Inferring gene regulatory networks using Kendall's tau correlation coefficient and identification of salinity stress responsive genes in rice

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Salinity is one of the most common abiotic stresses that limit the production of rice. Since salinity stress tolerance is controlled by many genes, identification of these stress responsive genes as well as to understand the underlying mechanisms is of importance from breeding point of view. In this direction, the reverse engineering of gene regulatory networks has proven to be successful. In this study, we construct the gene regulatory network using Kendall's tau correlation coefficient, in order to identify the stress responsive genes. The proposed approach was tested on a rice microarray dataset and 18 key genes were identified. Most of these key genes were found to be involved directly or indirectly in salinity stress, as evidenced from the published literature. Gene ontology analysis further confirmed the involvement of the selected genes in ion binding, oxidation-reduction and phosphorylation activities. These identified genes can be targeted for breeding salt-tolerant varieties of rice.

Keywords: Correlation coefficient, gene regulatory networks, rice, salinity.

RICE (*Oryza sativa* L.) is the most important cereal crop, and is the major food source for South Asia, India in particular[1]. However, the production of rice has been increasingly affected by salinity stress present in the soil and/or water[2]. Therefore, to increase the production of rice, which can play an important role for ensuring food security, it is essential to breed high salt-tolerant varieties through molecular breeding techniques[3]. For this, it is necessary to have information regarding salinity stress responsive genes as well as their underlying mechanisms. In this regard, the reverse engineering of gene regulatory networks (GRNs; networks that represent genes as well as the interactions among them) can be used as a tool to understand the molecular mechanisms of underlying biological process of the salinity stress response in rice.

Advancement in microarray technologies has made it feasible to draw inference about the pre-existing regulatory networks of biological processes, by allowing simultaneous measurements of the expression levels of thousands of genes. The nodes of GRNs are represented by genes and the edges between nodes represent interactions among genes[4]. Analysis of the GRNs helps in understanding the interactions among the genes and to identify the target genes for the breeding of tolerant varieties. Further, the reverse engineering of GRNs explicitly represents the regulatory interaction between genes and their products, which helps researchers to understand complex regulatory mechanisms in biological systems.

Several methods have been developed for inferring GRNs from microarray gene expression profiles, which vary in terms of precision and computational complexity[5]. These methods can be broadly categorized into two types based on the type of data used, i.e. discrete or continuous[6]. For the discrete gene expression data (expressed: 1, unexpressed: 0), models like Boolean network (BN), probabilistic Boolean network (PBN), etc. have been proposed[7–9], while for the continuous expression data, Pearson's correlation based algorithm[10,11], Bayesian network modelling[12], dynamic Bayesian network modelling approach[13], etc. have been developed for network inference. During discretization of gene expression data, there is always a chance of losing information, and hence the respective model may not provide the expected result. Further, Pearson's correlation-based approach may not detect the nonlinear relationship present among the genes. Though, Bayesian network-based methods provide higher degree of accuracy, their application in real experimental case is limited due to their computational complexity.

In the present study, we propose an approach to infer GRN on the basis of Kendall's tau correlation coefficient (KTCC). We preferred to use KTCC because it is non-parametric, captures nonlinear relationships, and involves less computational complexity. The proposed approach was applied in rice gene expression data for identifying the salinity stress responsive genes. The identified genes were further validated based on information available in the literature. Surprisingly, most of the identified genes were found to be involved directly or indirectly in salinity stress.

In this study, the rice microarray dataset GSE14403 (ref. 14) was used, collected from the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/GEO). The collected dataset has been generated from Affymetrix Rice Genome Array (GPL 2025 in GEO) and contains 57,381 probes, where each probe corresponds to an individual gene. Here 123 probe sets have been designed as control, and hence the remaining 57,258 valid probe sets have been used for further analysis. The raw CEL files were processed using the robust multichip average (RMA) algorithm available in the Affy package[15], which includes background correction, quantile normalization and summarization by the median polish approach[16]. The $\log_2$ scale data obtained after executing the RMA algorithm were then used for filtering out differentially expressed genes.

