PLP6636 FRONTIERS IN PLANT BIOTECHNOLOGY

FALL 2018

3 credit hours

1. Instructor:

Svetlana Y. Folimonova

Room 2565 Fifield Hall
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Course Time/ Location:

Lectures: Fifield Hall (room 2564), Tuesdays, periods 3 (9:35-10:25 AM) & 4 (10:40-11:30 AM)

Discussions: Fifield Hall (room 2564), Thursdays, period 3 (9:35-10:25 AM)

Course Materials Access: course website in Canvas

2. Office Hours:

Office hours are Wednesdays and Fridays between 9 AM and 12 PM by appointment at the instructor's office (2565) Fifield Hall.

3. Course Overview:

This course explores some of the exciting concepts in modern molecular biology that have been recently implicated in the development of novel cutting-edge genetic tools for use in the field of plant biotechnology. Special emphasis is given to small non-coding RNAs and their role in plant immunity along with RNA interference (RNAi)-based approaches in plant bioengineering, novel systems for targeted gene editing (such as CRISPR/Cas9 and others) and their practical applications. Other cutting-edge biotechnology advancements, including strategies for precise regulation of gene expression in plants, multigene engineering, and the use of plant pathogens as materials for nanotechnology, will be discussed. In this course, each two-hour lecture is followed by an in-class paper discussion, which gives the students an opportunity to improve the understanding of the fundamental concepts that have been discussed in the preceding lecture by reviewing and discussing a research paper that is selected accordingly to what has been taught during the earlier lecture.

Course Objectives: through this course, students will:

1. Become familiar with the advanced genetic tools that recently have become available in the field of biotechnology as a result of breakthrough discoveries in fundamental science and with their applications for plant improvement and production of new products in plants.
2. Learn some of the recently developed experimental procedures and methods that are used in the biotechnology research, their theory, applications, and limitations and learn how to properly select appropriate methodology while designing experiments.
3. Improve professional skills, including skills in developing a scientific idea as well as in critical reading of scientific literature and presentation skills.

Prerequisites: graduate standing and general knowledge of Genetics, Organic Chemistry and Biochemistry

4. Course schedule of topics and assignments:

DATE	TOPIC	SUGGESTED READING (tentative, subject to change)
Aug 23, Thu	Course overview: Overview of the Lecture and Discussion parts of the course	
Aug 28, Tue *Lecture 1*	New paradigms in biotechnology: progress enabled by the advancements in fundamental science	Newell-McGloughlin and Re (2007); Liu et al. (2013); Saurabh et al. (2014); Silva et al. (2018)
Aug 30, Thu *Discussion 1*	Paper discussion 1	Molnar et al. (2010)
Sep 4, Tue *Lecture 2*	Translating fundamental knowledge into applications I. Non-coding RNA. RNAi: Discovery and molecular mechanism I.	Montgomery (2004); Lindbo (2012); Carthew and Sontheimer (2009)
Sep 6, Thu *Discussion 2*	Paper discussion 2	Qutob et al. (2013)
Sep 11, Tue *Lecture 3*	RNAi: molecular mechanism II. RNAi in different systems	Axtell (2013); Bologna and Voinnet (2014); Castel and Martienssen (2013); Zhang and Unver (2018)
Sep 13, Thu	Paper discussion 3	Li et al. (2012)

Discussion 3		
Sep 18, Tue *Lecture 4*	Small RNAs in inter-kingdom communications	Weiberg et al. (2015); Wang et al. (2016)
Sep 20, Thu *Discussion 4*	Paper discussion 4	Weiberg et al. (2013)
Sep 25, Tue *Lecture 5*	Applications of RNAi to plant biotechnology	Saurabh et al. (2014); Katoch and Thakur (2013); Djami-Tchatchou (2017); Zotti et al. (2017)
Sep 27, Thu *Discussion 5*	Paper discussion 5	Cao et al. (2014)
Oct 2, Tue *Lecture 6*	The power and promise of RNAi technology in controlling citrus Huanglongbing disease. *The topic will be presented by Dr. Nabil Killiny, Associate Professor at the UF, CREC*	Bove (2006); Harmon (2013); El-Shesheny et al. (2013); Hajeri et al. (2014)
Oct 4, Thu *Discussion 6*	Paper discussion 6	Hajeri et al. (2014)
Oct 9, Tue *Exam*	***MID-TERM EXAM (take-home exam)***	
Oct 11, Thu *Discussion 7*	Paper discussion 7	Niu et al. (2016)
Oct 16, Tue *Lecture 7*	Translating fundamental knowledge into applications II. New approaches to gene manipulation. Strategies for targeted genome editing. CRISPR/Cas system: interference mechanisms and applications	Sampson and Weiss (2013); Upadhyay et al. (2013); Barrangou (2013); Belhaj et al. (2013); Ran et al. (2013); Mohanta (2017); Knott & Doudna (2018)
Oct 18, Thu *Discussion 8*	Paper discussion 8	Barrangou et al. (2007)

