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DIVISION OF AGRICULTURE

Department of
Crop, Soil, and Environmental Sciences

UNIVERSITY of ARKANSAS
DALE BUMPERS COLLEGE
of AGRICULTURAL, FOOD AND LIFE SCIENCES

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Prof. Aditya Mukherjee, Director
Jawaharlal Nehru Institute of Advanced Study
Jawaharlal Nehru University
New Delhi 110067, India

March 8, 2011

Dear Prof. Mukherjee,

I am herewith submitting my application for a long-term fellowship (3 months) at Jawaharlal Nehru Institute of Advanced Study. This application package consists of my curriculum vitae, a research/ teaching proposal, and names of two referees. I have communicated with Prof. Neera Bhalla-Sarin at the School of Life Sciences, JNU, on my proposed activities during this fellowship. As outlined in my CV, I have over 15 years of research experience in plant biotechnology, first as a postdoc then as a University professor. Since 2001, I have been leading my own research group as a faculty member (currently Associate Professor) in the University of Arkansas. I have mentored graduate students and postdocs in plant biotechnology projects funded by the US federal agencies such as National Science Foundation and USDA. My expertise is specifically in the development of precise transformation technologies for genetic engineering of plant genomes. In past 10 years, I have published over 20 peer-reviewed journal articles and two book chapters on this topic, and received a number of invitations to present seminars in both international and national conferences.

In the last page of my CV, I have given names of two referees, Drs. David Ow and Andy Pereira. Dr. Ow was my advisor during my tenure as postdoctoral fellow at the University of California-Berkeley. He recently moved to China to start a new institute, but I continue to collaborate with him. Dr. Pereira is a colleague and a senior professor in my department at the University of Arkansas. He is most familiar with my professional activities on day to day basis. Both, Drs. Ow and Pereira will be able to comment on my progress through years and current status.

I must say that the JNIAS fellowship would have a special meaning for me as I am an alumnus of JNU, where I received PhD degree under Prof. Sipra Mukherjee. I look forward to the opportunity for interacting with the students at SLS and collaborating on research projects with the faculty. If you have more questions or need any clarification, please contact me at vibhas@uark.edu.

Sincerely,

Vibha Srivastava

Vibha Srivastava, Ph.D.
Associate Professor of Plant Biotechnology

VIBHA SRIVASTAVA, Ph.D.

Department of Crop, Soil & Environmental Sciences,
and Department of Horticulture
University of Arkansas, Fayetteville, AR

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PROFESSIONAL EXPERIENCE

July 2006--: **Associate Professor** (appointed into two departments) Department of Crop, Soil and Environmental Sciences, Department of Horticulture, University of Arkansas, Fayetteville, AR.

2001-2006: **Assistant Professor** (appointed into two departments) Department of Crop, Soil and Environmental Sciences, Department of Horticulture, University of Arkansas, Fayetteville, AR.

1996-2001: **Post-doctoral Fellow**, Plant Gene Expression Center, University of California, Berkeley, CA. Supervisor: Dr. David W. Ow.

1993-1995: **Post-doctoral Fellow**, University of Florida, Gainesville, FL. Supervisor: Dr. Indra K. Vasil.

1989-1990: **Visiting Research Fellow**, Freie Universitat, West Berlin, Germany. Supervisor: Dr. Otto Schieder.

1986-1990: **Graduate Research Fellow**, School of Life Sciences, Jawaharlal Nehru University, New Delhi. Supervisor: Dr. Sipra Guha-Mukherjee.

EDUCATION

Ph.D. (1991) Plant Biotechnology, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India (Supervisor: Prof. Sipra Guha-Mukherjee)

M.Sc. (1985) Biochemistry, G.B. Pant Univ. of Agric. and Tech., Pantnagar, India (Supervisor: Dr. D. N. Mishra)

B. Sc. (1983) Chemistry (major), Dayalbagh Educational Institute (DEI), Agra, India.

FUNDED PROJECTS (last 5 years)

Federal:

1. NSF, Evolutionary and Developmental Mechanisms of Embryo Dormancy in Rice, $487,659.00; 2010 – 2013 (Role: co-PI).
2. USDA-NIFA-CBG, Terminator-less transgene constructs for efficient gene silencing in crop plants; $500,000; 2010 – 2013; Role: co-PI.
3. USDA-NIFA-BRAG, *Recombinase-Mediated Targeted Gene Integration and Excision for Marker-free Transgenic crops,* $365,214; 2010 – 1013; Role: PI
4. *NSF-EPSCoR* Role of Ascorbate in Mitigating ER and Cellular Stress Associated with Transient and Stable Plant-Based Protein Production. Total Amount: $75,953; 2008 – 2010; *Role: Co-PI*
5. *USDA-CSREES-BRAG*, *Recombinase-Mediated Targeted Gene Integration and Excision for Marker-free Transgenic crops.* Total amount: $324,137; 2006 – 2010; *Role: PI*.
6. *USDA- CSREES-NRI*, *Site-specific Gene Integration in Plants*. Total amount: $160,000; 2003 – 2007; *Role: PI.*

State:

1. Arkansas Bioscience Institute (ABI) competitive grant, *A novel gene suppression system for agricultural and medical applications*. Total amount: $44,885. 2009 - 2010. *Role: PI*.

2. Arkansas Bioscience Institute (ABI) competitive grant, *Unraveling Epigenetic Mechanisms of Gene Expression for Agricultural and Medical Applications*. Total amount: $224,577. 2006 - 2009. *Role: PI*.

3. Arkansas Bioscience Institute (ABI) competitive grant, *New Technologies for Precise Genetic Modification of Crop Plants.* Total amount: $128,844. 2002 - 2005. *Role: PI*.

PATENT

U.S. Patent No. 6,114,600 (Ow D and Srivastava V) *"Resolution of Complex Integration Patterns to Obtain Single Copy Transgenes".*

AWARDS/ FELLOWSHIPS

Visiting Associate Professor, University of Geneva, Switzerland (Sabbatical research in Prof. Jerzy Paszkowski's group), 2011 (pending)

John W. White Outstanding Research Team Award, Division of Agriculture, University of Arkansas, 2005.

Research Scholarship, Department of Biotechnology, Govt. of India, to work at Freie Universitat, West Berlin, Germany, 1989 -1990.

Senior Research Fellow, Council of Scientific and Industrial Research, India, 1988 -1990.

Junior Research Fellow, Council of Scientific and Industrial Research, India, 1986 -1988.

PROFESSIONAL ACTIVITIES

Editorship and Advisory

1. Associate Editor, Crop Science, 2006---present
2. Scientific Advisory Committee member, Public Intellectual Property Resources for Agriculture (PIPRA), a Rockefeller foundation funded project, 2004--

Memberships

1. American Society of Plant Biologists
2. Society of In Vitro Biology

Active Collaborations:

1. Inez Slamet-Loedin, IRRI, Philippines
2. David Gidoni, The Volcani Center, Bet-Dagan, Israel.
3. Xingyou Gu, South Dakota State Univ., Brookings, SD.
4. Kan Wang, Iowa State University, Ames, IA.
5. Jiangqi Wen, Noble Foundation, Ardmore, OK.
6. Mariya Khodakovskaya, University of Arkansas-Little Rock, AR
7. Muthusamy Manoharan, UA-Pine Bluff, AR

INVITED TALKS (last 5 years)

1. **Plant Transformation Technologies II**, Feb 18-22, 2011, Vienna, Austria. Title: FLP-*FRT* Mediated Site-specific Gene Integration In Rice.
2. **2011 symposium on Plants for the Future**, University of Missouri, May 24 – 27, 2011, Columbia, MO, (to be delivered, tentative title: Applications of site-specific recombination systems in plant transformation).
3. **JNU, Delhi,** UGC Network Programme Winter Workshop 2010 Modern Biology & Development Of Research Programme 13th - 16th December 2010. Title: Site specific recombination based transformation technologies.
4. **IAPB**, June 7th, 2010, St Louis, MO. Title: Gene Dosage Dependent Expression from Direct Repeat Locus in Rice Developed by Site-Specific Gene Integration.
5. **Syngenta Inc**., June 12th, 2009, Research Triangle Park, NC. Title: "Site-specific Recombination Based Plant Transformation Technologies".
6. **International Rice Research Institute** (IRRI), Las Banos, Philippines, May 12th, 2009. Title: "Rice transformation using Cre/*lox* Technology"
7. **University of Arkansas**, Little Rock, Department of Applied Sciences, Mar 9th, 2008. Title: "Role of DNA methylation in gene expression of Arabidopsis Phytochrome A gene".
8. **South Dakota State University**, Department of Plant Sciences-NCRAL seminar series, Brookings, SD, Oct 20th, 2008. Title: "Targeted gene integration and role of DNA methylation in epigenetic modification of plant genome".
9. **World Congress on In Vitro Biology**, Tucson, AZ, June 14 – 18, 2008. Title: "Plant Genome Manipulation using Cre/*lox* Technology".
10. **XVI Plant & Animal Genome Conference**, San Diego, CA, Jan 12 - 16, 2008. Title: "Site-Specific Recombination Mediated Genetic Engineering Of Rice".
11. **Oklahoma State University**, Department of Biochemistry and Molecular Biology, Stillwater, OK, Sept. 7th, 2007. Title: "Site-specific recombination for Precise and Clean Transgene Integration in Plant Genome".
12. **Plant Transformation Technology Conference**, Vienna, Austria, Feb 3 - 7, 2007. Title: "Site-specific recombination for precise and clean transgene integration in plant genome"
13. **24th Symposium in Plant Biology**, University of California-Riverside. Riverside, CA, Jan 18 - 20, 2007. Title: "Exon Methylation leads to Transcriptional Silencing".
14. **Society of In Vitro Biology Annual Conference**, Minneapolis, MN, June 3-7, 2006. Title: Cre/*lox*-mediated site-specific gene integration in plants. (**Plenary talk).**
15. **Arkansas State University**, College of Agriculture, Nov 9, 2005. Title: Gene Targeting in Plants for Biotechnological and Genomics Applications.
16. **47th Maize Genetics Conference**, Lake Geneva, WI, March 10-13, 2005. Title: Site-specific integration for consistent transgene expression in rice.

