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## Academic Record

<u>Name of Institution</u>	<u>Duration of Stay</u>	<u>Principal Subject</u>	<u>Degree Awarded</u>
Jawaharlal Nehru University	2004-2009	Plant Biotechnology	Ph.D.
Indira Gandhi Agricultural University	2001-2003	Biotechnology	M.Sc.(Ag.)
Banaras Hindu University	1997-2001	Agricultural Sciences	B.Sc. (Ag.)

## Research Experience

1. Completed Ph.D. in the field of abiotic stress signalling mediated by two-component system, phosphoglycerate kinase in plants at Jawaharlal Nehru University, New Delhi, India under the guidance of Dr. Ashwani Pareek.  
Thesis title: **Molecular and Functional Characterization of a Hybrid Type Histidine Kinase and Phosphoglycerate Kinase Genes from *Oryza sativa* L.**
2. M.Sc. Thesis on the project entitled “**Molecular Differentiation of Cytoplasmic Male Sterile and Fertility Restorer Genes of *Oryza sativa* L.**” at National Centre for Plant Biotechnology, Indian Agricultural Research Institute, New Delhi, India under the guidance of Dr. Trilochan Mohapatra.

## Publications

### Research Papers

1. **Karan R**, Singla-Pareek SL, Pareek A (2009) Histidine kinase and response regulator genes as they relate to salinity tolerance in rice. **Func. Integ. Genomics. 9:** 411-417
2. Punjabi-Sabharwal V, Kushwaha HR., **Karan R.**, Joshi R, Kumari S, Singla Pareek SL, and Pareek A (2009) Transgenic tobacco plants overexpressing class I metallothionein gene OsMT1e show tolerance towards multiple abiotic stresses besides heavy metal stress. **Plant Physiology (Communicated).**
3. **Karan R**, Singla-Pareek SL, Pareek A (2009) A hybrid type histidine kinase of rice acts as an osmosensor and provides salinity tolerance to rice. (ready to communicate).
4. **Karan R**, Joshi R, Singla Pareek SL and Pareek A (2009) OsPGK2 is differentially regulated in contrasting genotypes of rice under salinity stress and its overexpression provides salinity tolerance to transgenic tobacco. (under preparation).

## Book Chapters

1. **Karan R.**, Kumari S., Yadav SK, and Pareek A. (2006) RNAi : A tool for functional genomics. Eds. A. Verma and R. Oelmuller, In “Soil Biology **11**: 131-142 Springer Verlag, Berlin, Heidelberg.
2. Punjabi-Sabharwal V, **Karan R.**, Khan T, and Pareek A. (2009) Abiotic Stress Responses: Complexities in Gene Expression. Eds. Pareek A, Sopory SK, Bohnert H and Govindjee, In “Abiotic stress adaptation in plants: Physiological, Molecular and Genomic Foundation” Springer Dordrecht, Netherlands (**In Press**).
3. Joshi R, **Karan R.**, Singla Pareek SL, and Pareek A. (2009) MICROARRAYS: The highthroughput advantage. Eds. Kumar A., Arora S. and Ambani, S. In Biotechnology in medicine and agriculture: Principles and Practices (**In Press**).

## Patent

1. Hybrid-type Histidine Kinase Gene Isolated from Indica Rice IR64, and Clones Produced Thereby.  
Indian Patent Application No. **1896/DEL/2008** dated 11/08/08  
US Patent Application No. **PCT/IN2009/000444** dated 11/08/09

