

Dr. Rachana Verma

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Doctorate degree in Biotechnology having 7 years of research experience, strong scientific skills and expertise in CRISPR/Cas9 mediated Genome editing, Molecular, Physiological, Tissue Culture and Biochemical techniques.

Research project keywords

Micronutrient malnutrition, biofortification, phytase, abiotic stress tolerance, herbicide tolerance, CRISPR/Caso based genome editing, Base Editing, Prime Editing, NUE Nutrient-Use efficiency, PUE phosphate-use efficiency, genetic engineering, nutritional improvement, crops, plant-based multi-targeted drugs, human nutrition.

Worked as National Post Doctorate Fellow DST (SERB-NPDF) in Plant Molecular BiologyGroup, ICGEB, New Delhi

Employer International Centre For Genetic Engineering And Biotechnology, New

DelhiDuration : 1st April 2018 to 30.9.2020

Project

Developing CRISPR/Cas9 edited cisgenic Cajanus cajan lines for

improvised weed management and high yields

Description:

Weed infestation is one of the major biotic stress factors responsible for yield loss in crop plants. Glyphosate (N-[phosphonomethyl]-glycine) is potent and most widely used broad-spectrum herbicide that interferes with shikimate pathway by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). In all plants and most bacterial EPSPS enzymes there is a conserved motif crucial for binding phosphoenolpyruvate (PEP) or its competitive inhibitor glyphosate, the active ingredient in Roundup. Here, we report the developing of efficient herbicide resistant pigeonpea lines by sitespecific EPSPS gene fragment replacement with insertion of desired HDR template (Homology Donor Repair) through CRISPR/Cas9 system. The CRISPR/Cas9 system has been demonstrated to efficiently induce targeted gene editing in a variety of organisms, including plants. We designed a plasmid (pCAMBIA1300 backbone) that harbours cassettes expressing plant codon optimized Caso from S. pyogenes and two Cc-EPSPS-sgRNAs. Cc-EPSPS-gRNA cassette (539 bp) was synthesized and sub-cloned via 5' BsaI/ 3' BsaI into Cas9 plasmid by Golden gateway method, renamed plasmid as pPCOCAS9-gRNA (15,757 bp). The Cas9 was under the control of Ubiquitin promoter and two gRNAs were driven by U₃ & U₆ promoters. Homology donor fragment (1084 bp) contained several features for HDR mediated mutated EPSPS gene replacement assembled in pCcU3 vector. The CRISPR/Cas9 constructs were transformed into Cajanus cajan by using gene biolistic approach. Putative To transgenic pigeonpea lines (20) were transferred to greenhouse under controlled conditions. The sgRNAs+pCas9 and HDR constructs were transformed into pigeonpea calli by using biolistic approach. Putative T₀ edited pigeonpea lines were validated by Cas9 and mEPSPS nested PCR analyses. T₁ seedlings of edited lines grew happily on media with glyphosate. Further, 30-d-old edited pigeonpea lines tolerated up to with 6-8ml/L of Roundup Ready. Endogenous shikimate levels and necrosis were quantified in these edited lines by HPLC that exhibited low shikimate levels (280-2000 µmol/ml) in comparison to treated WT (6000 μmol/ml). T₂ pigeonpea edited plants maintained enhanced photosynthetic capacity, transpiration rates, chlorophyll content and other related photosynthetic parameters like electron transport rate, maximal photosystem II efficiency (Fv/Fm) in comparison to treated-WT. LC-MS analysis showed significant enhancement in aromatic amino acid profiles. Results revealed enhanced glyphosate resistance in our edited pigeonpea lines with no yield penalty.

Keywords: CRISPR/Cas9, EPSP synthase, Homology Donor Repair, pigeonpea, mutations, short guide RNA, glyphosate resistance, shikimate, aromatic amino acids

- Data Mining by Pluralistic Approach on CRISPR Gene Editing in Plants. Front Plant Sci 10(801):1-17
- **Rachana Verma**, Jyotsna Bharti, Tanushri Kaul[,] Sonia Khan Sony, Arulprakash Thangaraj, Murugesh Easwaran, Khaled Fathy, Rapid and highly efficient biolistic transformation of Pigeon pea (*Cajanus cajan* (L.) Millsp.) utilizing cotyledonary node and embryonal axis explants, BMC Plant method. Accepted for publication.