TEACHING AND ADVISING

Courses

1. Instructor: **Plant Genetic Engineering** (CSES 5233), a graduate level course taught in the spring semesters of odd years.
2. Instructor: **Plant Molecular Biology Journal Club**
3. Advisor, Undergraduate program, **Crop Biotechnology**.
4. Supervisor, Plant Tissue Culture core facility.
5. Frequent guest lecturer in Crop Science and Horticulture courses.

Graduate Students

1. Luis Maas, M.S., 2003. Moved to Cornell Univ for PhD.
2. Magnolia Ariza-Nieto, Ph.D., 2004. Moved to USDA, Ithaca, NY as postdoc.
3. Parthiban Radhakrishnan, M.S., 2004. Moved to Ceres Inc., Thousands Oaks, CA.
4. Abhilasha Khattri, M.S., 2006. Moved to M D Anderson Medical Center, Houston, TX.
5. Aydin Akbudak, Ph.D. currently a postdoc in my group
6. Scott J. Nicholson, Ph.D.; moved to USDA, Stillwater OK as posdoc.
7. Gulab Rangani, Ph.D. candidate since Aug 2006 (expected graduation date: May 2011)
8. Linh Nguyen, MS student since Sept 2010-

Postdoc and Research Technicians

1. M. Aydin Akbudak, 2010 ---
2. Jamie Underwood, Research Specialist, 2008--
3. Soumen Nandy, Postdoc, 2007 ---
4. Scott J. Nicholson, Postdoc, 2008—2010
5. Anjali More, Research Technician, 2006 - 2008
6. Rekha Chawla, Postdoc, 2004 – 2006.
7. Sarah Moore, Research Technician, 2002 – 2007.
8. Andrea Wilson, Research Technician, 2001 - 2004.

PUBLICATIONS (* corresponding author)

1. Nicholson SJ, Hoecker U, *Srivastava V (2011) A novel Phytochrome B allele in *Arabidopsis thaliana* exhibits partial mutant phenotype: a short deletion in N-terminal extension reduces Phytochrome B activity. ***Plant Growth Regulation*** DOI: 10.1007/s10725-011-9576-z.

2. Akbudak MA and *Srivastava V (2011) Improved FLP recombinase, FLPe, efficiently removes marker gene from transgene locus developed by Cre-lox mediated site-specific gene integration in rice. ***Mol. Biotechnol.*** 10.1007/s12033-011-9381-y.

3. Nandy S and *Srivastava V (2010) Site-specific gene integration in rice genome mediated by the FLP-FRT recombination system. ***Plant Biotech J***. doi: 10.1111/j.1467-7652.2010.00577.x

4. Khattri A, Nandy S, and *Srivastava V (2011) Heat-inducible Cre-lox system for marker excision in transgenic rice. ***J. Biosciences*** DOI 10.1007/s12038-011-9010-8.

5. *Srivastava V and Gidoni D (2010) Site-specific gene integration technologies for crop improvement. **In Vitro Cellular and Developmental Biology - Plant** 46:219–232.

