Auxin activity and root development in phosphate-starved and phosphate-sufficient *Nicotiana plumbaginifolia* seedlings

By
Hannah Briggs
April 2nd, 2012



Allegheny College
Department of Biology

Auxin activity and root development in phosphate-starved and phosphate-sufficient *Nicotiana plumbaginifolia* seedlings

By
Hannah Briggs
April 2nd, 2012

A Senior Comprehensive Project in Partial Fulfillment of the Requirements for a Bachelor of Science Degree from Allegheny College

I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code and to maintain the integrity of both myself and the College community as a whole.

_____
Pledge

Approval by the Senior Thesis Committee:

_____
Dr. Catharina Coenen, First Reader

_____
Dr. Tricia Humphreys, Second Reader

Table of Contents

Acknowledgements ……..……..4

Abstract…….…….....5

Introduction…….……..….5

Methods ……... ……...10

Results …….……...…….12

Discussion ……...…….....21

Literature Cited ....……..…..28

Appendix ...……..30

Acknowledgements

Dr. Coenen- A tremendous thank you for your extreme patience and understanding throughout the comp process! I would have been lost without your knowledge and guidance yet somehow you always made me feel like I was 'on top of my game'. Thanks for being a wonderful teacher and advisor!

Dr. Humphreys- Although I did not consult you much during the process, I do appreciate you taking the time to actually *read* my comp. I realize that you will probably be one of the few so thank you!

Allegheny 1939 Fund- Thanks for providing the financial backing!

Alexander Berry- Thank you for listening to my rants and *always* assuring me that everything will work out.

Mom and Dad- Your suggestions and edits were enormously helpful along the way, especially at the end. Not everyone's parents would help out like that, especially on short notice. I am immensely thankful for everything you do for me.

Meaghan- Thanks for being a wonderful friend, before during and after this comp season.

Michelle- Long dog-watching strolls around Allegheny and Robertson were the perfect ticket for a clear, happy mind. Thanks for always being up for a walk!

Coenen Comp Group- We made it to the end- congrats! Thanks for critiques, commiseration and encouragement and along the way.

Abstract

Phosphate is an essential nutrient for plants. The model plant *Arabidopsis thaliana* demonstrates a phosphate starvation response characterized by a shortened primary root and increased lateral root growth. This response is dependent on the plant hormone auxin. Auxin-responsive gene activity in *Arabidopsis* lateral root primordia oscillates with a six hour period, but phosphate effects on this oscillation have not been investigated. This study assessed phosphate-dependent auxin activity in roots of *Nicotiana plumbaginifolia* seedlings transformed with a *GH3::Luciferase* promoter::reporter gene construct. This construct uses the gene that codes for luciferase as a reporter for auxin-inducible *GH3* promoter activity: when auxin is active, the corresponding region of the plant will emit photons, or glow. This glow can be captured by luminescent photography. Changes in auxin activity were recorded in seedlings growing in phosphate-starved and phosphate-sufficient conditions. *N. plumbaginifolia* seedlings failed to exhibit a consistent phosphate starvation response *in vivo*. Auxin activity of a limited number of *N. plumbaginifolia* roots oscillated similar to *A. thaliana*, but with a longer period; however, this pattern was not repeatable. The role of auxin oscillations in determining species-specific differences in root architecture requires further investigation in plants with disparate root systems and phosphate starvation responses.

Introduction

Plants, like all forms of life, need phosphorus (P) to survive. P is a key element in the building of nucleic acids and phospholipids, as well as certain metabolic reactions that allow for growth (Schachtman et al., 1998). Although some form of P is present in most soil types, in both acidic and alkaline soil it can bind with cations to form insoluble complexes (Peret et al., 2011).

Plants are require soluble P and are unable to use these insoluble complexes. If there is not enough soluble P available plants change their metabolic activity and growth pattern into a 'P-starvation mode' (Ticconi et al., 2004; Desnos, 2008; Lin et al., 2009; Rouached et al., 2010). The goal of P-starvation mode is to reduce overall P use, recycle present P and re-aliquot present P to structures and processes that may acquire additional P. Plants in P-starvation mode exhibit an increase in the root to shoot ratio, often because of increased lateral root formation and growth, and increased root hair density and length (Desnos, 2008; Peret et al., 2011). The root to shoot ratio can be altered by restrained growth of the primary root meristem and stimulated growth and density of lateral roots and root hairs. New root hairs increase the root surface area and the plant's ability to encounter and absorb P (Desnos, 2008; Fang et al., 2009).

Plant growth is highly associated with the phytohormone auxin. Auxin regulates various cellular processes, including cell division, growth, and cell differentiation (Guilfoyle, et al., 1998). As a result of these activities auxin influences phototropism, apical dominance and growth and development of primary and lateral roots (Casimireo et al., 2001; Guilfoyle, et al., 1998; Peret et al., 2009). Auxin is synthesized through the action of one or several proteins. The amino acid tryptophan is the precursor to the most common form of auxin, indole-3-acetic acid (IAA), to which the remainder of this paper will refer. After synthesis, auxin is distributed from the shoot to roots by the active, unidirectional process of polar transport (Taiz and Zeiger 2006; Goldsmith, 1977). Once in the root, auxin can move from the base to the end of the root in an acropetal fashion and from the tip of the root back toward the base in a basipetal direction (Muday and Delong, 2001). Auxin moves into cells by diffusion or using a proton symport mechanism and out of cells by moving down its electrochemical gradient using efflux carrier

proteins (Taiz and Zeiger 2006; Wargo, 2011). Auxin distribution is a consequence of both synthesis and transport processes.

Although changes in lateral root development in low P conditions could be mediated by changes in auxin synthesis or allocation, evidence suggests that this response coordinates with changes in auxin sensitivity (Perez-Torres et al., 2008). Perez-Torres and colleagues used the genetic model plant *Arabidopsis thaliana*, to find that low phosphate conditions altered the expression of the auxin receptor Transport Inhibitor Response1 (TIR1). Auxin interacts with TIR1 to alter repressors and release auxin response families (ARFs) of transcription factors. Increased TIR1 expression was found in P-starved conditions, which allowed for greater alteration of repressors by existing auxin levels. In turn this released ARFs and stimulated proliferation of pericycle cells and lateral growth. Low P conditions may trigger auxin responses by changing the sensitivity to existing levels of auxin by increasing expression of the auxin receptor TIR1, not by changing levels of auxin (Perez-Torres et al., 2008; Nacry et al., 2005).

The majority of experimental studies on P starvation responses and auxin activity have been performed in *Arabidopsis thaliana*. The experiment outlined here studies these processes in a different model organism: Tex-mex tobacco, *Nicotiana plumbaginifolia*. *N. plumbaginifolia* is an appropriate organism to study root development because it's relatively fast growing and its seedlings develop leaves in rosettes; these attributes ensure that the seedlings remain small and may be studied *in vitro* for several weeks (Wargo, 2011).

The *GH3* gene family is used frequently for studying auxin because genes in this family respond very quickly to changes in auxin activity. This quick response time allows genes in the *GH3* gene family to be called auxin early response genes (Ruijter et al. 2003). Several reporter gene constructs have been made to take advantage of this early response to changes in auxin,

including the *GH3::Luciferase* promoter::reporter gene construct. In the *GH3::Luciferase* construct, expression of the luciferase gene is used as a reporter for *GH3* promoter activity. Transcription and translation of luciferase occurs within minutes after activation of the *GH3* promoter (Ruijter et al. 2003; Wargo, 2011). Luciferase breaks down its substrate luciferin to emit photons and luminesce, as seen in fireflies. When *GH3* is active and luciferin is provided, photons will be emitted by luciferase-catalyzed oxidation of luciferin and the area of auxin activity will glow. This luminescence can be captured by a camera sensitive to low light. The amount of glow can then be quantified to find the relative amount of auxin activity at that location (D'Amico, 2011; Wargo, 2011). This method allows for relatively easy real-time monitoring of auxin activity.

Areas where the *GH3* promoter is active represent areas where auxin is present and able to initiate one or several of the functions mentioned above. For instance, auxin regulates phototropism and gravitrophism and *GH3* is active on the bottom and dark sides of shoots (Li et al., 1999). Auxin is also necessary for plant growth and *GH3* was found to be active at regions of apical roots and lateral roots when dormant and throughout their development. Additionally, auxin activity is high at the ends of adventitious roots, which can develop in response to exogenously applied auxin.

Use of the *GH3::Luciferase* promoter::reporter gene construct has certain advantages over other available reporter constructs. Limited research using this construct indicates that the level of auxin activity changes, or pulses, throughout a period of growth and that auxin activity varies depending on the location of growth (Heisler et al., 2005; Laskowski et al., 2008; Moreno-Risueno et al., 2010). The *GH3::Luc* construct can detect the above fluctuations in auxin activity while other constructs cannot. Previous studies on *GH3* promoter activity have used *GH3::GUS*

reporter gene construct. The *GH3::GUS* construct codes for the enzyme beta-glucuronidase. When the construct is active and auxin is present, beta-glucuronidase converts a substrate to an insoluble blue dye (Ruijter et al. 2003). This dye is unable to fade or change over time so although the *GH3::GUS* construct accurately displays auxin activity at the moment of the assay, it is insensitive to changes in auxin activity. By contrast, the *GH3::Luc* construct is sensitive to changes in auxin activity because both the luciferase enzyme and its mRNA are unstable. Luciferase mRNA readily degrades and the luciferase enzyme is permanently inactivated during luciferase oxidation (Ruijter et al. 2003). Therefore, the glow produced when using *GH3::Luc* corresponds to an active *GH3* promoter at that time. This suggests that the *GH3::Luc* construct can be used to analyze changes in auxin activity over a period of time.