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A two-stage filtering procedure was applied to identify the genes that are differentially expressed in salinity stress conditions compared to normal condition. In the first stage, *t*-test was employed and the genes which were found to be significant at 0.5% level of significance were retained for the next stage of filtering. The test statistic for testing the significance of the *i*th gene expression profile is given by

$$t_i = \frac{\bar{y}_i - \bar{x}_i}{\sqrt{\frac{s_{yi}^2}{n_y} + \frac{s_{xi}^2}{n_x}}},$$

where $\bar{y}_i$ and $\bar{x}_i$ are the means, s_{yi}^2 and s_{xi}^2 are the variances, n_y and n_x are the sample sizes under salinity stress and normal conditions respectively. In the second stage of filtering, fold change (FC) measure was computed for the genes selected in the first stage. The genes having at least 2.5-fold change in their expression levels were selected for further analysis. For the *i*th gene expression profile, the FC measure was computed as $FC_i = \log_2 \bar{y}_i - \log_2 \bar{x}_i$.

After filtering out the differentially expressed genes through two-stage filtering procedure, the regulatory relationships among them were computed using KTCC as follows.

Let $(x_1, x_2, \ldots, x_n)$ and $(y_1, y_2, \ldots, y_n)$ be the expression profiles of x and y genes respectively. Then, there are $n(n-1)/2$ possible distinct pairs of (x_i, x_j) and (y_i, y_j), and such pairs of gene expression values are said to be concordant if ($x_i > x_j$ and $y_i > y_j$) or ($x_i < x_j$ and $y_i < y_j$), and discordant ($x_i < x_j$ and $y_i > y_j$), or ($x_i > x_j$ and $y_i < y_j$). KTCC was then computed as

$$\tau_{xy} = \frac{2(C-D)}{n(n-1)},$$

where C and D are the number of concordant and discordant pairs respectively. Based on the value of KTCC (τ_{xy}), the following interpretation was made.

If $\tau_{xy} > 0$, then gene y acts as an activator for gene x.

If $\tau_{xy} < 0$, then gene y acts as an inhibitor for gene x.

After the computation of KTCC, weakly correlated genes were eliminated based on a certain threshold value and the remaining highly connected genes were retained for constructing the regulatory networks. Based on KTCC, the GRN was constructed using RCytoscape package[17] of R software.

Unravelling the underlying mechanism of salinity stress in rice as well as the regulatory relationships among the salinity stress responsive genes will be of help for the breeder community to develop salt-tolerant varieties of rice. To this end, the GRN plays an important role. Though there are several approaches available in the literature for inferring GRN, each has its own advantages and disadvantages. In this study, we propose an approach to infer GRN on the basis of KTCC, which avoids some of the limitations of the earlier approaches, e.g. it does not make any assumption about the probability distribution, can captures nonlinear relationships, involves less computational complexity, etc. The proposed approach was applied on rice gene expression profile as mentioned earlier.

Using complete gene expression profile, the expected differentially expressed genes were selected using a two-stage filtering procedure. In the first stage *t*-test was used, where 2936 genes were selected with P values <0.005, which comprises 5.2% of the total number of genes. In the second stage, out of 2936 genes, 117 (0.2% of the total number of genes) with FC value ≥2.5 were selected. The regulatory relationships among these 117 genes were then computed using KTCC. Out of the 117 genes, the pairs with KTCC < 0.81 (50 genes) were excluded from the analysis to avoid weakly connected genes. Among the remaining 47 highly connected genes, 120 regulatory relationships were identified, which is shown graphically in Figure 1.

From the node-degree distribution of the constructed regulatory network (Figure 1), 18 genes are observed to have connection degree ≥ 6 (the average node-degree distribution), and therefore these are considered as the genes participating in the hub gene interaction. Among these 18 genes, *Os11g0293800*, *Os07g0418700*, *Os06g0647500*, *Os03g0826800*, *Os03g0809000*, *Os03g0184100*, *Os02g0685200*, *Os01g0775100*, *LOC_Os12g38770*, *LOC_Os10g39360*, and *LOC_Os07g12240* are found to have connection degree 10 (both in-degree and out-degree), and the rest have between 6 and 10. The genes having connection degree 10 are considered as hub genes.