6. Akbubak MA, More, A, Nandy S, and *Srivastava V (2010) Dosage-dependent gene expression from direct repeat locus in rice developed by site-specific gene integration. ***Mol. Biotechnol.*** 45:15–23.

7. Nicholson SJ and *Srivastava V (2009) Transgene constructs lacking transcription termination signal induce efficient silencing of endogenous targets in Arabidopsis. ***Mol. Genet. Genom***. 282: 319-328.

8. *Gidoni D, Srivastava V, and Carmi N (2008) Site-specific excisional recombination approaches for agricultural biotechnology. **In Vitro Cellular and Developmental Biology-Plant** 44: 457-467.

9. Srivastava V (2008) *Book review: Breeding major food staples.* ***J. Environ. Qual*****.** 37: 1666.

10. Moore SK and *Srivastava V (2008) *A bacterial haloalkane dehalogenase (dhlA) gene as conditional negative selection marker for rice callus cells.* **In Vitro Cellular and Developmental Biology –Plant 44: 468-473.**

11. Chawla R, Nicholson, SJ, Folta, KM, and *Srivastava V (2007) *Transgene-induced silencing of Arabidopsis phytochrome A gene via exonic methylation*. ***Plant Journal*** 52: 1105 – 1118.

12. Nicholson SJ, and *Srivastava V (2006) Cre/lox technologies for plant transformation. ***CAB Reviews***: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 2006 1, No. 034.

13. Moore SK and *Srivastava V (2006) Efficient Deletion of Transgenic DNA from Complex Integration Locus of Rice Mediated by Cre/lox Recombination System. ***Crop Sci.*** 46: 700 - 705.

14. Chawla R, Ariza-Nieto M, Wilson AJ, Moore SK, and *Srivastava V (2006) Transgene expression produced by biolistic-mediated, site-specific gene integration is consistently inherited by the subsequent generations. ***Plant Biotech J.*** 4: 209 – 218. **(Cover Article).**

15. Radhakrishnan P and *Srivastava V (2005) Utility of the FLP-FRT recombination system for genetic manipulation of rice. ***Plant Cell Rep.*** 23: 721 - 726.

16. *Srivastava V and Ow DW (2004) Marker-free site-specific gene integration in plants. ***Trends in Biotechnol.*** 12: 627 - 630.

17. *Srivastava V, Ariza-Nieto M and Wilson A (2004) Cre-mediated site-specific gene integration for consistent gene expression. ***Plant Biotech. J.*** 2: 169 -179.

18. *Srivastava V and Ow DW (2003) Rare instances of Cre-mediated deletion product maintained in transgenic wheat. ***Plant Mol. Biol.*** 52: 661 - 668.

19. Srivastava V and *Ow DW (2001) Site-specific gene integration in rice. ***Mol. Breed.*** 8: 345 - 350.

20. Srivastava V and *Ow DW (2001) Single copy primary transformants of maize obtained through the co-introduction of a recombinase-expressing construct. ***Plant Mol. Biol.*** 46: 561 - 566.

21. Srivastava V, Anderson OA, *Ow DW (1999) Single-copy transgenic wheat generated through the resolution of complex integration patterns. ***Proc. Natl. Acad. Sci. (USA)*** 96: 11117 - 11121.

22. Judova J, Ow DW, Srivastava V, McBride K, *Simuth J (1998) Agrotransformation of plants with cDNAs encoding honeybee proteins. Chemical Papers-**Chemicke Zvesti** 52: 584.

23. Srivastava V, Vasil V and *Vasil IK (1996) Molecular characterization of the fate of transgenes in transformed wheat. ***Theor. Appl. Genet.*** 92: 1031 - 1037.

24. Altpeter F, Vasil V, Srivastava V, *Vasil IK (1996) Integration and expression of the high-molecular weight glutenin subunit 1Ax1 gene into wheat. ***Nat. Biotechol.*** 14: 1155 - 1159.

25. Altpeter F, Vasil V, Srivastava V, Stoger E, *Vasil IK (1996) Accelerated production of transgenic wheat. ***Plant Cell Rep.*** 16: 12 - 17.

26. John SJ, Srivastava V, *Guha-Mukherjee S. (1995) Cloning and sequencing of chickpea cDNA coding for threonine deaminase. ***Plant Physiol.*** 107:1023-1024.

27. Vasil V, Srivastava V, Castillo AM, Fromm ME and *Vasil IK (1993) Rapid production of transgenic wheat plants by direct bombardment of cultured embryos. ***Bio/Technol.*** 11: 1553-1558.