The genes that code for auxin were found to oscillate with a regular pattern in *A. thaliana* (Moreno-Risueno et al. 2010). Auxin encoding genes in the elongation zone of the apical root pulsed with a period of approximately six hours. This oscillation provided a regular opportunity for lateral root formation by production of a prebranch site. Whether or not a lateral root developed from the prebranch site was dependent on the environmental conditions. Exogenous applications of auxin were able to increase lateral root growth; however they were unable to increased lateral root formation (Moreno-Risueno et al. 2010). This study suggested that the oscillation of auxin encoding genes produced an element, in addition to auxin, that was essential to lateral root formation.

My objective was to observe and quantify changes in auxin activity and root development in *N. plumbaginifolia* seedlings subjected to varied P and sucrose conditions. In attempt to elicit a P starvation response, the growth of tobacco seedlings was compared between P-sufficient, P-starvation and P-insoluble media. Insoluble P in the form of calcium phosphate was used to see

if *N. plumbaginifolia* can solubilize P on its own and to mirror development of the P-sufficient condition. Observation of P starvation responses in the laboratory often requires supplementation with a carbohydrate, such as sucrose, because photosynthetic activity is much lower in the incubator as compared to plants grown in the sunlight. Auxin activity, indicated by the *GH3::Luc* construct, was also recorded in the above conditions. In sum, I observed root growth patterns and auxin activity in *N. plumbaginifolia* seedlings grown under varying P and sucrose conditions.

Methods

Plant culture

Nicotiana plumbaginifolia seeds that contained the auxin-inducible *GH3::Luciferase* promoter reporter gene construct (Brazelton et al. 2008) were stored in a 4˚C refrigerator. To induce germination, seeds were sterilized with 35% Clorox bleach solution for 10 minutes, washed four times with sterile water and suspended in 1 mM gibberellic acid at 4˚C for one to two days (Brazelton, 2007; Wargo, 2011). Gibberellic acid was removed by washing the seeds four times with sterile water; seeds were plated on M-media with low P (10 µM KH_2PO_4) without organics on 100x15mm Petri dishes sealed with parafilm. Petri dishes were then placed in the incubator for 3-4 days with a 16 hours light/8 hours dark at 26˚C and 60% humidity to allow for germination. Germinated seeds were transferred to M-media with desired P and sucrose treatment at approximately 4 seedlings per plate. Plates were turned vertically to promote root growth down the diameter of the plate and placed back in the light/dark incubator under the same conditions. The plants were grown in the incubator for approximately 7 more days before initial

pictures were taken. Subsequent pictures were taken depending on the results on the initial photos.

Determination of auxin activity

N. plumbaginifolia seedlings were sprayed with a buffer solution (4mM luciferin, 100 mM potassium phosphate buffer (pH 6.5), sterile water). Each sample absorbed the buffer for approximately 10 minutes before it was imaged with the Kodak Image Station 4000MM Pro for luminescence. Settings were: CHEMI Ultra Sensitive, Standard Exposure for 7 minutes, 4x4 X:Y Binning, Luminescence, f-stop=2.51, Focal plane~10.52 mm, "Dana Protocol". Field of view was adjusted as necessary. Brightfield images were obtained by taking a screen shot of the plate while previewing the image. All images were viewed with set values for designation of black and white pixels (50 and 400, respectively); this ensured the criterion for black and white regions was uniform across samples. Areas of auxin activity were identified by creating 'auto ROI sets', which prompted the Image Station to automatically find regions of interest (ROI). ROIs were areas that had significantly higher luminescene than the background, and corresponded to regions in the plant where *GH3::Luc* was active. The Image Station provides a table that assigned quantitative measures to each ROI set (D'Amico, 2011). From the provided table, net intensity (the number of photons per pixel with the background light level subtracted out) was used as the standard to quantify and compare auxin activity.

In addition to luminescent photos, traditional photos were also taken with a Cannon Rebel Digital Camera.

Phosphorus and sucrose treatments

To study the effect of P starvation and observe whether sucrose was needed to elicit P-starvation effects, six varieties of M-media were prepared. Three P conditions were used: sufficient (1000 μM KH_2PO_4), insufficient (10 μM KH_2PO_4) and insoluble (1000 mM $Ca_3(PO_4)_2$) and each P condition was prepared with and without sucrose (3%) (Miura et. al, 2011; McGrogan, 2011). The final trial was run with two conditions, sufficient P with sucrose and insufficient P with sucrose, to increase number of replicates and decrease variability.

Time-course of auxin activity during root development

Germinated seeds were plated on sufficient P conditions without sucrose. To allow ample room for root development, two germinated seeds were plated per plate. Plants were allowed to grow for 4-5 days, then auxin activity was determined as described above for eight hours at approximately one hour intervals. Plants were re-sprayed with the luciferin buffer and allowed to absorb the buffer for ten minutes before each picture was taken. Luminescent and brightfield images were taken and total auxin activity was quantified by means of net intensity at each time point. Net intensity over time was also quantified for individual ROIs by use of their Y-position coordinate in the Image Station table.

Results

P-starvation Trials

The number of lateral roots and lateral root primordia were averaged for each plant (Fig 1). Variability within treatments was extremely high and no increased lateral root growth is evident in phosphate-starved plants.

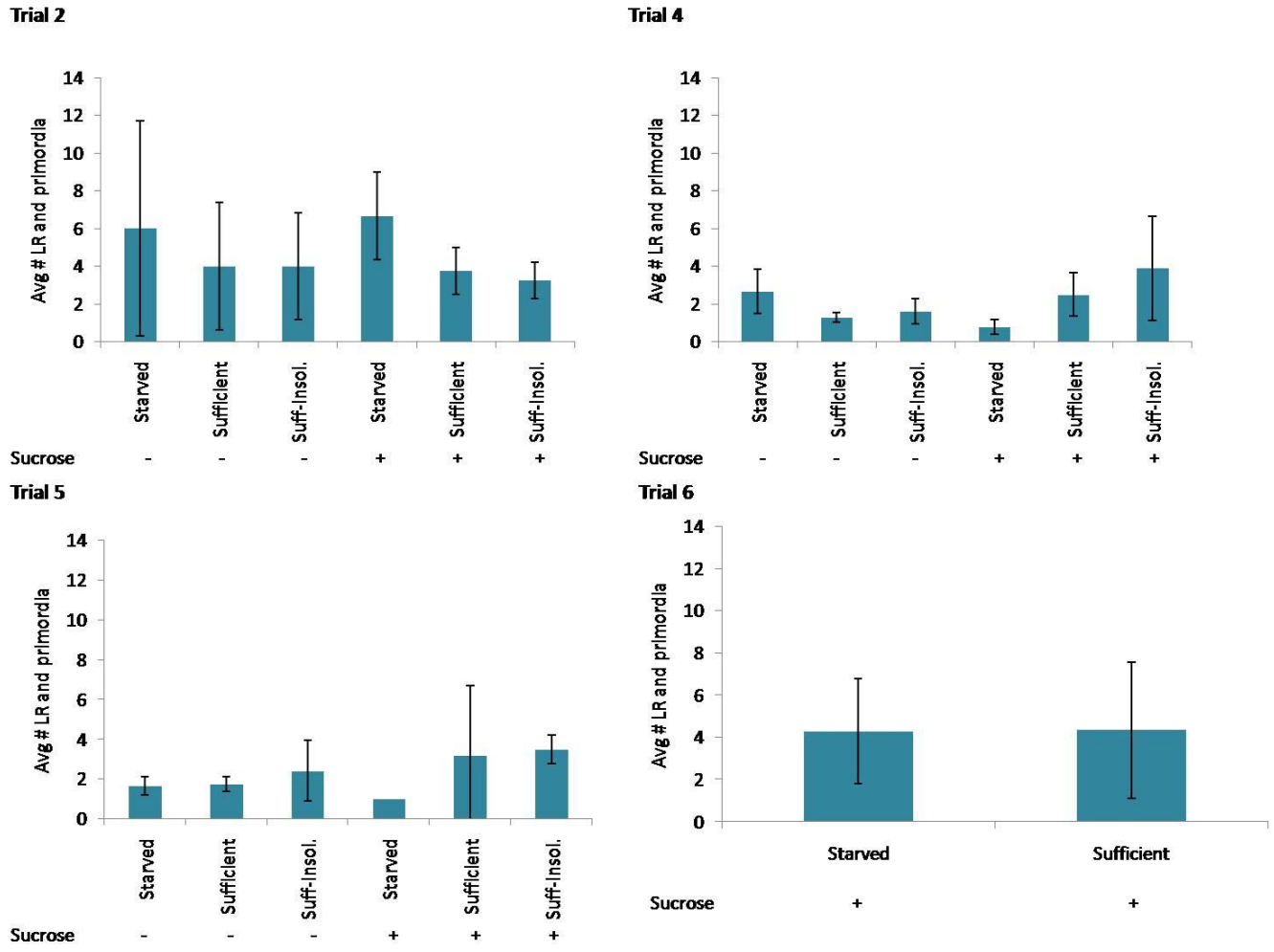

Figure 1: Combined average of lateral roots and lateral root primordia per plant. Seedlings were grown for 11, 8, 7 and 8 days in trials 2-6, respectively. Error bars represent standard deviation where N=4. No response to phosphate starvation is evident. Note that media for P-starved +S conditions in Trials 4 and 5 were deficient.