After analysing the regulatory network, the regulatory relationships among the hub genes were decided based on the sign of the KTCC (Table 1). From the table it can be seen that the gene *Os03g0184100* is activated by the genes *Os03g0826800*, *LOC_Os12g38770*, *Os03g0809000*, *LOC_Os10g39360*, *Os11g0293800*, and *LOC_Os06g36850*, and inhibited by the genes *Os07g0418700*, *Os06g0647500*, *Os01g0775100*, and *Os02g0685200*. Similarly, *Os07g0418700* is observed to be regulated by nine genes, namely *Os06g0647500*, *Os01g0775100*, *Os02g0685200*, *Os03g0826800*, *LOC_Os12g38770*, *Os03g0809000*, *LOC_Os10g39360*, *Os11g0293800* and *LOC_Os07g12240*. More interestingly, *Os11g0702400* which is not found to be activated by any other genes, seems to act as an inhibitor for genes *Os07g0525100*, *Os07g0525100*, and *Os01g0826000*, and an activator for *Os10g0370500*. Similar interpretation can be made for other genes as well.

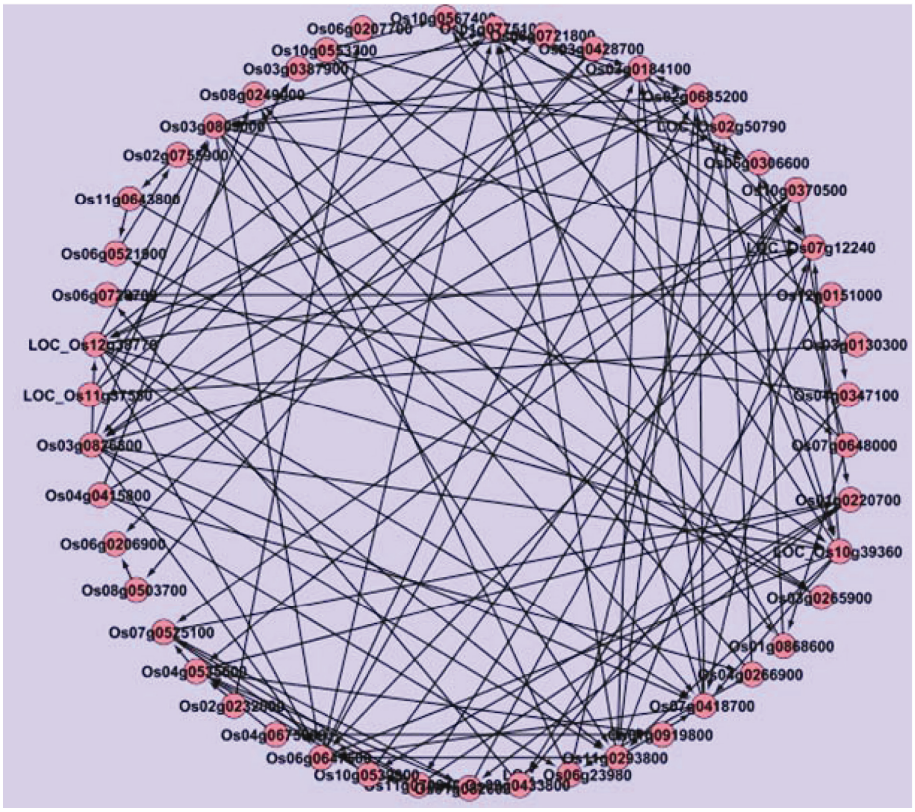

Figure 1. Inferred gene regulatory network in rice under salt stress condition.