28. Srivastava V and *Guha-Mukherjee S (1992) Polyamine levels in crown gall tumor and teratoma. ***Phytochem.*** 31: 3357 - 3358.

29. Srivastava V and *Guha-Mukherjee S (1992) Phosphatidylinositol turnover in crown gall tumor. ***Phytochem.*** 31: 773 - 774.

30. Reddy AS, Srivastava V and *Guha-Mukherjee S (1989) A tandemly repeated DNA sequence from B. juncea. ***Nucl. Acid. Res.*** 17: 5849.

31. Srivastava V, Reddy, AS and *Guha-Mukherjee S (1988) Transformation and regeneration of Brassica oleracea mediated by an oncogenic *Agrobacterium tumefaciens*. ***Plant Cell Rep.*** 7: 504 - 507.

Book Chapters:

1. *Srivastava V, Akbudak MA, Nandy, S. Marker-free plant transformation. In: **Plant Transformation Technology Revolution in Last Three Decades**. Bentham Science Publishers (Eds: Y. Dan and D. W. Ow). (in press).

2. *Srivastava V and Ow D. Site-specific recombination for precise and clean transgene integration in plant genome. **"Plant Transformation Technologies"** Blackwell publishing (Eds: C. Neal Stewart, Jr., Alisher Touraev, Vitaly Citovsky, and Tzvi Tzfira) (in press).

References:

(1) David W. Ow, Director, Gene Engineering Center, South China Botanical Garden, Guangzhou, China (Email: dow@scbg.ac.cn)

(2) Andy Pereira, Professor, Department of Crop Soil & Environmental Sciences, University of Arkansas, Fayetteville, AR, USA (Email: apereira@uark.edu)

Proposed activities during the JNIAS fellowship

(1) Research:

In collaboration with faculty at SLS, especially Prof. Neera Bhalla-Sarin, I would like to work on the following two focus areas. I have over 15 years of experience in developing plant genetic engineering technologies, which are useful for a wide range of plant species. In recent years, I have collaborated with scientists in the USA, Philippines, and Australia in implementing these technologies into their transformation platforms. As these technologies are not patented, there is no legal hurdle in transferring them to other countries/ agencies. The background and brief description of the proposed research and technology development is given below.

1.1 Focus area 1: plant biotechnology: The technologies for genetic modification of plants were developed more than 25 years ago; however, myths and fear among public towards genetically modified (GM) or transgenic crops has hampered the full-fledged application of GM plants in society. Many countries have developed strict regulations on the release of GM crops in field, which is indeed a good step towards preventing any mishap involving transgenic crops. While public at large stays skeptical about the potential of GM technologies, it is clear to scientists, economists, and policy makers that GM crops are essential for meeting the future needs of food, fiber, feed, and biofuel (*Borlaug, 2000, 2007 and references therein*).

This project is focused on developing 'clean' plant genetic engineering approaches (*Srivastava and Ow, 2010*). In the conventional genetic engineering methods, both 'intended' and 'unintended' modifications are introduced into the plant genome (*Chilton and Que, 2003; Tzfira et al., 2003; and references therein*). While the effect of the unintended modifications may not be apparent in the first generation transgenic plants, their presence may generate undesirable 'pleiotroic' effects in advanced generations. Therefore, it is best to keep them out. Further, the use of selectable marker genes is a common practice in plant genetic engineering as they play an important role in streamlining the process. However, their presence in the transgenic plants is undesirable (*Lemaux, 2009*). European governments mandate their removal prior to the release of the transgenic plants in the field (FDA guidance document, 1998); other countries are also supporting this move.

Within this project, we will test the use of site-specific recombination systems in the Indian crops such as *Vigna mungo* for developing 'clean' transformation technology. Specifically, the project will test the removal of selectable marker genes from transgenic plants using Cre-*lox* recombination system.

1.2 Focus area 2: plant genomics: Identification of agriculturally important genes (*aig*) is a pre-requisite for developing GM crops for future. For example, drought tolerant crops can be developed by introducing genes encoding drought tolerance from weedy species into cultivated species. Genome sequencing projects have annotated a number of *aig* from crop or weedy species. The next step is to validate their function before they can be used for genetic engineering. Again, plant transformation is the most effective approach for gene function validation, in which, the isolated gene is over-expressed in a model plant, and the resulting transgenic plant is tested for the expression of the novel trait. The problem associated with this

approach is that different transgenic plants express the foreign gene at different levels, and the additional 'unintended' modifications that occur during the process may interfere with the analysis of the gene function. This project proposes to develop an approach in which the incorporation of the foreign gene into the model plant genome is reversible. Therefore, if over-expression of the gene alone does not clearly establish the gene function, a subsequent gene-removal step can be taken to test if the trait would also be reversed. This method will allow establishing a clear association of the trait with the presence of the candidate gene.