Sites of auxin activity in P-starved, P-sufficient and P-sufficient insoluble treatments are visible by luminescent photographs (Fig 2-5), where each photographs represents a condition. Note that contrast in luminescent photos was reversed so that dark areas correspond to glowing areas and areas where auxin is active. In some instances the plant did not glow at all (Fig 2, right arrow). The high variability within treatments due to plant vitality and development is visually evident by comparing replicate plates. Shorter, less developed seedlings had more condensed auxin

activity than more developed seedlings, which were longer with separate spots of auxin activity (Fig 2-5; circled on Fig 3-4). Spots of auxin activity often, but not always, occurred with lateral root formation or growth (Fig 3 arrows, 4-5). Seedlings grown with sucrose appear larger and more developed than seedlings grown without sucrose (Fig 3 A vs. B; Fig 4 C vs. D, G vs. H). In trials where two time points were taken, auxin activity appeared to decrease from the earlier to the later time point (Fig 4 A-C vs. D-H; Appendix Fig 2).

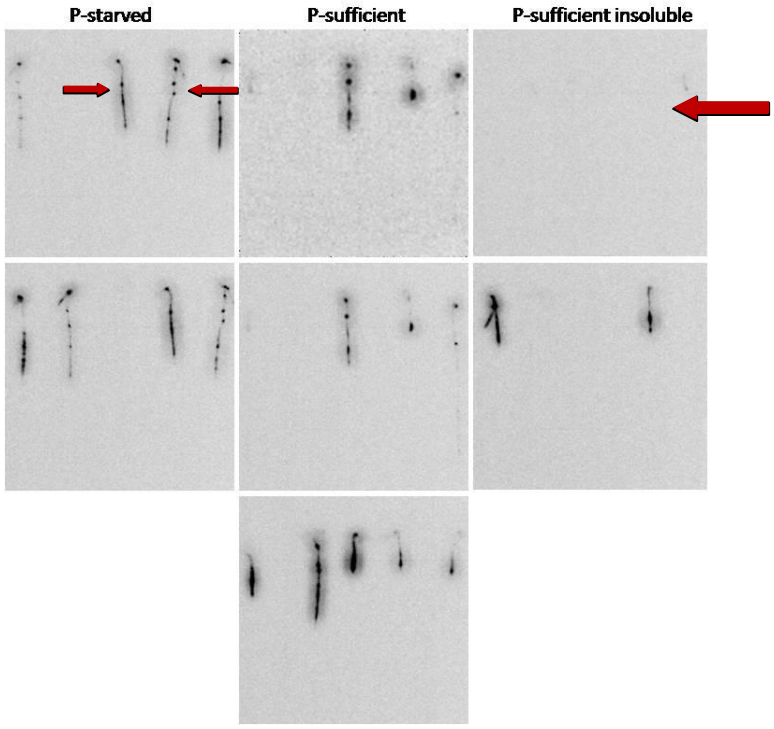

Figure 2: Luminescent photos indicating auxin activity in roots of six day old seedlings from Trial 1. Roots of less developed seedlings appear to be shorter with more condensed auxin activity than more developed seedlings, which are longer with spotted auxin activity (arrows comparing plants in top P-starved condition). Note variable plant vitality within and between treatments.

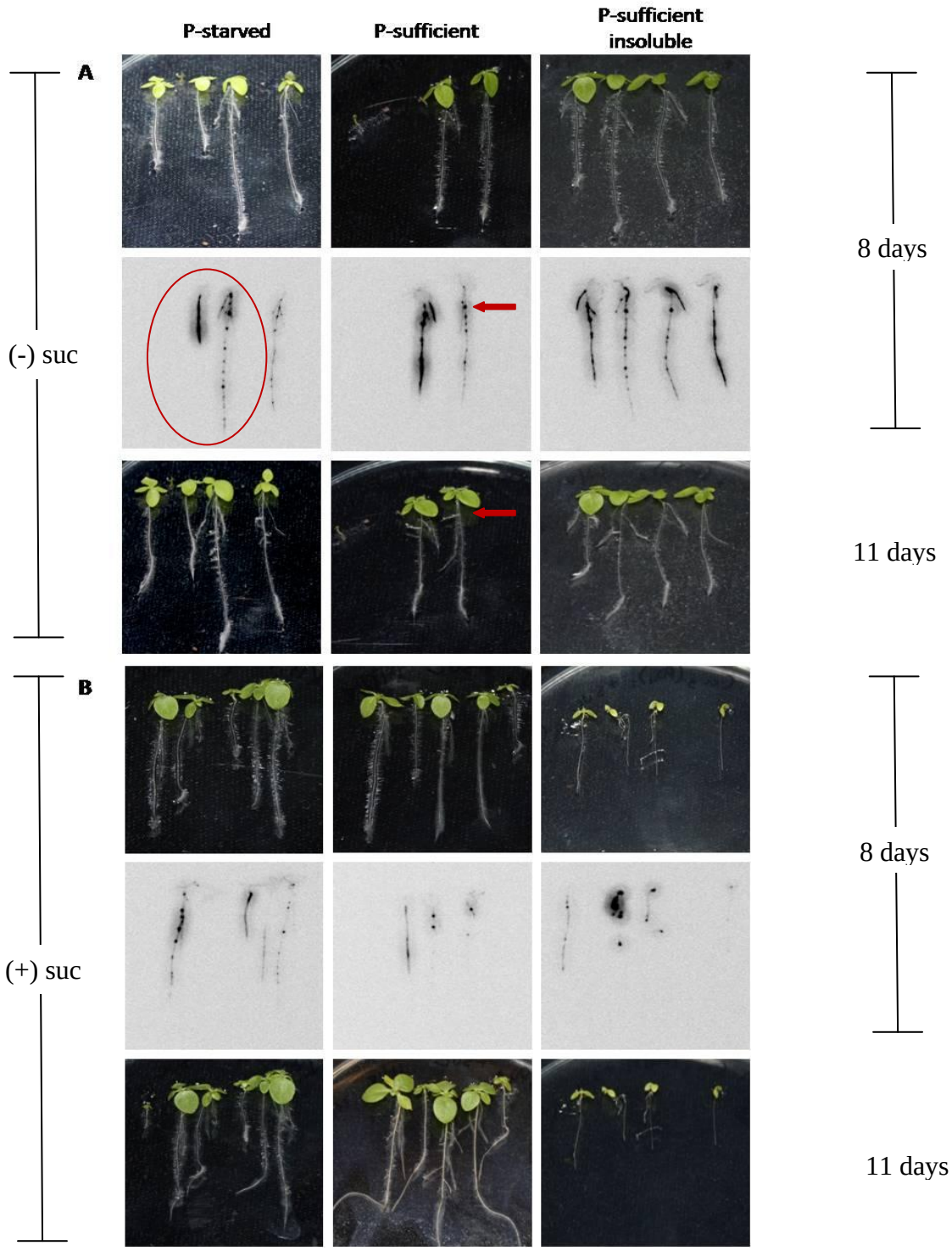

Figure 3: Luminescence and plant morphology from Trial 2. Presence or absence of sucrose is noted on the left while age of seedlings is indicated on the right. The P-sufficient insoluble media with sucrose was defective and resulted in poor growth of plants. Auxin activity is higher

after 8 days of growth as compared to eleven days of growth. Variation in plant development within treatments is greater than variation between treatments.