## Conferences Attended/Posters/Proceedings

1. **Karan R.**, Singla-Pareek S. L., Pareek A. A Hybrid Type Histidine Kinase from *O. sativa* L. cv IR64 is Regulated by Multiple Abiotic Stresses. Annual Meeting of American Society of Plant Biologists “Plant Biology 2009”. **Hawaii, USA**, 18-22<sup>nd</sup> July 2009
2. **Karan R.**, Singla-Pareek S. L., Pareek A. Exploring the Unexplored: Two-component System and Their Role in Salinity Stress in *O. sativa* L. International Conference on “Plant Abiotic Stress Tolerance 2009”. **Vienna, Austria**, 8-11<sup>th</sup> February 2009
3. **Karan R.**, Kumar G., Singla-Pareek S. L., Pareek A. Functional and Molecular Characterization of a Hybrid Type Histidine Kinase Gene from *O. sativa* L. 2<sup>nd</sup> International Conference on “Plant Cellular and Molecular Biology” **New Delhi, India**, 2008
4. Pareek A., Kumari S., Panjabi V., **Karan R.**, Singla-Pareek SL., Sopory SK. Delineating early and late salinity stress responses in contrasting genotypes on *O. sativa* L.: a comparative transcriptome data. The 5th International Symposium of Rice Functional Genomics. Epochal Tsukulba, Japan, October 2007
5. **Karan R.**, Kumar G., Singla-Pareek S. L., Pareek A. Functional characterization of a Hybrid type Histidine kinase form *O. Sativa* L.. 75<sup>th</sup> Annual meeting: Society of Biological Chemists, India 2006.
6. Pareek A., **Karan R.**, Kumari S., Kumar G., Purty RS., Panjabi V. Understanding the gears of signalling machinery operative under salinity stress response in crop plants. 75<sup>th</sup> Annual meeting: Society of Biological Chemists, India 2006
7. **Karan R.**, Singh A., Singla-Pareek S. L. and Pareek A. Raising of functional stress Responsive mutants in *O. Sativa* L. International Conference on Plant Genomics and Biotechnology “Challenges and Oppurtunities” Raipur, Chattissgarh, **India**, 26-28<sup>th</sup> October, 2005

## Summary (Ph.D. Thesis)

**Title:** Functional and Molecular Characterization of a Hybrid Type Histidine Kinase and Phosphoglycerate Kinase Genes from *Oryza sativa* L.

Protein kinases are known to be major players in regulating the signal transduction pathway operative in cellular system. In the present work, I have isolated, characterized a Hybrid type histidine kinase, a member of two component system and Phosphoglycerate kinase from *Oryza sativa* L. which is known to be involved in glycolysis and Calvin cycle.

Hybrid type histidine kinase is a member of two-component system (TCS) that has been shown to be involved in regulation of cellular processes in response to environmental stimuli in bacteria, yeast and *Arabidopsis*. In eukaryotes, a typical two-component system consists of a sensor hybrid type histidine kinase containing transmitter domain as well as a fused receiver domain (HK), histidine phosphotransfer protein (HPT) and a regulatory protein referred to as response regulator (RR). In *Saccharomyces cerevisiae*, the osmoregulatory system mediated by the hybrid type histidine kinase, SLN1 has been well characterized and reported to be involved in osmosensing. In *Arabidopsis*, ATHK1, a hybrid type histidine kinase has been reported to be involved in osmotic stress perception and response under different abiotic stresses and also provide osmotic stress tolerance to transgenic *Arabidopsis*. Rice genome analysis has revealed the presence of fourteen histidine kinases (OsHKs), 5 phosphotransfer genes (OsHPTs), and 32 response regulator genes (OsRRs), which led to a consensus of uniform nomenclature system (Pareek et al., 2006; Schaller et al., 2007). However, it still remains to be found which member of TCS pathway may be working as osmosensor in rice.