Research Experience

Employer : National Research of Plant Biotechnology (NRCPB), New Delhi

Duration : 1st April 2017 to 31st March 2018

Project : "Indo-UK centre for improvement of nitrogen use efficiency in wheat (INEW)"

Description : • Understanding molecular mechanism of nitrogen use efficiency in wheat

• Allele mining of different nitrogen metabolism genes to enhance NUE

Employer : Indian Institute of Maize Research, New Delhi

Duration : 9th July 2015 to 31 March 2017

Project : "Genetic enhancement for low moisture stress tolerance in maize"

Description : • Understanding cellular and molecular basis of abiotic stress

• Cloning of abiotic stress related transcription factors from maize genotypes

 Identification of novel drought responsible miRNA for development ofmarkers

Employer : Div. of Plant Physiology, Indian Agricultural Research Institute

Duration : 26th February 2013 to 30th September 2013

Project : **CSIR Funded** "Analyzing role of zeaxanthin cycle pigments for

hightemperature tolerance in chickpea"

Description : • Estimation of pigments for high temperature tolerance in chickpea

• Physiological and Biochemical analysis

TLC and HPLC

Employer : Div. of Plant Physiology, Indian Agricultural Research Institute

Duration : 1st October 2009 to 31st December 2012

Project : DST Funded "Cloning and overexpression of the genes encoding

induciblehigh affinity nitrate transporter genes in Brassica juncea and

wheat"

Description : • Identification of high affinity nitrate transporter gene from

BrassicaJuncea

Clonened and sequence analysed, 5'RACE PCR

• Overexpression of NRT genes in *Brassica* and *Arabidopsis thaliana*

· Functional analysis of Transgenic plant

Physiological and Biochemical characterization of transgenic plants

Employer : Div.of entomology, Indian Agricultural Research Institute

Duration : 16th Feb 2009 to 30st September 2009

Project : NAIP Project on "Designing and studying mode of action and bio-

safety ofnano-pesticides"

Description : • Isolation of different strain of spodeptra

· Mortality assay of different strain

• Quality control of pesticides

Research/Dissertation Title

Degree : Title of the thesis

Ph.D Cloning and overexpression of inducible high affinity nitrate

transporter gene from *Brassica juncea*. Thesis research work done

at Indian Agricultural Research Institute, New Delhi

M.sc : Done 6 month dissertation (From February, 2008 to June, 2008)

on research topic entitled "Overexpression of Divinyl Reductase

in Brassica juncea"

School of Life sciences, Jawaharlal Nehru University (JNU),

New Delhi, under the supervision of Dr. B.C. Tripathi

Paper Published

Pranjal Yadava1,Vikram Dayaman1, Astha Agarwal, Krishan Kumar, Ishwar Singh, **Rachana Verma**, Tanushri Kaul (2021). Fine-tuning the transcriptional regulatory model of adaptation response to phosphate stress in maize (Zea mays L.) **Plos one**

Tanushri Kaul, Sonia Khan Sony, **Rachana Verma**, Khaled Fathy ABDEL MOTELB, Arul T Prakash, Murugesh Eswaran ,Jyotsna Bharti , Mamta Nehra and Rashmi Kaul (2020). Revisiting CRISPR/Cas-mediated crop improvement: Special focus on nutrition. **Journal of Bioscience.**

Pranjal Yadava, Chetana Aggarwal, **Rachana Verma**, Krishan Kumar, Ishwar Singh **(2020)**. Effect of nitrogen-starvation on growth pattern and expression of nitrogen assimilation related genes in maize (*Zea mays* L.). **Indian journal of Agricultural Sciences**

Kaul T, Raman NM, Eswaran M, Thangaraj A, Verma R, Sony SK, Sathelly KM, Kaul R, Yadava