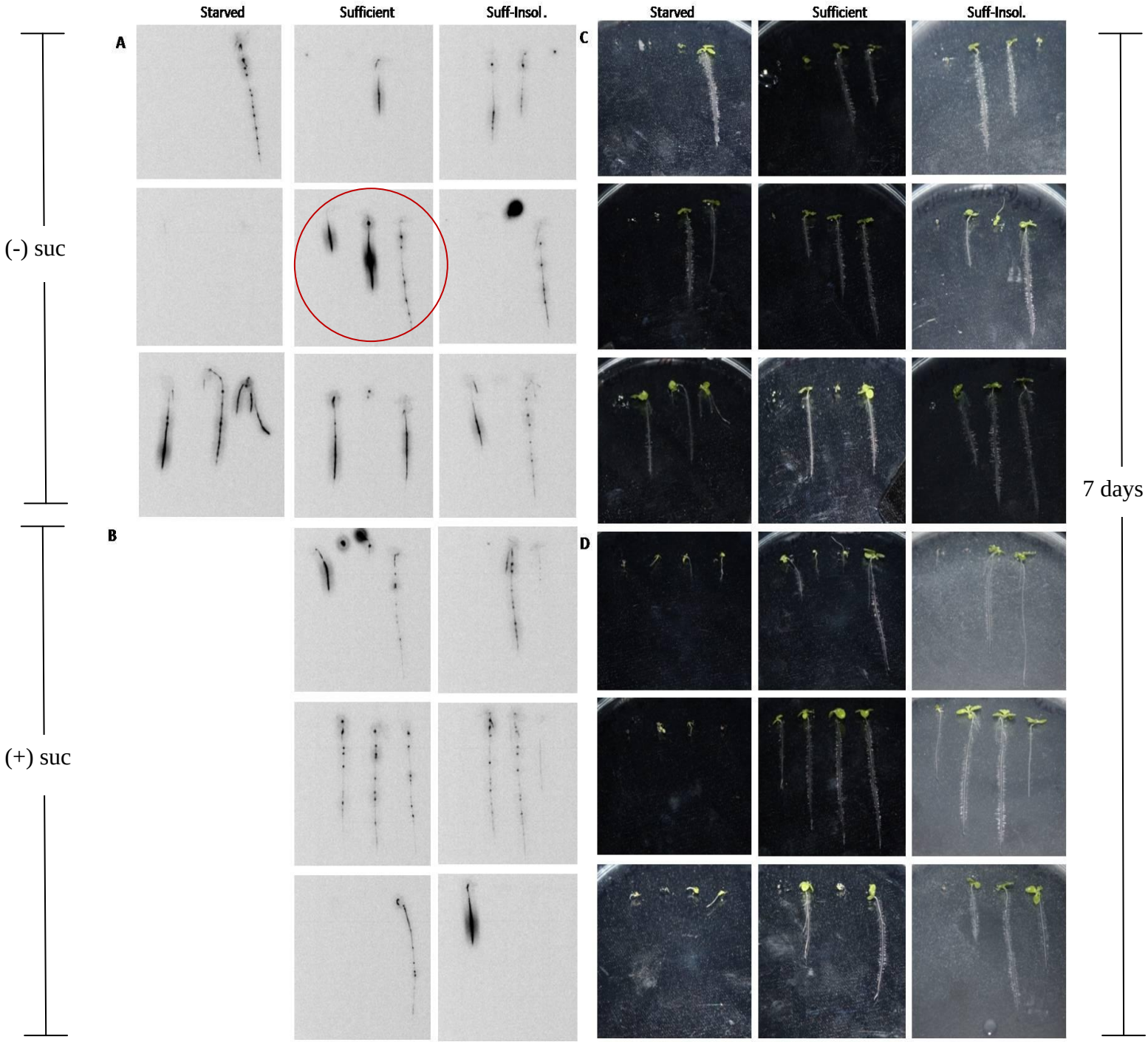

Figure 4 continued on next page.

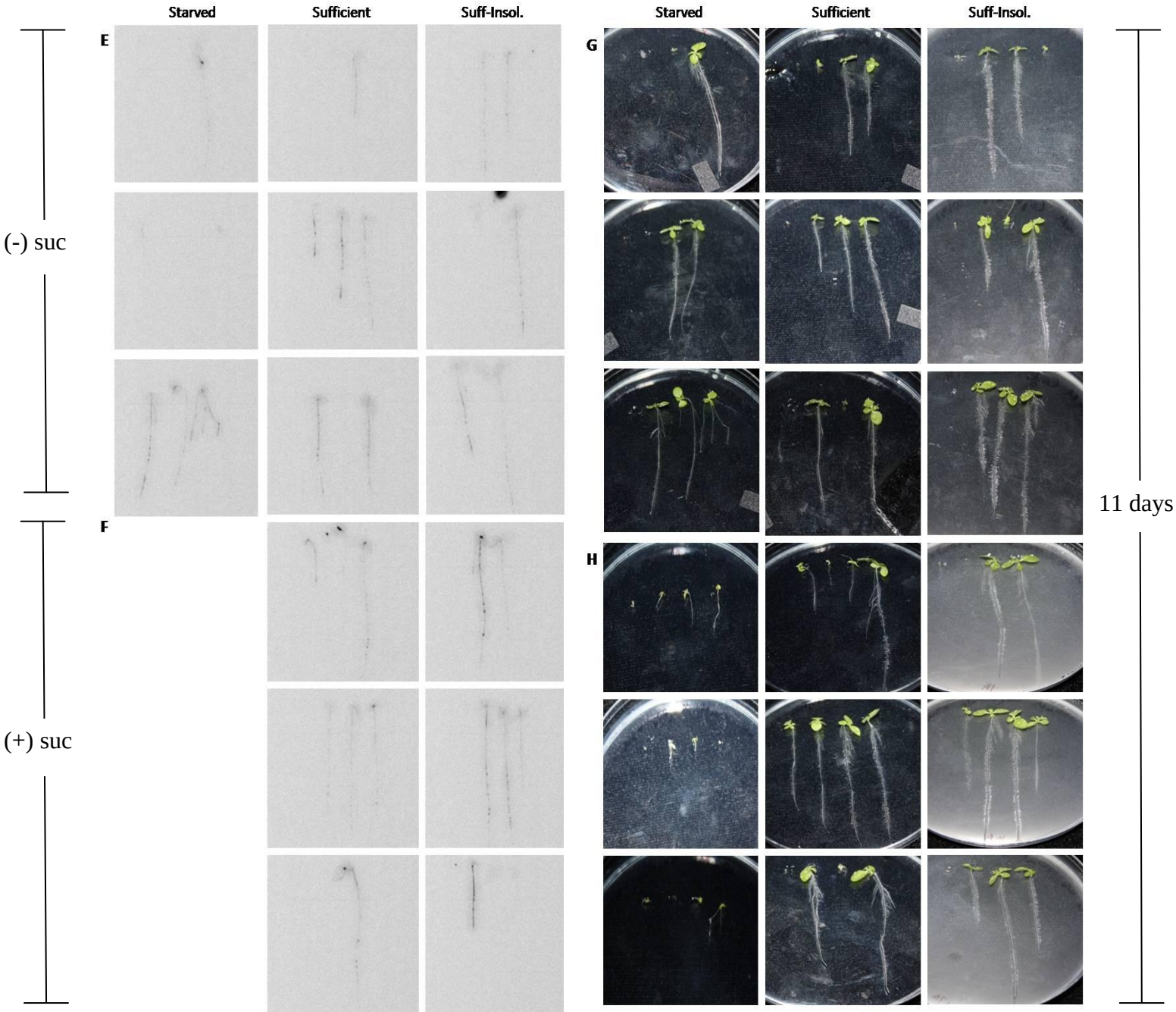

Figure 4: Luminescence and plant morphology from Trial 4. Presence or absence of sucrose is noted on the left while age of seedlings is indicated on the right. Media for P-starved condition with sucrose was defective so luminescent photos were omitted, and the traditional photos display unhealthy plants in this condition. Variability of the number of viable plants, the stage of development and the amount of auxin activity is greater between replicates than between treatments. Auxin activity, as indicated by darker regions in A and B compared to E and F, is higher in 7 day old seedlings than 10 days old seedlings.

In attempt to decrease variability, trial 6 was performed with only the P-starved and P-sufficient conditions, both containing sucrose. The number of viable plants (circled on Fig 5) and lateral roots (Fig 1D) remained highly variable.

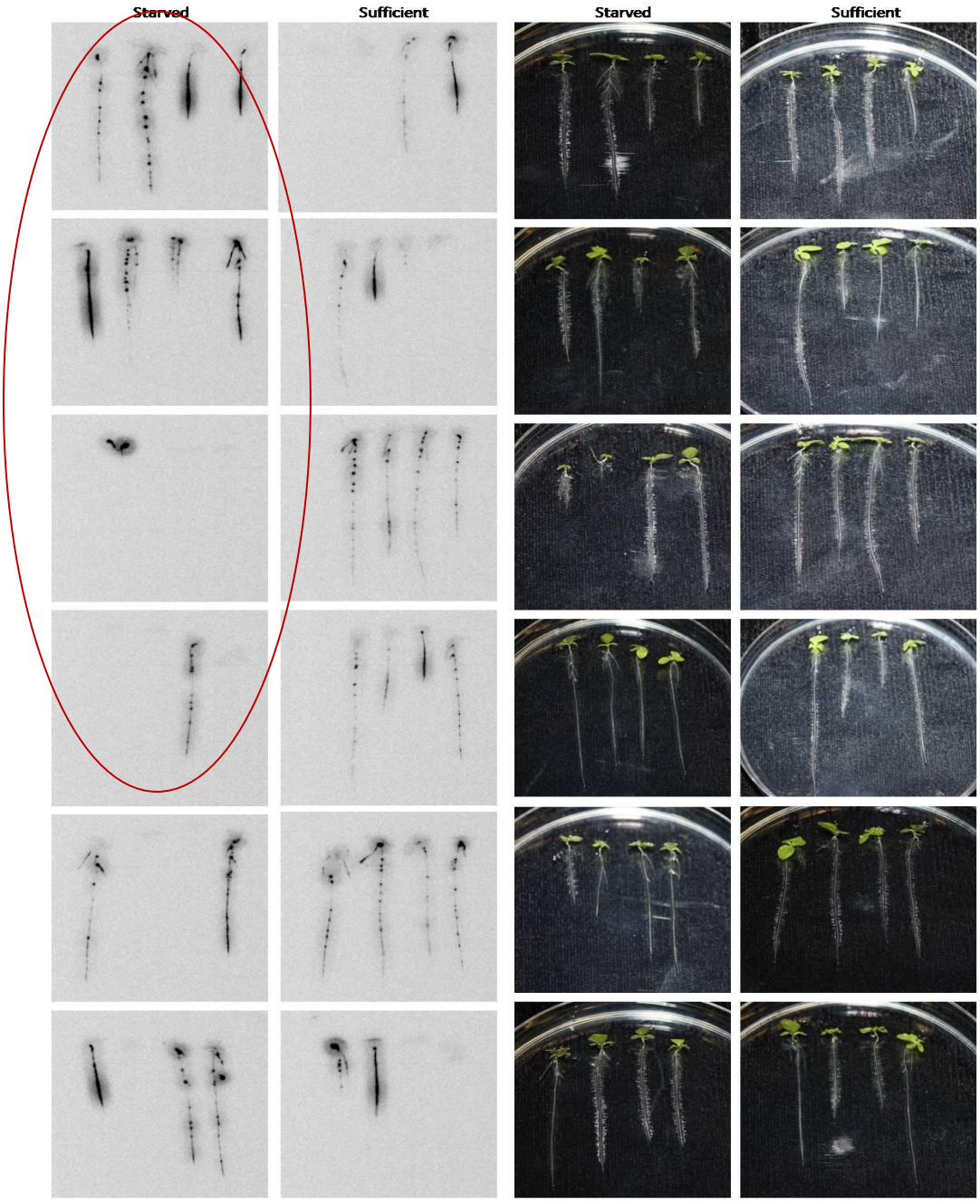

Figure 5: Comparison of luminescent and traditional photos of eight day old P-starved and P-sufficient seedlings grown with sucrose in Trial 6. High variability is present within treatments.

