Spring 4-26-2014

The Development of Polyamines throughout Brassica rapa over its Lifecycle

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The Development of Polyamines throughout *Brassica rapa* over its Lifecycle

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Abstract

Polyamines are naturally produced chemicals in plants involved in growth, development and stress response. The primary objective of my study is to create a profile of changes in the entire life of the plant, in every organ at all stages of development from seed germination to seed formation. We have analyzed polyamines putrescine, spermidine and spermine in all parts of *Brassica rapa*, a small, rapid growing plant. Parallel to the polyamines, we will also study changes in the activities of the polyamine biosynthetic enzymes and the expression of their genes in different organs at different times. In the next stage of the study, the expression of selected genes will be inhibited by RNAi constructs, allowing further analysis of their role in growth and stress response. Because polyamines play are important in development and lifecycle of plants, altering their presence may be useful in altering plant growth patterns, such as in seasonal crops.

Materials and Methods

Growing of plants

- Seeds of a rapid cycling plant *Brassica rapa* L. (genotype Aaa; Rcb) were obtained from the Crucifer Genetics Cooperative, University of Wisconsin, Madison, WI or from Carolina Biologicals.
- Seeds were germinated in styrofoam quads, petri dishes or plastic pots. Each contained soil mixed with fertilizer pellets from Carolina Biologicals.
- Three seeds were planted in each cell of a quad and covered with soil. The completed quads were watered from below in plastic trays.

Polyamine analysis

- Collection of tissue for polyamine analysis used a fixed ratio of 1:4 w:v of tissue to 5% perchloric acid (PCA).
- Tissues collected, included whole dry seeds, germinating seeds, cotyledons, first and second leaves, stems, and roots at different stages of growth and development. Three replicates were taken of each sample.
- The amount of tissue collected varied from 25 to 200 mg in 100 to 800 µl of PCA in 1.5 ml microfuge tubes.
- The samples were stored frozen at -20°C until the time of polyamine
- Polyamines were extracted from the tissues by repeated (3X) freezing and thawing and centrifugation at 13,500xg.
- The supernatant fraction was prepared for dansylation by addition of 0.04 mM (final concentration) heptanediamine that acted as an internal standard for quantification of polyamines.[3]
- Polyamines were quantified by High Performance Liquid Chromatography (HPLC). Results presented here are representative of two or three analysis.[4]

Current plant growing and sample collection follows a similar method, using HPLC analysis. However, additional steps will be taken to identify enzyme activity, and later to assess affects of inhibiting polyamine formation.

Acknowledgments

I express significant thanks to Dr. Subhash Minocha for taking me on in my last undergraduate semester and guiding me through this project. I appreciate all the time given to helping me through this research. I would also like to thank Jon Larsen for initiating this project and compiling most of the data presented here.

Introduction

Polyamines

- Polyamines are present in eukaryotes and most prokaryotes
- The most common plant polyamines are putrescine, spermidine and spermine. Putrescine is the simplest, and first to form.
- The biosynthesis of putrescine involves either the decarboxylation of ornithine or arginine.
- Putrescine is metabolized into spermidine and spermine by addition of aminopropyl groups.
- Ornithine decarboxylase (ODC), arginine decarboxylase (ADC) and S-adenosylmethionine decarboxylase (SAMDC) are key regulatory enzymes in the plant polyamine biosynthetic pathway.[1]

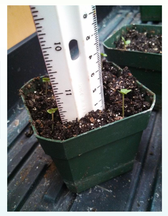
Current Research

- Polyamines presence varies during development and with environmental stimuli.[2]
- Polyamine development corresponds with ADC, ODC and SAMDC development; the extent of this association is under investigation.

Wisconsin Fast Plant

- *Brassica rapa* (Wisconsin Fast Plant) is rapid cycling plant, reaching senescence within 50 days
- Seeds germinate in under 24 hours
- Ideal growth when exposed to 24-hour light in controlled temperature environment.

Objective: This study aims to identify the changes in cellular polyamines and their biosynthetic enzymes in all parts of a plant, from seed germination to maturity and senescence. Two factors that made this study feasible are the short life cycle of *B. rapa*, and use of High Performance Liquid Chromatography.