We will use site-specific recombination system, Cre-*lox*, for developing a novel gene-function-validation system consisting of over-expression of candidate gene with the option of its removal from the host genome. If the expressed trait is reversed upon gene removal the function of the gene will be established beyond any doubt.

1.3 Research Design: A brief description of the project approach and research design is given below. Upon receiving approval from JNIAS and JNU, the detailed research plan will be developed in collaboration with Prof. Neera Bhalla-Sarin.

2.3.1 Plant biotechnology project: Prof. Sarin is working on developing transgenic plants of legumes (*Vigna mungo*) or *Brassica* species that express salt tolerance, a trait highly desirable for meeting the challenges of climate change. In past 10 years, I have developed an efficient 'clean' transformation method, based on Cre-*lox* recombination system, for plants using rice as the model (Srivastava and Ow, 2010; see the list of publication in my CV). Thus, I propose to introduce the 'clean' transformation technologies into Prof. Bhalla-Sarin's program. Collaboration with other SLS faculty will also be open. Specifically, I will provide materials and technical help in developing DNA vectors that contain Cre-*lox* components. Use of these vectors for introducing salt tolerance genes into plant species will allow removal of selectable marker genes to generate marker-free 'clean' transgenic plants.

2.3.2 Plant genomics project: Within this project, DNA vectors suitable for the over-expression of candidate genes for abiotic stress tolerance will be constructed. Again, components of Cre-*lox* recombination system will be integrated into this vector to allow excision or inversion of the candidate gene. The vector will have gateway cloning system to allow rapid cloning of a range of candidate genes. Most gene function validations are done on Arabidopsis or rice. Therefore, two vector lines will be developed suitable for dicotyledonous (Arabidopsis) and monocotyledonous (rice) plants. Presence of Cre-*lox* recombination elements in this vector will allow subsequent removal of the candidate genes to test if the trait is tightly associated with the gene.

(2) Teaching:

Besides helping in the wet laboratory, I also wish to deliver lectures on topics of current interest in the areas of plant molecular biology and biotechnology. Interaction with graduate students will be an important part of my JNIAS fellowship. To enable face-to-face interaction with a large number of students, I would like to be involved in teaching a course on plant molecular genetics. Depending on the duration and the time of the fellowship, I could either teach a part of the ongoing plant molecular biology course (in collaboration with the course instructor) or I could

develop a special course. In my past visit to SLS during December 2010, I observed that many students were enthusiastic about learning advanced topics in plant biotechnology. As I already teach a similar course in my home institution, University of Arkansas, (For the course syllabus and other details see: http://cses.uark.edu/1958.htm), it will be fairly simple for me to teach plant biotechnology in SLS, JNU.

References:

Borlaug NE (2000) Ending world hunger. The promise of biotechnology and the threat of antiscience zealotry. *Plant Physiol.* 124: 487-90.

Borlaug NE (2007) Feeding a hungry world. *Science* 318: 359.

Chilton M-D and Que Q (2003) Targeted integration of T-DNA into the tobacco genome at double-strand breaks: new insights on the mechanism of T-DNA integration. *Plant Physiol.* 133: 956–965.

Lemaux P (2009) Genetically engineered plants and foods: a scientist's analysis of the issues (Part II). *Annual Review of Plant Biology* 60: 511-559.

Srivastava V, Ow DW (2004) Marker-free site-specific gene integration in plants. *Trend Biotechnol*. 22: 627-629.

Srivastava V and Ow DW (2010) Site-specific recombination for precise and 'clean' transgene integration in plant genome. In: Plant Transformation Technologies, (Eds: C. Neal Stewart, Jr., Alisher Touraev, Vitaly Citovsky, and Tzvi Tzfira) Wiley Blackwell.

Srivastava V, Akbudak MA, and Nandy, S (2011) Marker-free plant transformation. In: Plant Transformation Technology Revolution in Last Three Decades (Eds: Y. Dan and D. W. Ow). Bentham Science Publishers.

Tzfira T, Frankmen, L, Vaidya M and Citovsky V (2003) Site-specific integration of Agrobacterium tumefaciens T-DNA via double-stranded intermediates. Plant Physiol. 133: 1011–1023.