Changes in Auxin Activity Trials

Total auxin activity was measured by net intensity, as quantified by the Image Station, over a period of 8-12 hours. The first time point was eliminated because it did not accurately represent auxin activity due to the presence of excess luciferase. When observed over a period of eight hours total auxin activity decreased consistently without recovery (Fig 6). When observations were extended to 11 hours four seedlings displayed similar patterns of auxin decrease then increase (Fig 7A). All four of the seedling reached a baseline level of auxin activity near zero around hour four. Around hour six three of the seedling began show increased auxin activity while the fourth remained near the baseline. Results were variable in the eleven hour period: total auxin activity in some plants displayed hints of a cyclic nature while the auxin activity of other plants simply decreased without recovery (Fig 7B).

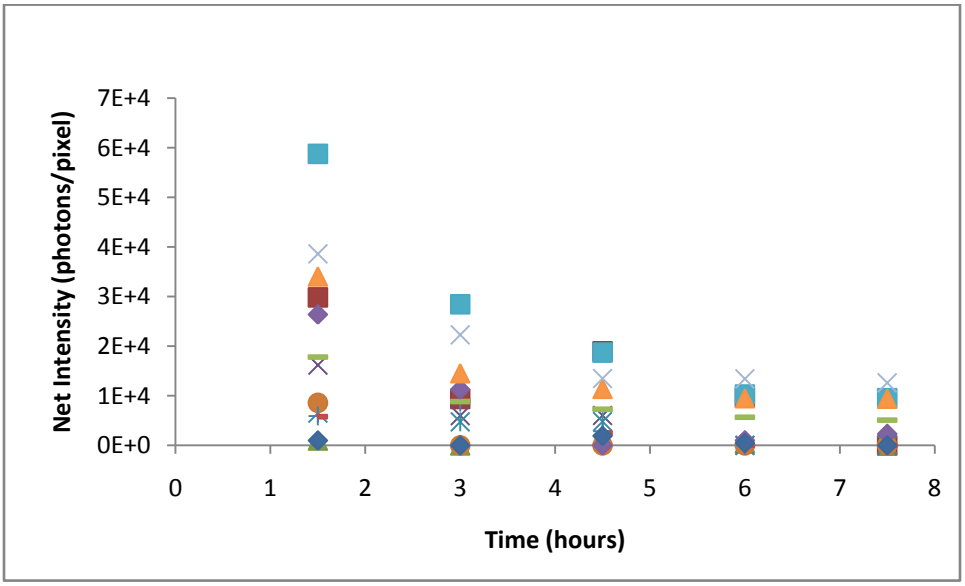

Figure 6: Total auxin activity, as measured by net intensity (total brightness per pixel – background brightness), uniformly decreased in five day old seedlings over 8 hours.

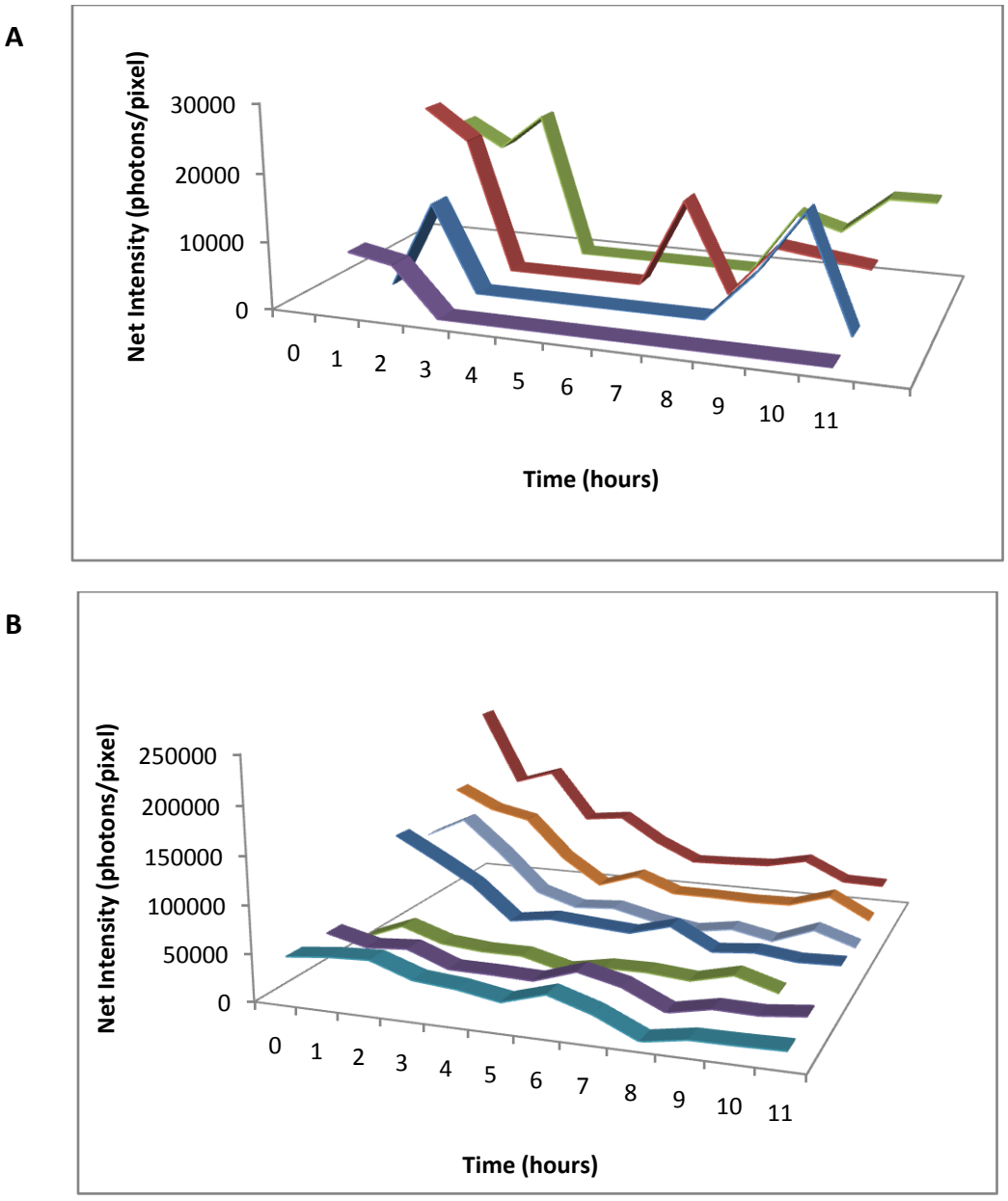

Figure 7: Total auxin activity, as measured by net intensity (total brightness per pixel – background brightness) of whole 8 day old seedlings' luminescence over 11 hours. **A.** Three of the four seedlings displayed a similar decrease then increase pattern. **B.** No clear pattern of auxin activity between seedlings.

Auxin activity was also quantified at specific regions of interest (ROIs) over time. Results were quite variable between plants, with assorted start points, end points and patterns in the middle (Fig 9).