My work involved transcriptome analysis of TCS members in contrasting cultivars of rice and identification and characterization of a putative osmosensor from rice and its role towards stress tolerance in rice. We found that all the TCS members are differentially regulated under salinity stress in two contrasting cultivars of rice and some of the members are co-localized within salinity related QTLs of rice (**Karan et al., Functional and Integrative Genomics, 2009**). Further, we have isolated OsHk3 (accession number FJ004641), a hybrid type histidine kinase of rice that functionally complemented the SLN1 mutant strain of yeast. When either conserved histidine or aspartate residue of OsHk3 is mutated then OsHk3 is unable to complement, indicating that OsHk3 is a histidine kinase and a conserved histidine in transmitter domain and aspartate residue in receiver domain is required for its activity. When histidine mutated and aspartate mutated OsHk3 is co-transformed into SLN1 mutant, they again complement the SLN1 mutant implies that the phosphotransfer reaction from histidine to aspartate occurs in trans between two OsHK3 molecules thus OsHk3 act as homodimer. As OsHk3b has a transmembrane domain, OsHk3-GFP fusion protein could be localized in plasma membrane of onion epidermal cell indicated the transmembrane property of OsHk3. OsHk3 transcript has been found to be inducible by multiple abiotic stresses such as salinity (200 mM), drought, high temperature (42°C), low temperature (4°C) as well as by exogenously applied ABA (100 µM) as revealed by qRT-PCR analysis. Translational regulation of OsHk3 by different abiotic stresses has been observed by Western using antibodies raised against the OsHk3. Developmental regulation of OsHk3 was also observed at the seedling, tillering and mature stage of field grown rice plants. As OsHk3 was found to be regulated by different abiotic stresses, 1.6 kb upstream sequence of OsHk3b was isolated and found to be inducible by different abiotic stresses when used for driving the expression of reporter gene (GUS) under salinity and drought stresses. To find out the in-plant role of OsHk3, overexpression and RNAi constructs for OsHk3 were made under the control of 35S promoter and transformed into rice callus to develop transgenic plants overexpressing and underexpressing OsHk3 respectively. Overexpressing rice plants were expressing more transcript and protein in comparison to wild type plants, whereas underexpressing (RNAi) plants were expressing less transcript and protein in comparison to wild type plants. We have also detected siRNA in RNAi lines which may be responsible for degrading the mRNA of OsHk3 further indicated the operation of RNAi machinery in rice. Leaf disc analysis of OsHk3 transgenic plants indicated the possible tolerance of transgenic rice plants towards salinity stress that was further confirmed by the analysis of various physiological parameters such as chlorophyll, proline and K<sup>+</sup>/Na<sup>+</sup> estimation. Microarray analysis of OsHk3 transgenic plants revealed the upregulation and downregulation of various abiotic stress related genes that may be responsible for providing stress tolerance to transgenic plants mediated by OsHk3. To find out the possible downstream member of OsHk3 in rice, Yeast two hybrid analysis was performed to find out which HPT of TCS may be involved in phosphorelay mediated by OsHk3. This analysis revealed the interaction of OsHk3 with its downstream TCS member, OsAHP2

(Histidine Phosphotransfer protein) indicated the possible phosphorelay of OsHk3-OsAHP2 in rice (**ready to communicate**).

Phosphoglycerate kinase (PGK) is a main player for the production of ATP during glycolysis and production of 1,3-bisphosphoglycerate to participate in the Calvin cycle for carbon fixation in plants. PGK protein has been reported to be highly upregulated under salinity stress in the seedlings of rice. Previous work in lab has reported a partial fragment of OsPGK in subtraction library constructed under salinity stress in rice, which was found to be highly regulated under salinity stress. I have further cloned a full length OsPGK from rice (accession number DQ899741) corresponding to the partial fragment. Whole genome analysis was performed using TIGR var. 5.0 of rice, revealed the presence of four OsPGK in the rice genome. OsPGK isolated from rice variety IR64 was identical to OsPGK2 of rice genome. OsPGK2 transcript was found to be differentially regulated in two contrasting genotypes of rice from 10 minutes upto 48 hours of salinity stress. OsPGK protein was also found to be differentially regulated as it could cross react with anti phosphoglycerate kinase antibodies of *Saccharomyces cerevisiae*. When OsPGK was overexpressed under 35S promoter into tobacco, it resulted into increased tolerance of OsPGK transgenic plants under salinity stress (200 mM NaCl) as could be observed by leaf disc and analysis of various physiological parameters such as chlorophyll, proline and  $K^+/Na^+$  estimation (**manuscript under preparation**).

### **Salient findings of my PhD work:**

- Most of TCS members are differentially regulated in two contrasting genotypes of rice under salinity stress.
- OsHk3 is putative osmosensor of rice.
- OsHk3 is transcriptionally and translationally regulated by multiple abiotic stresses.
- OsHk3 is localized in the plasma membrane of onion epidermal cell.
- OsHk3 transgenic rice plants harbouring overexpression and RNAi cassettes indicated the role of OsHk3 for osmotic stress tolerance.
- Microarray analysis of overexpression and RNAi rice plants indicated the altered expression of various stress responsive genes that may be regulated by OsHk3.
- OsPGK2 is a member of phosphoglycerate kinase gene family in rice.
- OsPGK2 is transcriptionally and translationally regulated by salinity stress in rice.
- Overexpression of OsPGK2 in transgenic tobacco plants enhanced tolerance towards salinity stress.

### **Summary (M.Sc. Thesis)**

**Title:** Molecular Differentiation of Cytoplasmic Male Sterile and Fertility Restorer Lines of *Oryza sativa* L.”

Development and use of hybrid rice technology can enhance the yield levels. One of the important requirements for suitable performance of rice hybrids is stability in fertility restoration behaviour across the locations. Therefore, it is necessary to transfer the fertility restorer gene (Rf) to widely adapted varieties, which produce heterotic hybrids when crossed with CMS (cytoplasmic male sterile) line. Transfer of restorer genes to desirable genetic background can be greatly facilitated by identifying markers linked to fertility restorer genes and using them in marker aided selection programme.