- P and Agrawal **(2019).** Data Mining by Pluralistic Approach on CRISPR Gene Editing in Plants. Frontier in Plant Sci. 10(801).
- Nitya Meenakshi Raman, Murugesh Eswaran, Jyotsna Bharti, Khaled Fathy Abdel Motalb, **Rachana Verma**, Rashmi Kaul, Tanushri Kaul **(2019).** Ushering in CRISPR/Cas Mediated Genome Engineering for Crops. Scholars Academic Journal of Biosciences. 10.21276/sajb.2019.7.7.5.
- **Rachana Verma,** Jyotsna Bharti, Tanushri Kaul, Sonia Khan Sony, Arulprakash Thangaraj, Murugesh Easwaran, Khaled Fathy (2019). Rapid and highly efficient biolistic transformation of Pigeon pea (*Cajanus cajan* (L.) Millsp.) utilizing cotyledonary node and embryonal axis explants. **BMC Plant method**.
- Ngursangzuala Sailo, **Rachana Verma**, Renu Pandey and Vanita Jain (**2013**). Effect of elevated carbon dioxide on nitrogen assimilation and mobilization in wheat and rye genotypes of different ploidy level. *Indian Journal of plant physiology* **18(4)**; 333-338.
- **Verma R,** Singh AK and Jain V. (**2014**). Expression study of high affinity nitrate transporter gene from *Brassica juncea* in *Arabidopsis* T-DNA "knockout". *Research Journal of Biotechnology*. **9(10)**; 73-79.
- **Verma R,** Pandey R, Singh AK, Jain V and Nilofar R. (2015). Cloning and molecular chracterization of high-affinity nitrate transporter gene *BjNRT2.1* from Indian mustard. *Indian Journal of plant physiology* 20(1); 63-71.
- Renu pandey, Krishna Kant Dubey, Atlaf Ahmed, Rakshanada Nilofar, **Rachana Verma**, Vanita Jain, Gaurav Zinta and Vikas Kumar (**2015**). Elevated Carbon dioxide improves growth and Phosphorus utilization efficiency in cereal species under sub optimal phosphorus supply. *Journal Of Plant Nutrition* **38(8)**; 1196-1217.
- Lekshmy Nair, Ngursangzuala Sailo, **Rachana Verma**, Renu Pandey and Vanita Jain **(2016)**. Growth under elevated carbon dioxide differentially affects the growth and nitrogen metabolism in wheat seedlings supplied with ammonium and nitrate ions. *Indian Journal of Agricultural Sciences* **86(1)**; 25-30.

Gene Submission

- Jain V, Verma R, Nilofar R and Pandey R. (2012). Cloning full length cDNA of high-affinity nitrate transporter from *Brassica juncea* (L.) Czern (Indian mustard). Acc #JQ305139.1; GI:374723164
- Yadava P, **Verma R** and Singh I. (2016). Isolation & Cloning of MYB releated transcription factor from maize inbred line. **Acc # KX528335**
- Yadava P, **Verma R** and Singh I. **(2016).** Role of growth regulating factor (Angustifolia- AN3) in drought tolerance in maize. **Acc # KX494923**

Abstract published

- Jain V, Pandey Renu, **Verma R**, Sailo N and Anand Kumar P. (2010). Mobilization of nitrogen in wheat plants grown under carbon dioxide enrichment. Abstract; 5th International Nitrogen Conference on *Reactive N Management for Sustainable Development Science, Technology and Policy*, **3rd 7th December 2010**, New Delhi, pp. 292.
- Pandey R, Jain V, Singh KP, **Verma R** and Nilofar R. (2009). Root morphology of maize hybrids grown under low soil phosphorus and its impact on physiological analysis of growth. *Abst:* Zonal *Seminar on Abiotic Stress Tolerance in Plants-Physiological and Biotechnological Approaches*, Centre for Plant Biotechnology, CCSHAU New Campus, Hisar, Haryana, **5**th **December 2009**, pp-15.
- Pandey Renu, **Verma R**, Jain V, Singh KP, Khetarpal S and Kushwaha SR. (2010). Influence of low soil phosphorus on physiological growth analysis and P utilization efficiency of maize (*Zea mays* L.) in relation to grain yield. Abstract. National Conference of Plant Physiology on *Physiological and Molecular Approaches for Crop Improvement under Changing Environment*, **25-27**th **November 2010**, Banaras Hindu University. Varanasi.
- Pandey R, Gautam A, Dubey KK, Nilofar R, **Verma R** and Jain V (2012) Kinetic parameters and gene expression analysis of high-affinity phosphate transporter in response to elevated CO2 and phosphorus nutrition. Abst: 01-02, National Seminar on "Physiological and Molecular Approaches for Development of Climate Resilient Crops" **12-14 December 2012**, Acharya NG Ranga Agricultural University, Rajendranagar, Hyderabad, pp. 3.
- Sailo N, **Verma R**, Pandey R and Jain V. (2012). Nitrogen assimilation and mobilization of nitrogen in wheat genotypes under rising atmospheric carbon dioxide. Abst: 01-06, National Seminar on "Physiological and Molecular Approaches for Development of Climate Resilient Crops" **12-14 December 2012**, Acharya NG Ranga Agricultural University, Rajendranagar, Hyderabad, pp. 6.
- **Verma R,** Jain V, Singh AK, Nilofar R and Pandey R. (2012). Isolation and characterization of high affinity nitrate transporter gene from *Brassica juncea* roots grown under low nitrate supply. Abst: 02-65, National Seminar on "Physiological and Molecular Approaches for Development of Climate Resilient Crops" **12-14 December 2012**, Acharya NG Ranga Agricultural University, Rajendranagar, Hyderabad, pp 68.