The selected genes were further validated based on information available in the literature and biological databases. Surprisingly, most of the genes were found to be involved directly or indirectly in salinity stress response mechanism in plants and other species, a brief description of which is provided in Table 2. More specifically, the hub gene *Os11g0702400* that encodes zinc finger DNA-binding protein is expected to have some role in salinity tolerance in rice[18] and *Arabidospis*[19]. Further, the genes *LOC_Os10g39360* and *Os01g0868600*, which are members of eukaryotic aspartyl protease family protein, are found to be involved in response to salt stress in *Arabidospis*[20]. The gene *Os10g0539300* that encodes aspartic proteinase nepenthesin-2 is expected to be linked to salinity stress tolerance in *Arabidospis*[21], rice[22] and *Poa annua*[23]. Also, the gene *Os01g0826000*, which represents heavy metal-associated domain containing protein in halophytic plant *Atriplex canescens*, confers tolerance to iron and other abiotic stresses[24].

To be more confident, the selected genes were further subjected to Gene Ontology (GO) enrichment analysis. This was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID)[25], which is a biological knowledge base and analytic tool aimed at systematically extracting biological meaning from gene lists. Table 3 provides results of GO analysis. From the table it can be seen that the chosen genes are over-represented in the categories of cation binding, and ion binding, metal ion binding, iron ion binding, transition metal ion binding, all of which may be active due to the high concentration of ionic salts in soil and water. The behaviour of the genes in electron carrier activity may be related to cell membrane disruption due to high concentration of salt ions, thereby inhibiting the activities of membrane associated electron carriers and enzymes[26,27]. Further, they may be involved in transporting the ions outside the cell to maintain proper pH in the cell. In biological processes, the selected genes are represented in two categories, i.e. reduction–oxidation (redox) and phosphorylation. The behaviour of these genes in redox activities may be related to electron transport in chemical reaction that ensures the balance of charges during ion transport[22]. It may also be linked to the generation reactive oxygen species that are produced in response to oxidative stress due to water deficit during salinity stress, which is lethal and can cause cell death[28].

The role of some genes in phosphorylation activity for salt stress condition is detected in GO analysis that supplements the recent report on the active role of phosphorylation activity in salinity stress[22]. It has been reported that biological processes like phosphorylation activity have a key role in ion homeostasis under salinity stress in *Arabidopsis*[29].

The complex gene–gene interactions underlying the salinity response mechanism may be due to perturbations in the GRNs. Therefore, identification of salinity-responsive

Table 1. Interaction between genes involved in salinity stress response in *Oryza sativa*

Target	Regulator	Activate (+)/ repress (–)
Os03g0184100	*Os03g0826800, LOC_Os12g38770,Os03g0809000, LOC_Os10g39360,Os11g0293800, LOC_Os06g36850*	+
	Os07g0418700, Os06g0647500, Os01g0775100, Os02g0685200	–
Os07g0418700	*Os06g0647500, Os01g0775100, Os02g0685200*	+
	Os03g0826800, LOC_Os12g38770, Os03g0809000, LOC_Os10g39360, Os11g0293800, LOC_Os07g12240	–
Os06g0647500	*Os01g0775100*	+
	Os03g0826800, LOC_Os12g38770, Os03g0809000, Os11g0293800, LOC_Os07g12240, LOC_Os10g39360	–
Os03g0826800	*LOC_Os12g38770, Os03g0809000, LOC_Os10g39360, Os11g0293800, LOC_Os07g12240*	+
	Os01g0775100	–
Os01g0826000	*Os10g0370500, Os07g0525100*	+
	Os04g0535600, Os11g0702400, Os01g0220700, Os10g0539300	–
Os07g0525100	*Os01g0220700, Os10g0539300*	+
	Os10g0370500, Os04g0535600, Os11g0702400	–
Os01g0220700	*Os10g0539300*	+
	Os10g0370500, Os04g0535600, Os11g0702400	–
Os10g0370500	*Os04g0535600, Os11g0702400*	+
	Os10g0539300	–
Os01g0775100	*Os02g0685200*	+
	LOC_Os12g38770, Os03g0809000, LOC_Os10g39360, Os11g0293800, LOC_Os07g12240	–
LOC_Os12g38770	*Os03g0809000, LOC_Os10g39360, Os11g0293800, LOC_Os07g12240*	+
	Os02g0685200	–
Os03g0809000	*LOC_Os10g39360, Os11g0293800,LOC_Os07g12240*	+
	Os02g0685200	–
Os11g0702400	*Os10g0539300*	–
Os11g0293800	*LOC_Os07g12240*	+
	Os02g0685200	–
LOC_Os10g39360	*Os11g0293800, LOC_Os07g12240*	+
	Os02g0685200	–

+, represents the activation; –, represents the inhibition.