Oct 23, Tue *Lecture 8*	CRISPR/Cas system: interference mechanisms and applications (cont.)	Sampson and Weiss (2013); Upadhyay et al. (2013); Barrangou (2013); Belhaj et al. (2013); Ran et al. (2013); Mushtaq et al. (2018); Zhang et al. (2017); Borrelli et al. (2018)
Oct 25, Thu *Discussion 9*	Paper discussion 9	Sternberg et al. (2014)
Oct 30, Tue *Lecture 9*	The roles of CRISPR-Cas systems beyond adaptive immunity	Barrangou (2015); Westra et al. (2014); Jaganathan et al. (2018)
Oct 31-Nov 1, Thu *No class*	***Florida Genetics Symposium:*** students are encouraged to attend talks and poster presentations	
Nov 6, Tue *Lecture 10*	Applications of CRISPR-Cas editing in tomato. *The topic will be presented by Tong Geon Lee, Assistant Professor, Dept. of Horticultural Sciences*	
Nov 8, Thu *Discussion 10*	Paper discussion 10	Sapranauskas et al. (2011)
Nov 13, Tue *Lecture 11*	Bacterial applications of CRISPR-Cas editing. *The topic will be presented by Chris Reisch, Assistant Professor, Dept. of Microbiology and Cell Science*	
Nov 15, Thu *Discussion 11*	Paper discussion 11	Nekrasov et al. (2013)
Nov 20, Tue *Lecture 12*	Strategies for targeted genome editing: ZFNs and TALENs	Gaj et al. (2013)
Nov 22, Thu	**HOLIDAY**	
Nov 27, Tue *Lecture*	Targeted genome editing in citrus: applications and perspectives. *The topic will be presented by Dr. Nian Wang, Professor at UF, CREC*	
Nov 29, Thu *Discussion*	Summary of research papers read in class: discussion and conclusions	
Dec 4, Tue *Exam*	***EXAM***	

REFERENCES FOR THE LECTURE SUPPLEMENTARY READING MATERIALS:

Axtell MJ (2013) Classification and comparison of small RNAs from plants. *Annu. Rev. Plant Biol*. 64:137-159.

Barrangou R (2013) CRISPR-Cas systems and RNA-guided interference. *WIREs RNA* 4:267-278.

Barrangou R (2015) The roles of CRISPR-Cas systems in adaptive immunity and beyond. *Curr. Opin. Immunol.* 32:36-41.

Belhaj K, Chaparro-Garcia A, Kamoun S, Nekrasov V. (2013) Plant genome editing made easy: targeted mutagenesis in model and crop plants using the CRISPR/Cas system. *Plant Methods* 9:39.

Bologna NG and Voinnet O. (2014) The diversity, biogenesis, and activities of endogenous silencing small RNAs in *Arabidopsis*. *Annu. Rev. Plant Biol*. 65:473-503.

Borrelli VMG, Brambilla V, Rogowsky P, Marocco A, Lanubile A. (2018) The Enhancement of Plant Disease Resistance Using CRISPR/Cas9 Technology. *Frontiers in Plant Science*. 9:1245. doi:10.3389/fpls.2018.01245.

Bove JM. (2006) Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. *J. Plant Pathol.* 88:7-37.

Carthew Richard W., and Sontheimer, Erik J. (2009) Origins and Mechanisms of miRNAs and siRNAs. *Cell* 136, 642–655

Castel SE and Martienssen RA (2013) RNA interference in the nucleus: roles for small RNAs in transcription, epigenetics and beyond. *Nature Reviews* 14:100-112.

Dalton M. (2014) DuPont's Corn to Take Root. *The Wall Street Journal* Feb. 12, 2014.

Dawson WO, Folimonova SY. (2013) Virus-Based Transient Expression Vectors for Woody Crops: A New Frontier for Vector Design and Use. *Annu. Rev. Phytopathol.* 51:321-337.