Prize and Awards

- Selected for National Post Doctorate Fellowship (DST-SERB), 2017-2020
- Oualified ICAR-NET, 2016
- Invited Oral Presentation entitled "Rapid and highly efficient biolistic transformation of Pigeon pea (Cajanus cajan (L.) Millsp.) utilizing the cotyledonary node and embryonal axis explant at the 5th International Conference on Plant Genetics and Genomics in NASC Complex, PUSA, New Delhi, 17th-18th October, 2019.
- Presented Poster entitled "Unravelling low phosphorus stress responses in maize" in "Plant Stress Biology and Food Security" ICGEB Workshop, 18-19th April, 2019.
- Presented Paper on "Understanding Mechanism Underlying Maize Response to Low Phosphorus Stress"in 4th International Plant Physiology Congress, 2nd -5th December 2018 at NBRI, Lucknow.

In addition I am actively involved in

Create and conduct experiments

- · Process and analyse results and data
- Communicate results to the scientific community via published papers
- Collaborate with industry/academia to apply the results of research and develop new techniques, products or practices
- Present on going work and findings to colleagues at academic conferences, and summarise the nature of the research, methodology and results
- Carry out field work to inform research
- Teach, demonstrate to or supervise students (in academia) and train and supervise other members of staff
- Devise or help to draw up new research proposals and apply for funding
- Work in multidisciplinary teams, in different faculties or schools in academia, and in different functions of the business in industry
- Carry out peer reviews of written publications and presentations to validate theories and inform research keep up to date with the work of other scientists attend academic conferences across the world and regularly read industry.

Lab skill

Microbiology techniques, Enzyme assay, Plant genomic DNA isolation, RNA isolation, Plasmid isolation, Restriction digestion, Gene cloning, Southern hybridization, SDS-PAGE, PCR, Real time PCR, Tissue Culture and bioinformatics techniques

Masters

Passed Master of Science (M.Sc.) course in Biotechnology from T. M Bhagalpur University, Bihar with <u>76%</u> in the year 2008. This course comprises papers in Cell Biology, Genetic Engineering, Animal Cell Science & Technology, Biological Macromolecules and Basic Enzymology, Virology and Immunology, Microbial Diversity, Physiology and Genetics, Plant Biotechnology, Bioprocess Engineering and Technology, Bioresource and Environmental Biotechnology.

Bachelor's Degree

Completed Bachelor of Science (B.Sc.) from B.R Ambedkar, Bihar University with $\underline{67\%}$ in the year 2004 having, Chemistry (Honours), Botany, Zoology as major subjects.

Senior Secondary

Passed Senior Secondary school examination with <u>64%</u> from Central Board of Secondary Education (CBSE) in 1998. The papers in this course were Physics, Chemistry, Biology and English.

Secondary

Passed Secondary school examination with <u>65%</u> from Central Board of Secondary Education (CBSE) in 1996. The papers were English, Hindi, Science, Mathematics and Social Studies.

Personal Details

Name : Dr. Rachana Verma

Sex : Female

Marital status : Married

Husband's Name : Shreyansh

VermaNationality : Indian

Date of Birth : April 12, 1981

Language known : Hindi (R/W/S), English (R/W/S)

Strengths : Hardworking, Innovative, Believe in perfection, Quick learner from

mistake, Good coordination

Address : Flat no-95, Qutab View Apartment, ShaJeet Singh Marg, New

Delhi-110016

Reference

Dr. Madan Pal	Dr. Tanushri Kaul	Dr.Vanita Jain
Principal Scientist	Group Leader	Principal Scientist
	Plant Biology and Biotechnology	
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	Engineering and Biotechnology (ICGEB), New Delhi	
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Declaration

I hereby solemnly affirm that the information furnished above are true and complete to the best ofmy knowledge, please consider my humble request.