

Plant Tissue Culture and Engineering

11:776:452 (3 credits)
Spring Semester (yearly)

CONTACT INFORMATION

Instructor: Dr. Rong Di
Office Location: 222B Foran Hall, 59 Dudley Rd., New Brunswick, NJ 08901
Phone: 848-932-6350
E-mail: di@aesop.rutgers.edu
Office Hours: by arrangement

Lab Assistant: Dr. Tiemi Curry
Office Location: 237B Foran Hall, 59 Dudley Rd., New Brunswick, NJ 08901
Phone: 848-932-6374
E-mail: curry@aesop.rutgers.edu
Office Hours: by arrangement

COURSE DESCRIPTION

Principles and culture techniques of cells, callus, organs, pollen, anthers, embryos, and protoplasts. The applications in clonal propagation and research in breeding, physiology, and pathology.

COURSE WEBSITE, RESOURCES AND MATERIALS

- 3-ring binder should be used to keep these lecture materials

PREREQUISITE

11:776:210 Principles of Botany OR 11:776:211 Introduction to Horticulture OR 11:776:242 Plant Science

COURSE LEARNING GOALS

(Link to Plant Biology Undergraduate Program Goals: <http://plantbiology.rutgers.edu/undergrad/plantbiology/>)

By the end of this course, the student will be able to:

1. Comprehend and describe various protocols of plant cell, tissue, and organ culture as well as the scientific principles underlying the protocols (addresses program goal 1)
2. To apply plant tissue culture technology for clonal propagation, assisting plant breeding and plant improvement, recovering plants from transformed cells, and production of valuable plant biochemical (addresses program goal 1)
3. Explain and demonstrate various protocols of plant gene transfer technology (addresses program goal 1)
4. Describe the underlying principles of each step of the plant gene transfer protocols (addresses program goal 1)
5. Describe the methods and principals for the validation of transgene integration into plant genome (addresses program goals 2 and 3)

ASSIGNMENTS/RESPONSIBILITIES AND ASSESSMENT

Grading (100 points)

- Mid-term Exam: 30 points
- Final Exam: 30 points
- Laboratory notebooks (graded 4 times at 5 points each): 20 points
- Laboratory report (tobacco transformation): 10 points
- Lecture attendance: 5 points
- Laboratory attendance: 5 points
- Scale: 90-100% = A; 80-89 = B; 70-79 = C; 60-69 = D

Laboratory notebook: Laboratory exercises are grouped into 7 projects. Each student is required to bring a 3-ring binder to keep notes; notes will be recorded and grouped for each project. Notes include: date, lab exercise protocol, medium type, plant tissue, number of dishes/flasks, morphology of tissues, development of tissues, results, etc. Each student will include one page of photos for each project in the lab notebook. Notebooks will be graded 4 times (after labs 4, 7, 10, and 13).

Laboratory report: Each student will submit a report, in publication format, on Lab project #3: Production of GUS-transgenic tobacco via *Agrobacterium*-mediated transformation. Contents include: introduction, methods, results, and references.

Learning goals assessment: Specific questions on exams and quizzes will be used to assess student knowledge of all course learning goals. Students will integrate knowledge of plant gene transfer protocols in graded laboratory exercises and the laboratory report (learning goal 5). The percentage score on these assessments will determine the level of mastery: >90% outstanding; 80-89% good; 70-79% satisfactory; <69% unsatisfactory.

PARTICIPATION GRADE AND ABSENCE POLICY

A maximum of 2 excusable absences are allowed. Points are deducted for each unexcused absence. Students unable to attend must contact the instructor via e-mail prior to the missed class or may use the University absence reporting website (<https://sims.rutgers.edu/ssra/>) to indicate the date and reason for the absence. An e-mail is automatically sent to the instructor.

COURSE SCHEDULE

Lecture/Lab	Topics
1	Introduction Preparation of media MS media with different hormones
2	Plant growth regulators; organogenesis Project #1 – Effects of plant hormones on regeneration; organogenesis (follow up in 5 weeks)
3	Plant genetic engineering Project #2 – <i>In planta</i> transformation of <i>Kalanchoe pinnata</i> with <i>Agrobacterium tumefaciens</i> and <i>A. rhizogenes</i> (follow up in 2 months)
4	More on <i>Agrobacterium</i> , Ti plasmid, vir genes; selection of transgenics Project #3 – Transformation of tobacco leaves with <i>A. tumefaciens</i> -GUS (follow up in 1.5 to 2 months) Notebook grading #1

