT) during the third week of germination for certain plants, such as pea, but not for others in the *Brassicaceae.*

If one poisons the pea and cabbage seeds with a translational inhibitor, like cycloheximide, very few of the seeds will germinate. In one week old poisoned embryos, the expressed proteins (greater than 25kD) do not resemble the scans of embryos or control seedlings. In addition, there is an increased supply of low molecular proteins (less than 25kD).

Acknowledgments

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