Djami-Tchatchou AT, Sanan-Mishra N, Ntushelo K, Dubery IA. (2017) Functional Roles of microRNAs in Agronomically Important Plants—Potential as Targets for Crop Improvement and Protection. *Frontiers in Plant Science*. 8:378. doi:10.3389/fpls.2017.00378.

El-Shesheny I, Hajeri S, El-Hawary I, Gowda S, and Kiliny N (2013) Silencing Abnormal Wing Disc Gene of the Asian Citrus Psyllid, *Diaphorina citri* Disrupts Adult Wing Development and Increases Nymph Mortality. *PLOS One* 8: e25677.

Gaj T, Gersbach CA, Barbas III CF. (2013) ZFN, TALEN, and CRISPR/CAS-based methods for genome engineering. *Trends in Biotechnology* Vol. 31-No.7:397-405.

Gleba YY, Giritch A. (2011) Plant viral vectors for protein expression. *In Recent Advances in Plant Virology,* ed. C Caranta, MA Aranda, M. Tepfer, JJ Lopez-Moya. pp. 387-412. Norfolk, UK: Caister Acad. Press. 412pp.

Hajeri, S., Killiny, N., El-Mohtar, C., Dawson, W.O., Gowda, S. (2014) Citrus tristeza virus-based RNAi in citrus plants induces gene silencing in Diaphorina citri, a phloem-sap sucking insect vector of citrus greening disease (Huanglongbing). *J. Biotechnol.* 176, 42-49.

Harmon A. (2013) A Race to Save the Orange by Altering Its DNA. *The New York Times*, July 27, 2013.

Hefferon KL. (2012) Plant virus expression vectors set the stage as production platforms for biopharmaceutical proteins. *J. Virol.* 433:1-6.

Hefferon K. (2014) Plant virus vector development: new perspectives. *BioMed Research International* Vol. 2014, Article ID 785382.

Jaganathan D, Ramasamy K, Sellamuthu G, Jayabalan S, Venkataraman G. (2018) CRISPR for Crop Improvement: An Update Review. *Frontiers in Plant Science*. 9:985. doi:10.3389/fpls.2018.00985.

Katoch R. and Thakur N. (2013) Advances in RNA interference technology and its impact on nutritional improvement, disease and insect control in plants. *Appl. Biochem. Biotechnol.* 169:1579-1605.

Knott GJ, Doudna JA. (2018) CRISPR-Cas guides the future of genetic engineering. Science 361:866-869.

Lindbo J. A. (2012) A historical overview of RNAi in plants. *Methods Mol. Biol.* 984:1-16.

Liu W, Yuan JS, Stewart Jr CN. (2013) Advanced genetic tools for plant biotechnology. *Nature Rev. Genet.* 14:781-793.

Montgomery M. K (2004) RNA interference: historical overview and significance. *Methods Mol. Biol.* 265:3-21.

Mohanta TK, Bashir T, Hashem A, Abd_Allah EF, Bae H. (2017) Genome Editing Tools in Plants. *Genes*. 8(12):399. doi:10.3390/genes8120399.

Mushtaq M, Bhat JA, Mir ZA, Sakina A, Ali S et al. (2018) CRISPR/Cas approach: A new way of looking at plant-abiotic interactions. Journal of Plant Physiology. 224–225:156-162, https://doi.org/10.1016/j.jplph.2018.04.001.

Newell-McGloughlin M. and E. Re. (2007) The evolution of Biotechnology. From Natufians to Nanotechnology. Springer. The Netherlands.

Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. (2013) Genome engineering using the CRISPR-Cas9 system. *Nature protocols* 8:2281-2308.

Sampson TR, Weiss DS. (2013) Exploiting CRISPR/Cas systems for biotechnology. *Bioassays* 36:34-38.

Saurabh S, Vidyarthi AS, and Prasad D. (2014) RNA interference: concept to reality in crop improvement. *Planta* 239:543-564.

Silva MS, Arraes FBM, Campos M deA, Grossi-de-Sa M, Fernandez D et al. (2018) Potential biotechnological assets related to plant immunity modulation applicable in engineering disease-resistant crops. Plant Science. 270:72-84, https://doi.org/10.1016/j.plantsci.2018.02.013.

Upadhyay SK, Kumar J, Alok A, Tuli R. (2013) RNA-Guided Genome Editing for Target Gene Mutations in Wheat. *Genes, Genomes, Genetics* Vol. 3:2223-2228.

Wang M, Weiberg A, Lin F-M, Thomma B P H J, Huang H-D, Jin H. (2016) Bidirectional cross-kingdom RNAi and fungal uptake of external RNAs confer plant protection. Nature Plants 2, article 16151.