In the present investigation, an attempt was made to differentiate the two parental lines viz., IR58025A (CMS line) and PRR78 (Basmati fertility restorer line) using microsatellite (STMS) and expressed sequence tag (EST) markers. Markers that showed polymorphism were further used for co-segregation analysis of F<sub>2</sub> population derived from crossing of IR58025A and PRR78 to find out linkage between markers and fertility restorer gene. Out of 10 EST primers, none could show parental polymorphism. RT-PCR product revealed no difference between the parents with respect to the expression of the genes corresponding to the ESTs located in the Rf-1 region on chromosome 10 indicating the sequence conservation of ESTs in this region of the chromosome. A total of 12 rice microsatellite markers present in the Rf gene of chromosome 10 was used in the study. Among the 12

STMS markers, two markers namely RM6100 and RM6737 were found to be polymorphic between parents as well as between fertile and sterile bulks of F<sub>2</sub> derived population. The STMS marker RM6100 was found to be tightly linked being 8.7 cM from the fertility restorer locus Rf-1.

The tightly linked STMS marker, RM6100 for the fertility restorer gene should facilitate the marker aided selection for identification of Basmati germplasm carrying the fertility restorer gene which will enable to design crosses for exploitation of heterosis in basmati hybrids.

## Awards/Scholarships

- 2009, Awarded International travel grant by CSIR (Council for Scientific and Industrial Research), Government of India to participate in International Conference on “Plant Abiotic Stress Tolerance 2009” Vienna, Austria.
- 2005-2008, Awarded Senior Research Fellowship by The University Grants Commission, Govt. of India.
- 2003-05, Awarded the Junior Research Fellowship by The University Grants Commission, Govt. of India.
- **2004, Qualified NET (National Eligibility Test) for Eligibility of Lectureship, conducted by ICAR (Indian Council for Agricultural Research), India.**
- **2003, Qualified NET-JRF (Council for Scientific and Industrial Research - University Grants Commission), Ministry of Human Resource Development, Govt of India.**
- 2003, Qualified National level entrance examination conducted by Jawaharlal Nehru University, India for admission into M.Phil/Ph.D. programme at School of Life Sciences, New Delhi, India.
- 2001-2003, Awarded DBT merit fellowship during M.Sc.
- 2001, Qualified National level entrance examination conducted by Jawaharlal Nehru University, India and awarded fellowship during M.Sc. (Ag) Biotechnology (2001-2003) by DBT (Department of Biotechnology), Government of India.
- 1997, Qualified National level entrance examination conducted by Banaras Hindu University, India and awarded University Merit Scholarship for the third year of B.Sc. (Ag.).

## Techniques Known

### ***Molecular Biology Techniques***

Total RNA isolation, mRNA isolation, small RNA isolation, cDNA preparation, Gene and promoter isolation and cloning into suitable binary vector, Cloning of gene into yeast expression vectors and RNAi vectors, Cloning of genes into Yeast two hybrid vectors, Plasmid isolation, Promoter analysis by transient and stable integration of gene, RT-PCR, Real time PCR, Genomic DNA isolation, Southern, Northern, Molecular analysis of transgenics, Microbiological practices related to Molecular biology.

### ***Biochemical and Protein related Techniques***

Expression of cDNA in *E.coli*, Protein purification by Ni-NTA, protein estimation, SDS and Native-PAGE analysis.

### ***Yeast Related Experiments***

Yeast transformation, Yeast complementation, Yeast two hybrid for interacting proteins by Filter Lift assay.

### ***Immunological Techniques***

Raising polyclonal antibodies in Rabbit, Western Blotting.

### ***Physiological Techniques***

Chlorophyll a, Chlorophyll b measurements, Proline estimation, K<sup>+</sup> and Na<sup>+</sup> estimation.

### ***Plant Based Techniques***

*Agrobacterium* mediated transformation of Rice and Tobacco, Onion peel transformation using Gene gun, Seeds plating and antibiotic selection, Seedlings analysis.

### ***Computer Knowledge***

MS office, Photoshop, Bioedit, Jalview, MayDay, Gene sequence submission at NCBI, Primer designing, Microarray data analysis, Use of different databases including NCBI, Use of EndNote.

## References

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