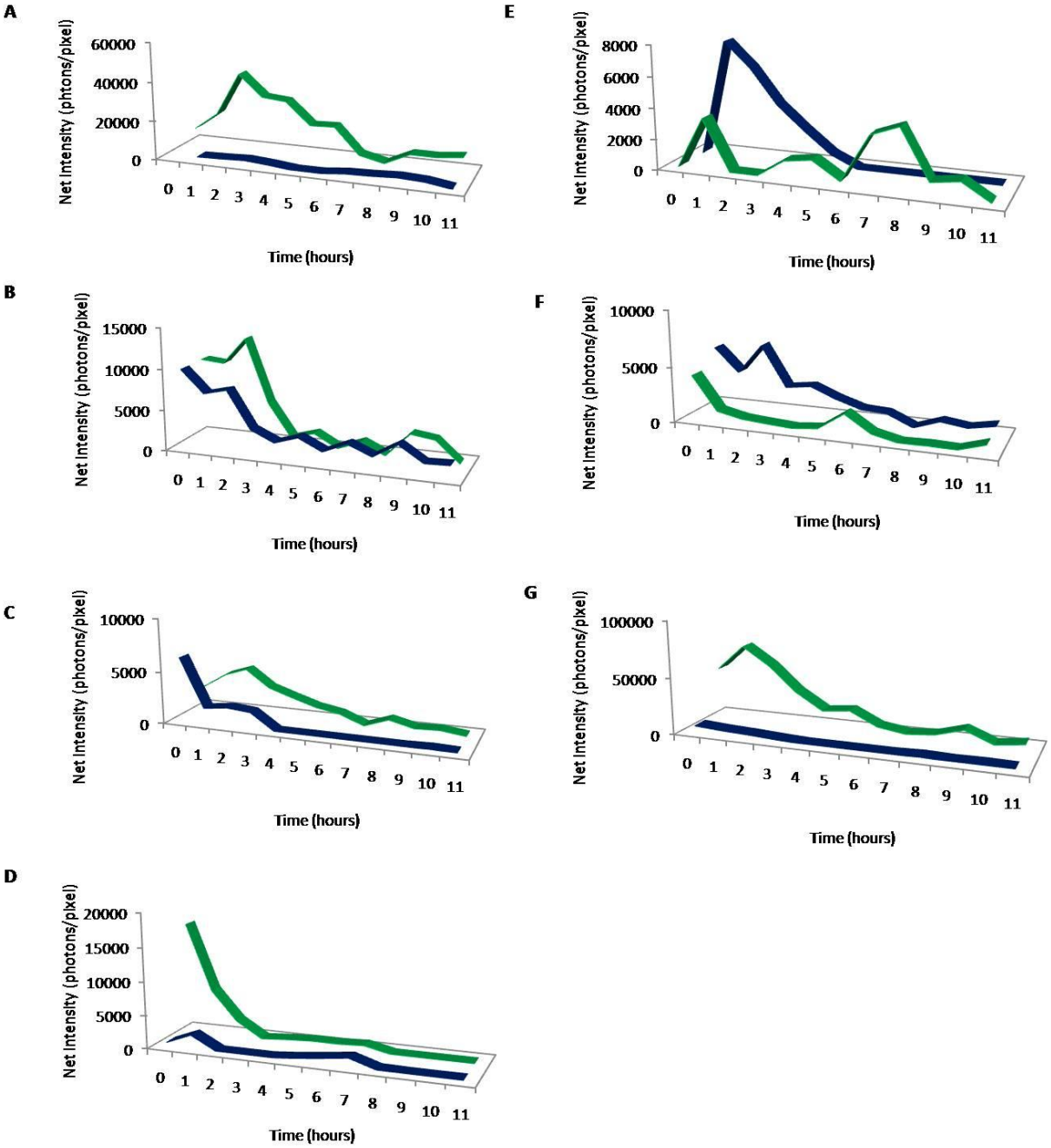

Figure 8: Variable auxin activity of specific ROIs (opposed to total auxin activity) over a period of 11 hours. Each graph represents a different plant and each line represents the changes in auxin activity at a specific region over 11 hours.

Discussion

The P starvation response of *Arabidopsis thaliana* includes changes in the root to shoot ratio, increased lateral root growth and increased auxin activity in regions of lateral root growth. P-starved plants halt elongation of the primary root and direct more energy to lateral development which increases root surface area and the ability to encounter and take up sources of P in the soil (Peret et al., 2011; Desnos, 2008; Fang et al., 2009).

Similar P starvation experiments on *N. plumbaginifolia* did not repeat the response seen in *A. thaliana* (Fig 1). The first trial suggested that P-starved seedlings had higher numbers of lateral roots but the results were highly variable (Fig 1A) and replications of the experiment showed no such trend (Fig 1B-D). The P-starved media with sucrose was defective and P-starvation cannot be compared in the sucrose treatments (Fig1B, C). Seedlings grown in media without sucrose showed no difference between P treatments (Fig1B, C). Trial 6 (Fig 5) was performed with only P-starved and P-sufficient conditions, yet variability remained high and a P-starvation response was not present (Fig 1D). The number of lateral roots in P-starved and P-sufficient seedlings was nearly the same, and both were highly variable. These data suggest that in *N. plumbaginifolia* P-starvation does not result in more lateral roots, in contrast to the pattern in observed *Arabidopsis thaliana* (Desnos, 2008; Peret et al., 2011).

The large variability in the lateral root data is evident in the luminescent and digital photographs. Contamination was present in the early trials (Fig 2-3) and could account for some variability, especially differences in plant vitality. However, inconsistencies in development occurred in subsequent trials as well (Fig 4-5). Seedlings exhibited auxin activity (indicated as luminescence resulting from an active *GH3::Luc* promoter::reporter gene construct) with considerable variation in time and space as well. No consistent patterns were observed.

Auxin activity was consistently higher in younger seedlings. Comparison of photos taken from early and late time-points suggests that auxin activity decreases as the plant grows (Fig 4; Fig 5-6; Appendix). This could reflect more overall growth in younger seedlings as compared to older seedlings. However, auxin activity in seedlings that were the same age remains quite variable. For example, the seven day P-starved seedlings grown without sucrose (Fig 3A) display a range of plant development and auxin activity even though the seedlings were the same age. The plant furthest to the left is least developed of the three and displays a relatively uniform glow in its primary root; the middle plant is most developed displays a nodal glow throughout the length of its primary root; the plant on the right is slightly less developed than the middle plant and displays a lighter, less aggregated glow along its primary root. These inconsistencies in plant development within the same plate make it difficult to recognize patterns between the treatments. However, these data also suggest that auxin activity differs depending on the seedling's stage of development.

More developed (larger) seedlings displayed localized patterns of auxin activity, with luminescence occurring as nodes along the primary root. Less developed, shorter, seedlings displayed a more condensed glow pattern that extended along the length of the primary root (Fig 3A; Fig 2-5). Given auxin's association with growth (Guilfoyle, et al., 1998), this suggests that older seedlings have nodes of auxin activity in areas that may develop into lateral roots; while younger seedlings have higher auxin activity throughout their apical root because the entire primary root is growing. Comparison of localized auxin activity in developed seedlings over the two times of observation suggests that nodal glow is often associated with lateral root development and/or growth (Fig 4).

If the localized pattern of auxin activity had been seen at an earlier time point in P-starved seedlings than the seedlings in other treatments it could suggest that P-starved seedlings had stopped primary root elongation and advanced to lateral root formation earlier than normal (Desnos, 2008; Peret et al., 2011). However, this pattern is not clear (Figures 3-4) and is not supported by the lateral root data (Fig 1). Variability in number of viable plants and stage of development is apparent in all treatments in most trials; some plates only supported the growth of one plant from the four or five seeds planted, while others supported the growth of all of the plated seeds.

It is unclear whether *N. plumbaginifolia* seedlings were able to solubilize their own P in the P-sufficient insoluble treatment. If they were able to, seedlings in that condition would mirror seedlings of the P-sufficient condition. This was difficult to determine in conditions without sucrose because unique characteristics of either condition were lost in replicate variability (Fig 3-4). Comparison of P-sufficient and P-sufficient insoluble conditions grown with sucrose suggest that these plants were similar in terms of size and overall health (Fig 4D, H); however, because the P-starved treatment was defective this comparison is incomplete.

Seedlings grown on plates containing sucrose showed clearer patterns as they appeared to be consistently more developed and healthier within replicates (Fig 3-5). This observation led to the final comparison of P-starved and P-sufficient seedlings, both grown with sucrose (Fig 1D, Fig 5). This experiment echoed earlier difficulties of variability and found no measurable difference between the P-starved and P-sufficient plants .

These results suggest that a P starvation response is difficult to elicit in *N. plumbaginifolia.* A possible reason for this is that the Tex-Mex strand of *N. plumbaginifolia* grows primary in the deserts of the southwestern US where P is not a limiting nutrient (Lajtha,

1987). If P is not limiting in *N. plumbaginifolia*'s natural habitat, a strong P starvation response would not have been selected for and therefore, would not be evident in the lab. Alternatively *A. thaliana* is a weed that takes hold in whatever environment it can, including those low in P. This lifestyle makes it advantageous for *A. thaliana* to develop a strong P-starvation response in attempt to reduce, recycle and find more P when trapped in a low P environment. No conclusions on patterns of number of lateral roots or auxin activity between P-starved and P-sufficient seedlings can be made from the collected data because of variability between replicates. This variability prompted an examination of another question: how auxin activity changed as a root system developed in *N. plumbaginifolia.*

In *A. thaliana* auxin activity in the basal meristem and elongation zone was found to oscillate with a period of about six hours due to oscillations in gene expression (Moreno-Risueno et al, 2010). *N. plumbaginifolia* may display similar oscillating activity but with a longer period. Over a period of eight hours total auxin activity was found to decrease with no indication of oscillation (Fig 6). The same experiment was repeated with a longer observation period and showed that total auxin activity decreased to a baseline level near zero after about three hours and then increased after approximately ten hours (Fig 7A). This pattern was found for three of the four plants observed; the fourth plant showed no increase in total auxin activity after the 12 hour period. This suggests that *N. plumbaginifolia* may have a cyclic pattern of total auxin activity with a period of about ten hours. However, repetition of this experiment failed to give the same results with seedlings showing an overall decrease in total auxin activity over eleven hours with little or no recovery (Fig 7B). This experiment must be repeated before any conclusions can be made.