Weiberg A, Bellinger M, and Jin H. (2015) Conversations between kingdoms: small RNAs. *Curr. Opin. Biotechnol.* 32:207-215.

Westra ER, buckling A, and Fineran PC (2014) CRISPR-Cas systems: beyond adaptive immunity. *Nature Reviews Microbiology* 12:317-326.

Zhang K, Raboanatahiry N, Zhu B, Li M. (2017) Progress in Genome Editing Technology and Its Application in Plants. *Frontiers in Plant Science*. 8:177. doi:10.3389/fpls.2017.00177.

Zhang B, Unver T. (2018) A critical and speculative review on microRNA technology in crop improvement: Current challenges and future directions. Plant Science, 274:193-200, https://doi.org/10.1016/j.plantsci.2018.05.031.

Zotti, M., dos Santos, E. A., Cagliari, D., Christiaens, O., Taning, C. N. T., and Smagghe, G. (2017). RNAi technology in crop protection against arthropod pests, pathogens and nematodes. Pest Manag. Sci. 74, 1239–1250. doi: 10.1002/ps.4813

PAPER SUGGESTIONS FOR DISCUSSIONS:

Small RNAs as crucial regulators, mechanism and applications

1. Molnar A, Melnyk CW, Basset A, Hardcastle TJ, Dunn R, Baulcombe DC. (2010) **Small Silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells.** *Science* 328:872-875.

2. Qutob D, Chapman BP, Gijzen M. (2013) **Transgenerational gene silencing causes gain of virulence in a plant pathogen**. *Nature Communications* 4:1349, DOI: 10.1038/ncomms2354.

3. Li F, Pignatta D, Bendix C, Brunkard JO, Cohn MM, Tung J, Sun H, Kumar P, and Baker B. (2012) **MicroRNA regulation of plant innate immune receptors**. *PNAS* 109: 1790–1795.

4. Weiberg A, Wang M, Lin F-M, Zhao H, Zhang Z, Kaloshian I, Huang H-D, Jin H. (2013) **Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways**. *Science* 342: 118-123.

5. Cao M, Du P, Wang X, Yu Y-Q, Qiu Y-H, Li W, Gal-On A, Zhou C, Li Y, Ding S-W. (2014) **Virus infection triggers widespread silencing of host genes by distinct class of endogenous siRNAs in *Arabidopsis***. *PNAS* 111:14613-14618.

6. Hajeri S, Killiny N, El-Mohtar C, Dawson WO, Gowda WO (2014). **Citrus tristeza virus-based RNAi in citrus plants induces gene silencing in Diaphorina citri, a phloem-sap sucking insect vector of citrus greening disease (Huanglongbing).** *Journal of Biotechnology* 176:42-49.

7. Niu D, Lii YE, Chellappan P, Lei L, Peralta K, Jiang C, Guo J, Coaker G, Jin H. (2016) **miRNA863-3p sequentially targets negative immune regulator ARLPKs and positive regulator SERRATE upon bacterial infection.** *Nature communications* 7:11324.

New approaches to gene manipulation. Strategies for targeted genome editing. CRISPR/Cas systems

8. Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D.A., and Horvath, P. (2007). **CRISPR provides acquired resistance against viruses in prokaryotes**. *Science* 315, 1709–1712.

9. Samuel H. Sternberg, Sy Redding, Martin Jinek, Eric C. Greene & Jennifer A. Doudna (2014). **DNA interrogation by the CRISPR RNA-guided endonuclease Cas9.** *Nature* 507:62-67.

10. Sapranauskas, R., Gasiunas, G., Fremaux, C., Barrangou, R., Horvath, P., and Siksnys, V. (2011). **The Streptococcus thermophilus CRISPR/Cas system provides immunity in Escherichia coli.** *Nucleic Acids Res.* 39, 9275–9282.

11. Vladimir Nekrasov, Brian Staskawicz, Detlef Weigel, Jonathan D G Jones & Sophien Kamoun (2013). **Targeted mutagenesis in the model plant *Nicotiana benthamiana* using Cas9 RNA-guided endonuclease.** *Nature biotechnology* 31(8): 691-693.