Table 2. List of genes involved in salinity stress response in rice

Genes	Brief description	Organism	Reference
LOC_Os10g39360, Os01g0868600	Eukaryotic aspartyl protease family protein	AT	20
LOC_Os07g12240	EF hand family protein	OS	30
Os11g0293800	Phosphatidylethanolamine-binding protein	HV	31
Os07g0418700	Vegetative cell wall protein	HG	32
Os03g0809000	Dirigent-like protein	SO	33
Os01g0775100	Plus-3 domain containing protein	–	
Os11g0702400	Zinc finger DNA-binding protein, putative	AT, OS	18, 19
Os10g0539300	Aspartic proteinase nepenthesin-2 precursor	AT, OS	21, 22
Os01g0826000	Heavy metal-associated domain containing protein	AC	24
Os01g0220700	Nodulin MtN3 family protein	AT	34
Os08g0249000	CONSTANS-like protein CO8	AT	35
Os06g0306600	Gibberellin receptor GID1L2	OS	22
Os04g0415800	protease inhibitor/lipid transfer protein	–	36
Os12g0151000	Serine/threonine protein	–	37
Os11g0643800	Sugar transporter family protein	SC	38
Os04g0347100	Similar to basic endochitinase precursor	AT	39

Genes are represented by their uni-gene id or locus id. 'Organism' indicates the one in which the genes are reported.
AT, *Arabidospis thaliana*; OS, *Oryza sativa*; AC, *Atriplex canescens*; HV, *Hordeum vulgare*; SO, *Saccharum officinarum*;, Sc, *Saccharomyces cerevisiae*; HG, *Halogeton glomeratus*.

hub genes and their regulators in GRN is a step forward for developing salinity-tolerant varieties in rice. A directed regulatory network like GRN is a legitimate way of representing gene–gene interactions responsible for such mechanism and also facilitating the activatory and inhibitory relationship between gene pairs. Here we present a simple approach to infer GRN based on KTCC, this has been applied to rice gene expression profile to extract the salinity stress responsive genes in rice. Based on the proposed approach, 18 hub genes have

Table 3. GO term enrichment analysis of selected genes

GO term	Description	Number in the selected gene set	*P*-value	Ontology
GO:0009055	Electron carrier activity	4	6.00E-02	MF
GO:0043169	Cation binding	8	6.10E-02	MF
GO:0043167	Ion binding	8	6.10E-02	MF
GO:0046872	Metal ion binding	7	1.30E-01	MF
GO:0005506	Iron ion binding	3	2.30E-01	MF
GO:0046914	Transition metal ion binding	5	3.30E-01	MF
GO:0055114	Oxidation–Reduction activity	5	4.00E-01	BP
GO:0016310	Phosphorylation activity	4	2.34E-02	BP

MF, Molecular function, BP, Biological process. Number in the selected gene set is the number of genes in the query gene list. *P* value represents the significance of the gene enrichment test.

been identified and are expected to have some role in salinity response mechanism in rice, as evidenced from the available literature and GO analysis. The regulatory interactions between gene pairs in case of salinity stress condition in rice are not freely available in databases and the literature, which limits the realistic validation of these interactions. The utility and validity of the results obtained in this study further need wet-lab experimental validation. The breeder can target these genes (validated genes) to develop salt stress-tolerant varieties of rice.

Competing interests: The authors declare that they have no competing interests.