***B. rapa* 4 days after planting**

***B. rapa* after 13 days of growth**

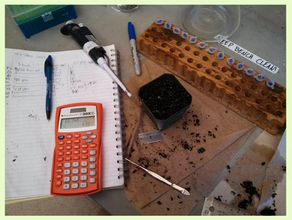
***B. rapa* after 25 days of growth**

Discussion and Current Direction

- From the data collected it has been shown that the polyamines change significantly during the development of different plant organs.
- The levels started off very high in most of the tissues and dropped as the tissues matured and developed more.
- Different plant organs vary widely in the cellular content of the three common polyamines.
- In developing flowers there were also varying polyamine levels in the different flower components (data not shown).
- Polyamine concentration in each organ seems to have a strong correlation to development of different organs. Stem and root polyamines seem to peak around time of flower blooming. As this is an essential stage in reproduction, the increased polyamines may be present to promote growth and environmental stress resistance.
- This research will aid in understanding the physiological functions of polyamines in the life of a plant, which is the primary goal of the Minocha lab research.
- Current research seeks to advance knowledge about enzyme activities for the biosynthesis polyamines, and the expression of genes related to biosynthesis.
- The planned next step is to inhibit each of the relevant genes through RNAi to see the effect of down-regulation of polyamine biosynthesis at various stages of development in the plant.
- Such alterations would allow control over plant maturation and thus flower and fruit production. Controlling this timing could be useful in agricultural development.

Results

- Total spermidine and putrescine increased over time in seedlings while spermine remained low. Spermidine showed the greatest presence **(Figure 1).**
- In cotyledons, from days 1 to 10 spermidine levels rose sharply, then gradually declined **(Figure 2)**. Putrecine fluctuated but remained relatively low, and spermine was not present in significant amounts.
- Similar to cotyledons, first leaves (**Figure 3)** and all subsequent true leaves had exceptionally high spermidine at time of leaf formation (approx. day 12 for first leaves), and decreased till near senescence.
- Around day 48 all polyamine levels rose again slightly **(Figure 3)**. This pattern was similar to the polyamine development in 2nd and presumably subsequent leaves.
- The stems **(Figure 4)** showed an early increase of all polyamines (1-2 d after the appearance of stem).
- A sharp decline followed by day 5 and another peak of high polyamines around 7-8 d, and again around 20 days. Spermidine had by far the highest presence, and spermine again had the lowest.
- In roots, spermidine and putricine showed Initially higher levels which led to a drop around day 10 and then a peak in all polyamines around day 22 **(Figure 5)**.
- This was followed by a somewhat smaller peak around day 40 and another increase when the leaves were showing signs of senescence. Spermidine was the most abundant polyamine, and spermine was again almost absent.



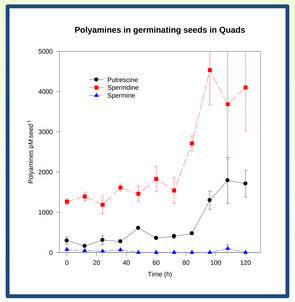


Figure 1
Cellular polyamine content of germinating seeds on a per seed basis.

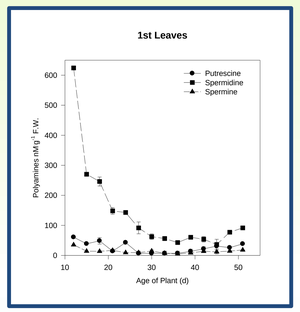


Figure 3
Polyamine content of first true leaves.

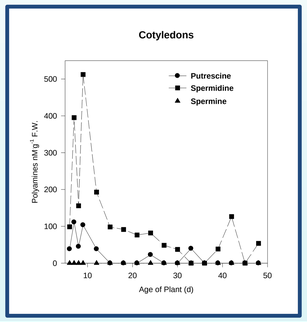


Figure 2
Polyamine content in cotyledons of *B. rapa* over 50 days

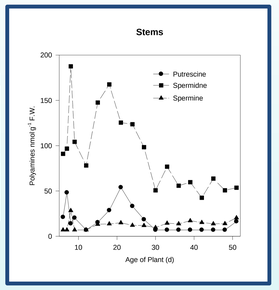


Figure 4
Polyamine content of stems.

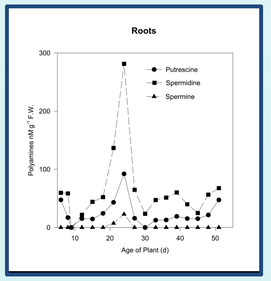


Figure 5
Polyamine content of the roots.

Reference

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