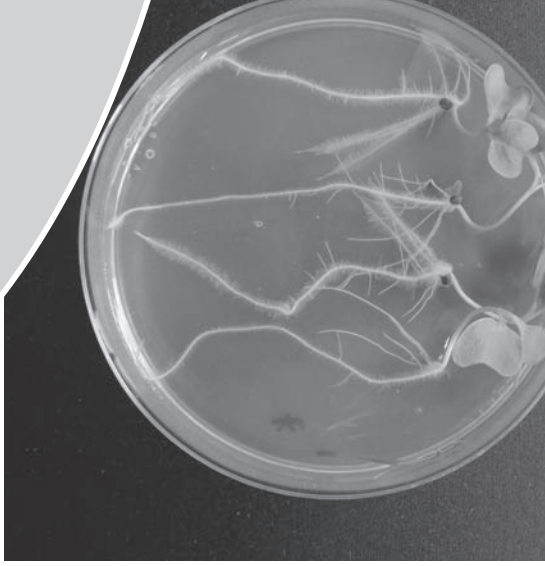
TIPS, TRICKS & TECHNIQUES

Visualizing Nutrient Effects on Root Pattern Formation



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Abstract

This lab gives students hands-on experience with visualizing the root architecture of plants exposed to varying concentrations of the vital nutrient phosphorus. By maintaining Brassica *sp. seedlings in the presence of different quantities of phosphate, students can quantify changes in the number of lateral roots as an example of how the environment influences plant pattern formation. Additional variables in the experimental design, such as the use of plant mutants altered in plant regulator action or the presence of plant regulators in the plant growth medium, allow for exploration of how plant growth regulators are involved in root development. The quantitative and qualitative nature of this nine-day activity provides instructors opportunities to introduce students to various data analyses in botanical study. Additional ties to plant anatomy and the agricultural use of plant growth regulators that alter root development make this activity a rich source of exploration for broadening student exposure to plants and their development.*

Key Words: *Root development; plant pattern formation; plant nutrition; plant growth regulators.*

○ Introduction

The phenomenon of pattern formation is a universal theme addressed in the study of developmental biology. Examples of this theme abound in the study of animals, but students often fail to appreciate how pattern formation occurs in plants. Many developmental biology textbooks emphasize animal developmental processes instead of detailing plant-based phenomena. Furthermore, since plant roots typically develop underground, their pattern formation is somewhat cryptic to most students. The following accessible laboratory experience was developed to help students observe firsthand an example of how plants exhibit pattern formation: development of root architecture. Students of biology observe that the basic differences in root anatomy between monocot and eudicot plants involve the arrangement of the vascular tissue and the presence or absence of a taproot, but they often overlook the plasticity of root architecture during development (Urry et al., 2017). By the branching of roots, plants can increase the absorptive surface available for water and nutrient uptake. Plant growth regulators such as auxins or auxin-like compounds such as 2,4-D govern lateral root development in plants by influencing the activity of the pericycle (Torrey, 1950). Understanding of this phenomenon has led to the commercial use of auxin-like compounds (e.g., Rootone, FastRoot) to promote lateral root formation from plant stem cuttings. Formation of lateral roots and root hairs allows the plant to interface with a greater soil volume, thus maximizing the absorption of key minerals such as phosphate. This activity is based on the well-documented effect of phosphate availability on root development (Niu et al., 2013). The use of *Arabidopsis* as a model plant in studying this effect (because of the availability of mutants with altered root architecture and hormone response) has generated a body of literature that can help students see the context of this laboratory activity (Williamson et al., 2001; Linkohr et al., 2002; Lopez-Bucio et al., 2002). By providing hands-on experiences with plants, educators can strengthen botanical knowledge in their students and encourage interest in these vital components of the environment. Previous work in elementary school classes demonstrated that guided exploration of plants increased students' interest in plants (Strgar, 2007). Analysis of the biological curriculum taken by students prior to college has raised concern over how botany is presented and documented the opportunity for educators to increase student appreciation of plants (Uno, 1994).

○ Procedure

In this exercise, students maintain germinating *Brassica* sp. seedlings on mineral salts agar (MSA) varying in phosphate content and observe the resulting root development. Depending on grade level, students can consider the type of seed used and the range of MSA phosphate levels as they design the experiment (additional variables for more advanced students are suggested below; see "Teaching Tips"). Wisconsin Fast Plant (Carolina Biological Supply