5. Assessment:

Exams – Mid-term exam (30% of student's grade); Final exam (45% of grade)

Participation in class discussions – 25% of grade

6. Attendance and Make-up Policy:

Students are expected to attend all course lectures and in-class discussions as well as complete required assignments on time. Students should arrive to the class on time. No cell phone use is allowed in the class. Along with the fact that these are firm requirements for participating in the course, the ability to fulfill these expectations reflects your professional characteristics. If you unable to attend a class due to illness or an emergency, you must notify the instructor as soon as possible, preferably prior to the scheduled class. If you miss an exam for a valid and documented reason, a make-up exam will be scheduled with permission from the instructor. These requirements for class attendance and make-up exams, assignments, and other work in this course are consistent with university policies that can be found at:

https://catalog.ufl.edu/ugrad/current/regulations/info/attendance.aspx

7. Accommodations for Students with Disabilities

The Disability Resource Center coordinates the needed accommodations of students with disabilities. This includes registering disabilities, recommending academic accommodations within the classroom, accessing special adaptive computer equipment, providing interpretation services and mediating faculty-student disability related issues. The respective students should first register with the Disability Resource Center at 0001 Reid Hall, 352-392-8565, www.dso.ufl.edu/drc/ and provide appropriate documentation.

8. **Required and Recommended Textbooks:**

Although there is no required textbook, students are expected to read all the reading materials provided by the instructor, which will include review and research articles. Those will be provided in electronic format. Among those materials will be papers selected specifically for in class discussions that students are expected to study in details in order to actively participate in discussions.

9. Grades and Grade Points:

In accordance with current University of Florida policy, grade points will be assigned as follows:

Letter Grade	Grade Points	Percentage
A	4.0	90 or above
A-	3.67	87-89
B+	3.33	84-86
B	3.0	80-83
B-	2.67	77-79
C+	2.33	74-76
C	2.0	70-73
C-	1.67	67-69
D+	1.33	64-66
D	1.0	60-63
D-	0.67	57-59
E	0.0	56 or below
WF	0.0	NA
I	0.0	NA
NG	0.0	NA
S-U	0.0	NA

Detailed and up-to-date information on UF grades and grading policies can be found at https://catalog.ufl.edu/ugrad/current/regulations/info/grades.aspx

10. On-line course evaluation:

According to the UF Policy on Course Syllabi, "students are expected to provide feedback on the quality of instruction in this course by completing online evaluations at https://evaluations.ufl.edu. Evaluations are typically open during the last two or three weeks of the semester, but students will be given specific times when they are open. Summary results of these assessments are available to students at https://evaluations.ufl.edu/results/."

11. Materials and supplies fees:

None

12. Academic Honesty

As a student enrolled at the UF, you committed yourself to the highest standards of honesty and integrity required by the honor code. You are expected to be consistent with this commitment. The following is the UF Honor Pledge: "We, the members of the University of Florida community, pledge to hold ourselves and our peers to the highest standards of honesty and

integrity." As it is stated by the UF student honor code, "all work submitted for credit by students at the university, the following pledge is either required or implied: On my honor, I have neither given nor received unauthorized aid in doing this assignment. The university requires all members of its community to be honest in all endeavors. A fundamental principle is that the whole process of learning and pursuit of knowledge is diminished by cheating, plagiarism and other acts of academic dishonesty. In addition, every dishonest act in the academic environment affects other students adversely, from the skewing of the grading curve to giving unfair advantage for honors or for professional or graduate school admission. Therefore, the university will take severe action against dishonest students. Students should report any condition that facilitates dishonesty to the instructor, department chair, college dean, Student Honor Council or Student Conduct and Conflict Resolution in the Dean of Students Office." (Source: 2013-2014 Undergraduate Catalog).

It is expected that you will complete all work independently unless the assignment is designed as a group project as explicitly indicated by the instructor.

This policy will be firmly upheld at all times during this course.

For more information regarding academic honesty and student responsibilities, please see: http://www.dso.ufl.edu/sccr/process/student-conduct-honor-code/

Software Use:

All faculty, staff and students of the university are required and expected to obey the laws and legal agreements governing software use. Failure to do so can lead to monetary damages and/or criminal penalties for the individual violator. Because such violations are also against university policies and rules, disciplinary action will be taken as appropriate.

Campus Helping Resources

The university's counseling resources are available for students experiencing personal problems that interfere with their general well-being and/or academic performance. The Counseling & Wellness Center provides confidential counseling services at no cost for students that are currently enrolled with the university.

- University Counseling & Wellness Center, 3190 Radio Road, 352-392-1575, www.counseling.ufl.edu/cwc/
    - Counseling Services
    - Groups and Workshops
    - Outreach and Consultation
    - Self-Help Library
    - Training Programs
    - Community Provider Database

- Career Resource Center, First Floor JWRU, 352-392-1601, www.crc.ufl.edu/