Lecture/Lab Topics

- 5 Callus and cell culture
Project #4 – Carrot somatic embryogenic cell culture (follow up in 2 to 4 weeks)
Project #5 – Gene gun bombardment of bentgrass calli (follow up in 1.5 months)
- 6 Meristem culture; vegetative propagation
Project #6 – Vegetative propagation
1. Potato tuberization (follow up in 3 weeks)
2. Fern propagation via spores and runners (follow up at end of semester)
- 7 Production of biochemical via cell culture
Project #4 – Initiation of carrot cell culture (follow up: transfer weekly)
Notebook grading #2
- 8 **Mid-term exam**
Spring recess
- 8 Somatic embryogenesis
1. Project #1 – Observe African violet and tobacco organogenesis; transfer shoots to rooting medium
2. Project #3 – Transfer regenerated tobacco shoots to MSO+Kan100
3. Project #4 – Carrot somatic embryogenic cell culture
5. Project #5 – Transfer bentgrass calli
4. Project #6 – Observe potato tuberization
- 10 Protoplast
1. Project #7 – Moss protoplast preparation and culture (follow up – observe weekly)
2. Project #3 – Observe GUS-transformed tobacco leaves
- 11 Gene gun transformation
1. Project #5 – Gene gun transformation of bentgrass calli-GUS (follow up – GUS staining in one week)
2. Project #4 – Observe carrot somatic embryogenic culture
Notebook grading #3
- 12 Validation of transgene integration
Project #5 – GUS staining of bombarded bentgrass calli
- 13 Expression of transgenes
1. Project #3 – Validation of transgenic tobacco with kanamycin resistance assay and GUS staining
2. Project #6 – Observe potato tuber formation and propagated fern
3. Project #7 – Observation of moss protoplast culture
- 14 Discussion
1. Project #2 – Observe *in planta* transformed *Kalanchoe pinnata*
2. Project #3 – GUS-transgenic tobacco: genomic DNA isolation; validation of GUS transgene by PCR
Notebook grading #4
Reading day: Continuation of unfinished projects
Lab report due

Final Exam

FINAL EXAM DATE AND TIME

The Online Final exam Schedule: <http://finalexams.rutgers.edu/>

ACCOMODATIONS FOR STUDENTS WITH DISABILITIES

Please follow the procedures outlined at <https://ods.rutgers.edu/students/registration-form>. Full policies and procedures are at <https://ods.rutgers.edu/>

ACADEMIC INTEGRITY

The university's policy on Academic Integrity is available at <http://academicintegrity.rutgers.edu/>

The principles of academic integrity require that a student:

- Properly acknowledge and cite all use of the ideas, results, or words of others.
- Properly acknowledge all contributors to a given piece of work.
- Make sure that all work submitted as his or her own in a course or other academic activity is produced without the aid of impermissible materials or impermissible collaboration.
- Obtain all data or results by ethical means and report them accurately without suppressing any results inconsistent with his or her interpretation or conclusions.
- Treat all other students in an ethical manner, respecting their integrity and right to pursue their educational goals without interference. This requires that a student neither facilitate academic dishonesty by others nor obstruct their academic progress.
- Uphold the canons of the ethical or professional code of the profession for which he or she is preparing.

Adherence to these principles is necessary in order to ensure that:

- Everyone is given proper credit for his or her ideas, words, results, and other scholarly accomplishments.
- All student work is fairly evaluated and no student has an inappropriate advantage over others.
- The academic and ethical development of all students is fostered.
- The reputation of the University for integrity in its teaching, research, and scholarship is maintained and enhanced.

Failure to uphold these principles of academic integrity threatens both the reputation of the University and the value of the degrees awarded to its students. Every member of the University community therefore bears a responsibility for ensuring that the highest standards of academic integrity are upheld.

STUDENT WELLNESS SERVICES

Just In Case Web App <http://codu.co/cee05e>

Access helpful mental health information and resources for yourself or a friend in a mental health crisis on your smartphone or tablet and easily contact CAPS or RUPD.

Counseling, ADAP & Psychiatric Services (CAPS)

(848) 932-7884 / 17 Senior Street, New Brunswick, NJ 08901/ www.rhscaps.rutgers.edu/

CAPS is a University mental health support service that includes counseling, alcohol and other drug assistance, and psychiatric services staffed by a team of professional within Rutgers Health services to support students' efforts to succeed at Rutgers University. CAPS offers a variety of services that include: individual therapy, group therapy and workshops, crisis intervention, referral to specialists in the community and consultation and collaboration with campus partners.

Violence Prevention & Victim Assistance (VPVA)

(848) 932-1181 / 3 Bartlett Street, New Brunswick, NJ 08901 / www.vpva.rutgers.edu/

The Office for Violence Prevention and Victim Assistance provides confidential crisis intervention, counseling and advocacy for victims of sexual and relationship violence and stalking to students, staff and faculty. To reach staff during office hours when the university is open or to reach an advocate after hours, call 848-932-1181.

Disability Services

(848) 445-6800 / Lucy Stone Hall, Suite A145, Livingston Campus, 54 Joyce Kilmer Avenue, Piscataway, NJ 08854 / <https://ods.rutgers.edu/>

Rutgers University welcomes students with disabilities into all of the University's educational programs. In order to receive consideration for reasonable accommodations, a student with a disability must contact the appropriate disability services office at the campus where you are officially enrolled, participate in an intake interview, and provide documentation: <https://ods.rutgers.edu/students/documentation-guidelines>. If the documentation supports your request for reasonable accommodations, your campus's disability services office will provide you with a Letter of Accommodations. Please share this letter with your instructors and discuss the accommodations with them as early in your courses as possible. To begin this process, please complete the Registration form on the ODS web site at: <https://ods.rutgers.edu/students/registration-form>.

Scarlet Listeners

(732) 247-5555 / <http://www.scarletlisteners.com/>

Free and confidential peer counseling and referral hotline, providing a comforting and supportive safe space.