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Company, Burlington, North Carolina) seed has typically been used, but other *Brassica* sources such as kale from garden suppliers may be suitable alternatives. *Arabidopsis* seed is available to schools from Ohio State University (http://abrcoutreach.osu.edu). Students rinse seed in tap water for 30 seconds, followed by disinfestation in 95% ethanol for five minutes and thorough rinsing in autoclaved distilled water. Students then transfer seed to 1% water agar plates for pregermination for 48 hours at 4°C prior to transfer to MSA plates. This results in synchronized seed germination producing sprouts of uniform radicle length. Students sow three to five pregerminated seeds per MSA plate. To avoid bias in data collection, students should not know the phosphate level in their plates (this can be accomplished by the instructor coding plates varying in phosphate level). Plates are secured with tape (so that the developing shoot does not displace the lid) and placed under fluorescent lights at room temperature for seven days. Plates are positioned at an angle of ~45 degrees to allow the downward development of the root system. After growth, students record the number of lateral roots present on each plant. Total student time for this task is ~30 minutes. Data from the entire class are then used to construct a graph representing the mean number of lateral roots per plant for each phosphate concentration. Figure 1 shows the growth of Fast Plant and kale seedlings on MSA with and without 1mM phosphate. Note the abundance of root hairs present on roots and clearly identified lateral roots in plate A. Figure 2 shows student data pooled from four years of experiments. Note the trend of increasing lateral root number as phosphate levels decrease and variability in the data as evidenced by the standard deviations of each average.

○ Teaching Tips

One variation of this protocol is to utilize MSA plates with varying levels of auxin. See Lopez-Bucio et al. (2002) for suggested concentrations of 2,4-D. As resources and seed availability allow, using auxin-insensitive mutants of *Arabidopsis* (Williamson et al., 2001) is an interesting sequel to this basic exercise. Asking students