Auxin activity was also examined at specific regions of interest (ROIs) where it appeared to be acyclic in nature, typified by an initial decrease from a variable start point then fluctuation around a baseline value between zero and 2000 photons/pixel (Fig 8). There was no pattern to auxin activity in these ROIs. This suggests that auxin activity was not periodic in specific areas of growth and that it varies even within a single plant. There is not enough evidence to support or undermine the idea that total auxin activity in the plant has a periodic nature.

In conclusion, P-starvation of *N. plumbaginifolia* seedlings did not result in a consistent growth response or pattern of auxin activity as has been found in *A. thaliana*. In one experiment total auxin activity showed oscillation with a period of about 10 hours; however, this result was not reproduced in the second experiment. Auxin activity at specific regions did not display a cyclic nature, or any discernable pattern. Similar experiments should be repeated in *N. plumbaginifolia* and other plants to determine whether the cyclic pattern found in *A. thaliana* is unique or universal. Repetitions with *N. plumbaginifolia* will identify whether the above findings were atypical; repetition in other plants will determine how common an oscillation of auxin activity is. It is possible that *A. thaliana*, because of its extended history as a model organism, has developed a unique pattern of auxin activity, which might account for differences in auxin activity between it and *N. plumbaginifolia*.

In order to repeat auxin activity experiments with the *GH3::Luc* promoter::reporter construct more accurate methods of quantifying auxin activity are required. The above experiment used computer generated, or auto, ROI to quantify areas of auxin activity. Total auxin activity for a plant was found by adding up the net intensity of all of its ROIs. This process was uniform across the samples and provided accurate numbers; however, the same approach limited the accuracy of finding changes in auxin activity at individual ROIs. This

inaccuracy arose because auxin activity changed over the measured time, which changed the presence and location of ROIs. The auto ROI function found different regions for each hour, which made it difficult to compare the same ROI over time. Use of a manual ROI function negated this issue by allowing one to choose the location of the specific ROI, thereby ensuring the same ROI was identified each hour. However, this approach relied on the exact same placement of the plant each hour or else the same ROI will not be followed throughout the experiment. Another flaw with manual ROI use was imprecision. Manual selection of ROIs of the same size was difficult, which resulted in varied measurements of net intensity, i.e. auxin activity, even when repeated at the same location. For these reasons, the auto ROI function was used for all of the above experiments. Future studies could perfect manual ROI measurements by designating an area on the camera screen for plants to be placed, ensuring they are in the same location, and taking the average of multiple measurements of the same ROI for any one time point.

References

Brazelton, J., Pfeufer, E., Sweat, T., Gardener, B. and Coenen, C. 2008. 2,4-Diacetylpholoroglucinol alters plant root development. MPMI. 21:1349-1358..

D'Amico, D. 2011. Luminescent photos: comparing brightness. Personal laboratory notebook.

Desnos, T. 2008. Root branching responses to phosphate and nitrate. Current Opinion in Plant Biology. 11:82-87.

Fang, Z., Shao, C., Meng, Y., Wu, P. and Chen, M. 2009. Phosphate signaling in *Arabidopsis* and *Oryza sativa*. Plant Science. 176:170-180.

Goldsmith, M. 1977. The polar transport of auxin. Annual Review of Plant Physiology. 28:439-478.

Guilfoyle, T., Ulmasov, T. and Hagen, G. 1998. The ARF family of transcription factors and their role in plant hormone-responsive transcription. Cellular and Molecular Life Sciences. 54:619-627.

Heisler, M., Ohno, C., Das, P., Sieber, P., Reddy, G., Long, J., and Meyerowitz, E. 2005. Patterns of auxin transport and gene expression during primordium development revealed by live imaging of the *Arabidopsis* inflorescence meristem. Current Biology. 15:1899-1911.

Lajtha, K. 1987. Nutrient reabsorption efficiency and the response to phosphorus fertilization in the desert shrub *Larrea tridentate.* Biogeochemistry: 4:265-276.

Laskowski, M., Grieneisen, V., Hofhuis, H., ten Hove, C., Hogeweg, P., Maree, A. and Scheres, B. 2008. Root system architecture from coupling cell shape to auxin transport. PLoS Biology. 6:2721-2735.

Li, Y., Wu, Y., Hagen, G. and Guilfoyle, T. 1999. Expression of the auxin-inducible GH3 promoter/GUS fusion gene as a useful molecular marker for auxin physiology. Plant Cell Physiology. 40:675-682.

Lin, W., Lin, S. and Chiou, T. 2009. Molecular regulators of phosphate homeostasis in plants. Journal of Experimental Biology. 60:1427-1438.

Miura, K., Lee, J. Gong, Q., Ma, S., Jin, J., Yoo, C., Miura, T., Sato, A., Bohnert, H. and Hasegawa, P. 2011. *SIZ1* regulation of phosphate starvation-induced root architecture remodeling involves the control of auxin accumulation. Plant Physiology. 55:1000-1012.

Moreno-Risueno, M, Van Norman, J, Moreno, A, Zhang, J, Ahnert, S and Benfey, P. 2010. Oscillating gene expression determines competence for periodic *Arabidopsis* root branching. Science. 239:1306-1311.

Muday, G. and DeLong, A. 2001. Polar auxin transport: controlling where and how much. *TRENDS* in Plant Science. 6:535-542.

Nacry, P., Canivenc, G., Muller, B., Axmi, A., Onckelen, H., Rossignol, M. and Doumas, P. 2005. A role for auxin redistribution in the responses of the root system architecture to phosphate starvation in *Arabidopsis*. Plant Physiology. 138:2061-2074.

Peret, B., Clement, M., Nussaume, L. and Desnos, T. 2011. Roots developmental adaptation to phosphate starvation: better safe than sorry. Trends in Plant Science. 18:442-450.

Peret, B., Rybel, B., Casimiro, I., Benkova, E., Swarup, R,, Laplaze, L., Beeckman, T. and Bennett, M. 2009. *Arabidopsis* lateral root development: an emerging story. Trends in Plant Science. 14:399-408.

Perez-Torres, C-A., Lopez-Bucio, J., Cruz-Ramirez, A., Ibarra-Laclette, E., Dharmasiri, S., Estelle, M. and Herrera-Estrella, L. 2008. Phosphate availability alters lateral root development in *Arabidopsis* by modulating auxin sensitivity via a mechanism involving the TIR1 auxin receptor. Plant Cell. 20:3258-3272.

Rouached, H., Arpat, A. and Poirier, Y. 2010. Regulation of phosphate starvation responses in plants: signaling players and cross-talks. Molecular Plant. 3:288-299.

Ruijter N., Verhees J., van Leeuwen W., van der Krol A. 2003. Evaluation and comparison of the GUS, LUC and GFP reporter system for gene expression studies in plants. Plant Biology 5:103-115.

Schachtman, D., Reid, R. and Ayling, S. 1998. Phosphorus uptake by plants: from soil to cell. Plant Physiology. 116:447-453.

Taiz and Zeiger (2006) Plant Physiology, 4th edition, Sinauer Associates, Sunderland, Ma 19: 467-507.

Ticconi, C. and Abel, S. 2004. Short on phosphate: plant surveillance and countermeasures. Trends in Plant Science. 9:548-555.

Wargo, C. 2011. Glow with the flow: monitoring auxin response patterns through Luciferase activity in *Nicotiana plumbaginifolia*. Allegheny College Senior Comprehensive Project.

Appendix:

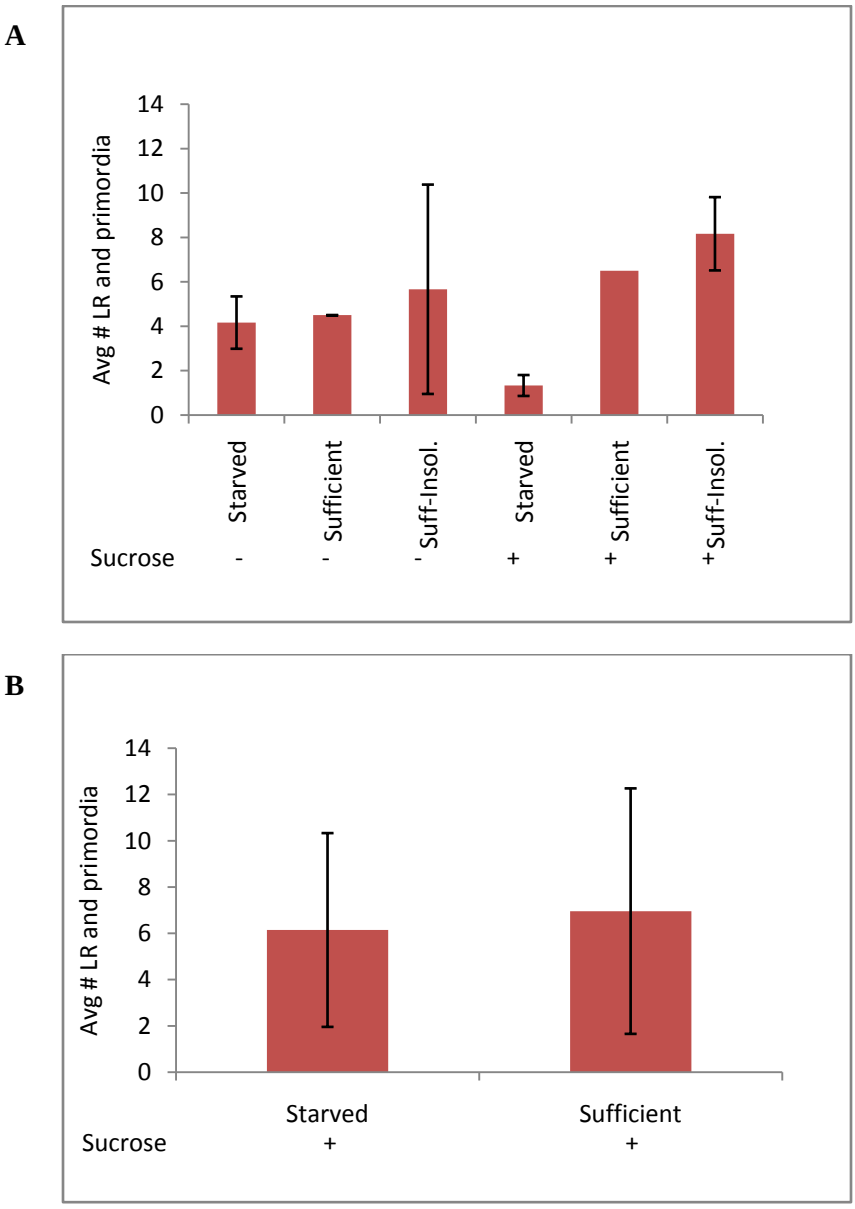

Figure 1: Later time points of Figure 1C& D in results-- A and B are10 day old seedlings from Figure 1C& D, respectively. High variability within treatments prevents comparison between treatments, as seen in Fig 1. Note that the P-starved medium +S was deficient in A.

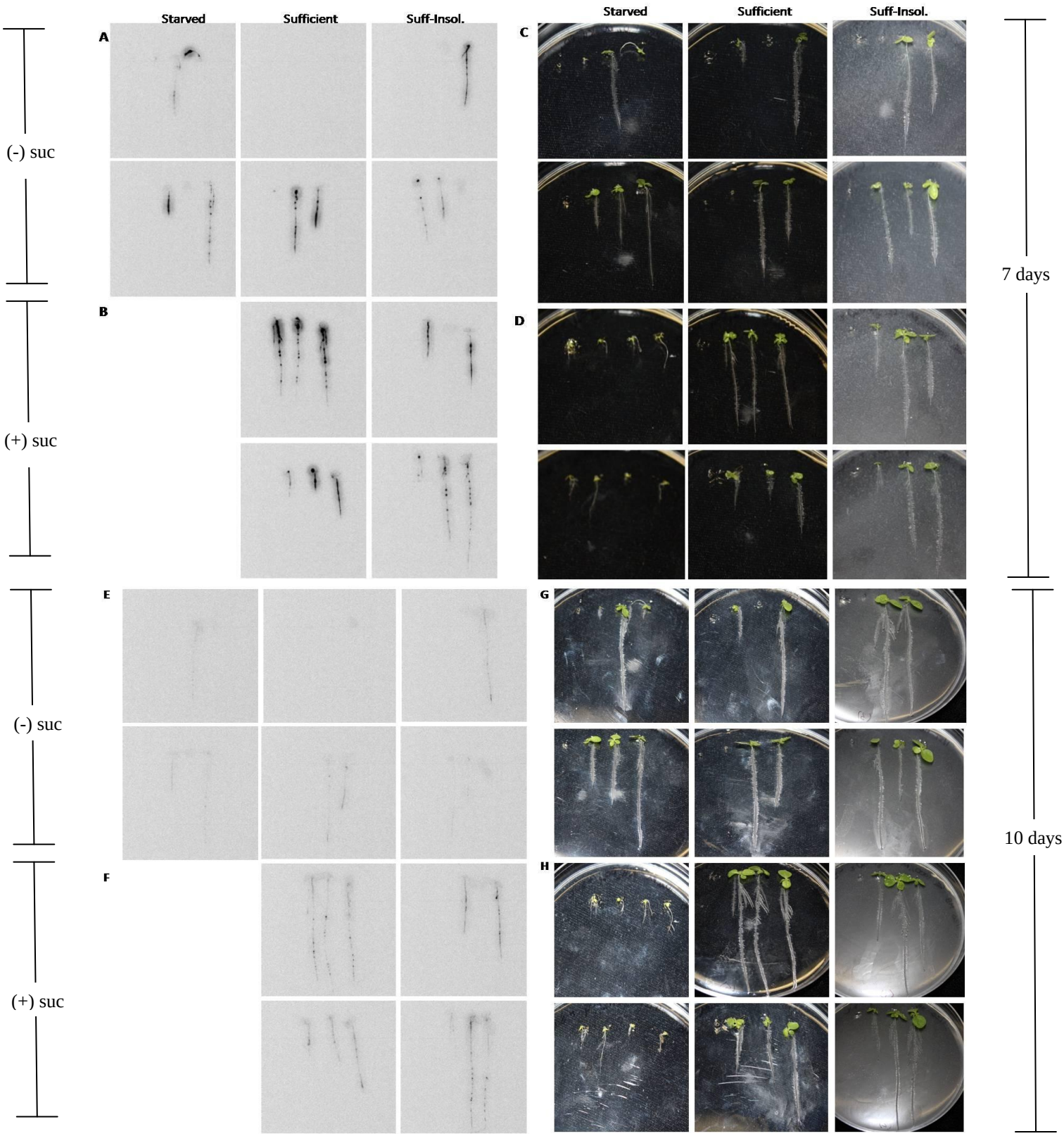

Figure 2: P-starvation Trial 5. Replicate of Trial 4 that yielded similar results (great variability).

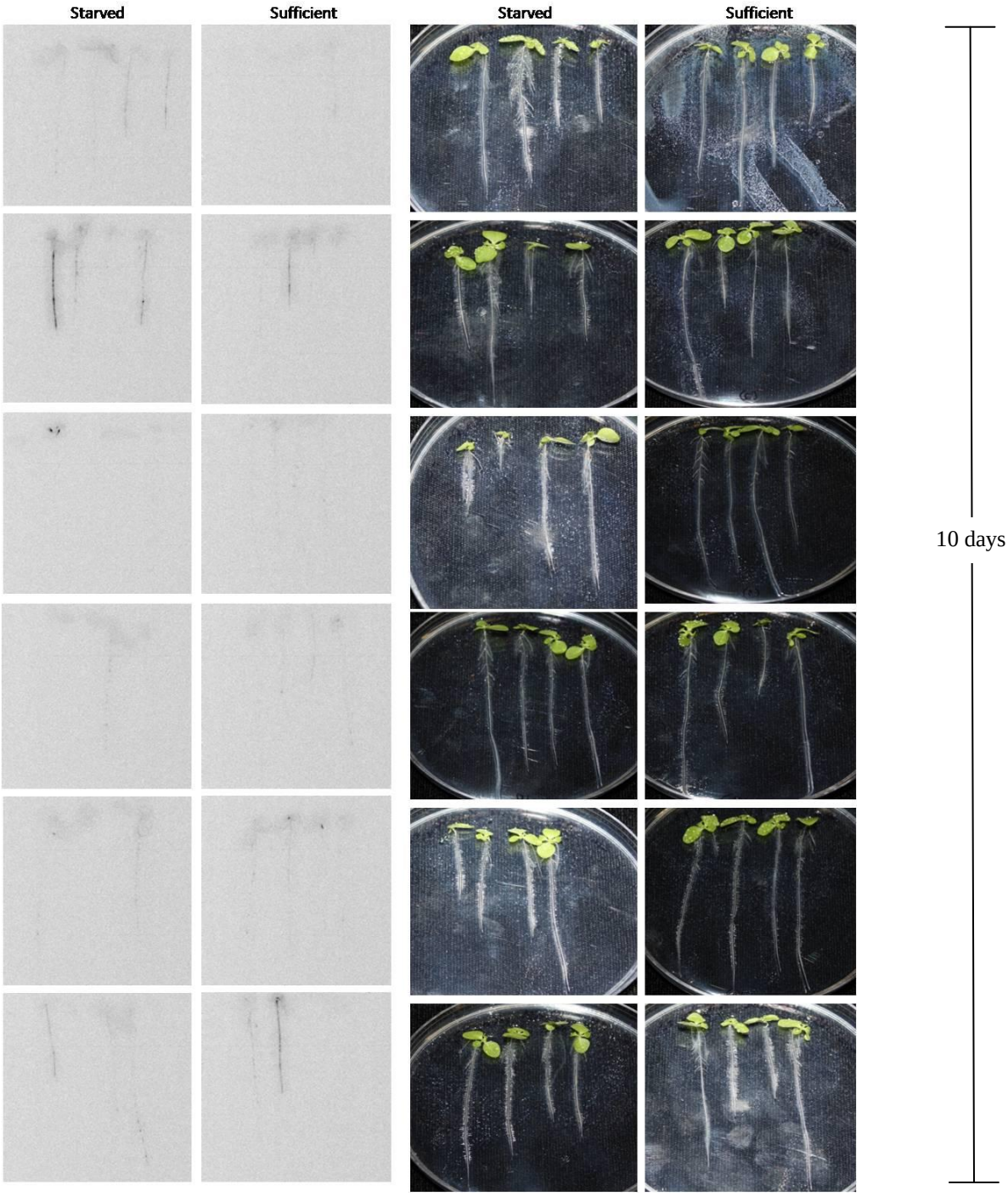

Figure 3: Older time point of P-starvation Trial 6 (Fig 5).