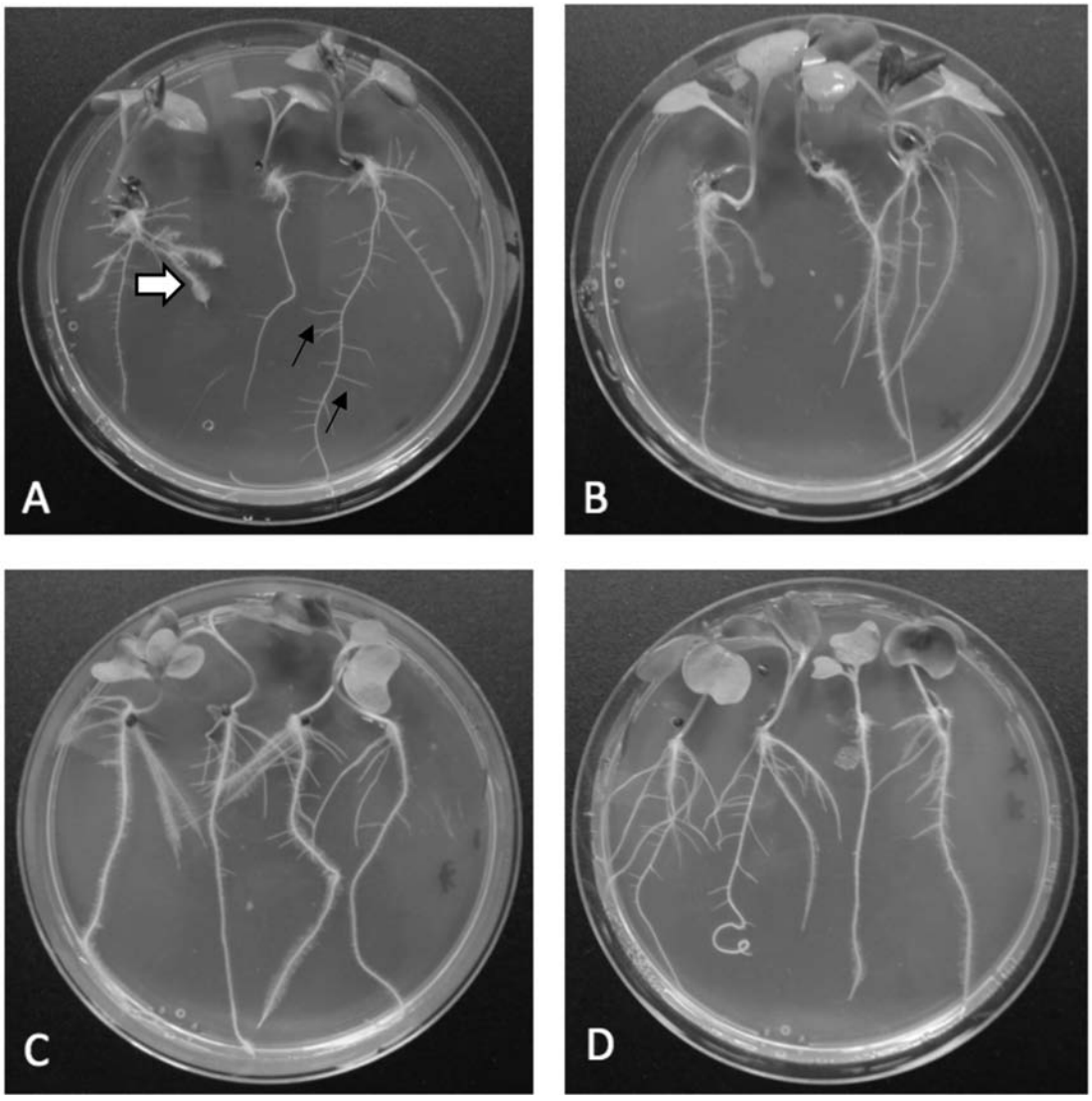

Figure 1. Seven-day-old Fast Plant (**A, B**) and kale (**C, D**) seedlings on mineral salts agar growing in the absence (**A, C**) and presence (**B, D**) of 1 mM sodium phosphate. White arrow indicates root hairs; black arrows indicate lateral roots.

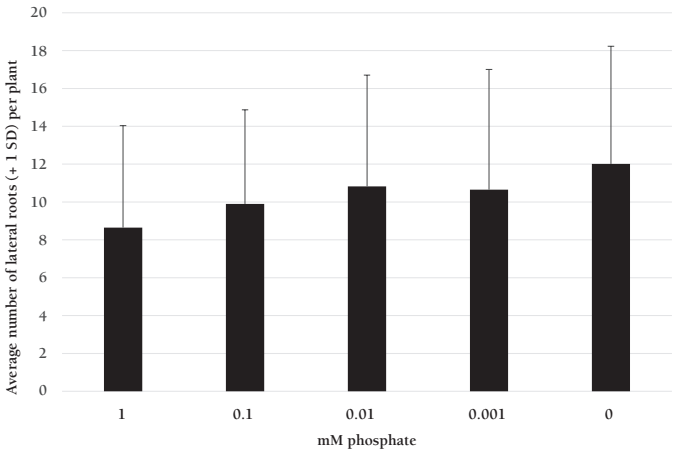

Figure 2. Effect of phosphate levels on lateral root numbers of *Brassica* sp. Each bar represents the average of 75–89 plants from four years of student data collection.

how to quantify root hair development on the root systems allows for student creativity and problem solving. In light of the results of this experiment, students are asked to reflect on the following questions (answers in italics):

1. How would a plant benefit from changing its root architecture in a low-phosphate environment? *A plant relies on its root as the primary organ of absorption of water and nutrients from the environment (typically soil). If nutrients are scarce (such as low phosphate levels), the plant can explore more soil by increasing the surface area of its root system via branching and root hair development, resulting in more root surface area for uptake.*
2. What roles does phosphorus play in the life of a plant? *Phosphorus is a key component of ATP, nucleic acids, and phospholipids of plant cells.*
3. How do plant growth regulators influence root architecture? *Auxins are the primary plant growth regulators that promote lateral root formation. The action of auxins in root development involves ethylene and strigolactones. Cytokinins suppress lateral root initiation.*
4. What soil factors influence phosphorus availability to plants? *Due to fixation to soil cations, phosphorus availability is optimal at pH values between 6 and 7.5. Phosphorus can be lost to soil via erosion. Release of phosphorus from decaying soil organic matter is an important source of input for phosphorus.*
5. Describe how lateral roots arise. *Lateral root growth is a type of primary plant growth that originates from meristematic cells of the pericycle, the outermost layer of the vascular cylinder of the root.*

○ Some Tips to Consider

- Crowded root systems on the agar plates make it difficult to count lateral roots. Use three seeds per plate and ensure they are well spaced to make it easier to count lateral roots.
- Different seed sources may require different conditions of pregermination. For example, kale seeds typically pregerminate slowly at 4°C in comparison to Fast Plant seeds.
- Don't confuse root hairs and lateral roots. Root hairs appear as tiny fuzzy growth along a root, whereas lateral roots are distinct branches off of another root (see Figure 1A). Root hair development may vary between phosphate concentrations, so this can be a point of discussion with the students.

○ Mineral Salts Agar

Composition and preparation of MSA is as follows (modified from Murashige & Skoog, 1962): 2 mM NH_4NO_3, 1.9 mM KNO_3, 0.3 mM $CaCl_2$-$2H_2O$, 0.15 mM $MgSO_4$-$7H_2O$, 0.1 mM $FeSO_4$-$7H_2O$, 1% agar, pH 5.7; a stock solution of 10 mM $Na_2H_2PO_4$ is prepared and sterilized separately; aliquots of the stock solution are added to quantities of MSA, resulting in agar varying in phosphate concentration from 0 to 1 mM. Prepare all solutions in distilled water, sterilize via autoclaving, and dispense the resulting agar into 100-mm-diameter Petri plates (teacher preparation time: three hours).

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