1. Das, S., Pandey, P., Rai, A. and Mohapatra, C., A computational system biology approach to construct gene regulatory networks for salinity response in rice (*Oryza sativa*). *Indian J. Agric. Sci.*, 2015, **85**(12), 1546–1552.
2. Wu, R. and Garg, A., Engineering rice plants with trehalose-producing genes improves tolerance to drought, salt and low temperature. ISBN News Report, 2003; http://www.isb.vt.edu
3. Kole, C. *et al.*, Application of genomics-assisted breeding for generation of climate resilient crops: progress and prospects. *Front. Plant Sci.*, 2015, **6**, 563.
4. Schaffter, T., Marbach, D. and Floreano1, D., GeneNetWeaver: *in silico* benchmark generation and performance profiling of network inference methods. *Bioinformatics*, 2011, **27**, 2263–2270.
5. Bansal, M., Belcastro, V., Ambesi-Impiombato, A. and di Bernardo, D., How to infer gene networks from expression profiles. *Mol. Syst. Biol.*, 2007, **3**, 78.
6. Karlebach, G. and Shamir, R., Modelling and analysis of gene regulatory networks. *Nature Rev. Mol. Cell Biol.*, 2008, **9**, 770–780.
7. Akutsu, T., Miyano, S. and Kuhara, S., Inferring qualitative relations in genetic networks and metabolic pathways. *Bioinformatics*, 2000, **16**, 727–734.
8. Ching, W., Fung, E., Ng, N. and Akutsu, T., On construction of stochastic genetic networks based on gene expression sequences. *Int. J. Neural Syst.*, 2005, **15**, 297–310.
9. Hirose, O., Nariai, N., Tamada, Y., Bannai, H., Imoto, S. and Miyano, S., Estimating gene networks from expression data and binding location data via Boolean networks. *Lecture Notes Comput. Sci.*, 2000, **3480**, 349–356.
10. Zhang, B. and Horvath, S., A general framework for weighted gene co-expression network analysis. *Stat. Appl. Genet. Mol. Biol.*, 2005, **4**, 17.
11. Raza, K. and Jaiswal, R., Reconstruction and analysis of cancer specific gene regulatory networks from gene expression profiles. *Int. J. Bioinformat. Biosci.*, 2013, **3**(2), 25–34; doi:10.5121/ijbb.2013.3103.
12. Imoto, S., Goto, T. and Miyano, S., Estimation of genetic networks and functional structures between genes by using Bayesian network and nonparametric regression. *Pac. Symp. Biocomput.*, 2002, **7**, 175–186.
13. Perrin, B. E., Ralaivola, L., Mazurie, A., Bottani, S., Mallet, J. and D'Alche-Buc, F., Gene networks inference using dynamic Bayesian networks. *Bioinformatics*, 2003, **19**(2), 138–148.
14. Cotsaftis, O., Plett, D., Johnson, A. A. and Walia, H., Root-specific transcript profiling of contrasting rice genotypes in response to salinity stress. *Mol. Plant*, 2011, **4**(1), 25–41.
15. Gautier, L., Cope, L., Bolstad, B. M. and Irizarry, R. A., Affy – analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics*, 2004, **20**, 307–315.
16. Irizarry, R. A., Hobbs, B., Collin, F., Beazer-Barclay, Y. D., Antonellis, K. J., Scherf, U. and Speed, T. P., Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics*, 2003, **4**, 249–264.
17. Shannon, P. T., Grimes, M., Kutlu, B., Bot, J. J. and Galas, D. J., RCytoscape: tools for exploratory network analysis. *BMC Bioinformat.*, 2013, **14**, 217–226.
18. Huang, X. *et al.*, A previously unknown zinc finger protein, DST, regulates drought and salt tolerance in rice via stomatal aperture control. *Genes Dev.*, 2009, **23**, 1805–1817; doi:10.1101/gad.1812409.
19. Sun, J., Jiang, H., Xu, Y., Li, H., Wu, X., Xie, Q. and Li, C., The CCCH-type zinc finger proteins AtSZF1 and AtSZF2 regulate salt stress responses in *Arabidopsis*. *Plant Cell Physiol.*, 2007, **48**(8), 1148–1158.
20. https://www.arabidopsis.org/servlets/TairObject?id=46633&type=array_ele_pre_exp
21. Seki, M., Narusaka, M., Ishida, J., Nanjo, T. and Fujita, M., Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. *Plant J.*, 2002, **31**(3), 279–292; doi:10.1046/j.1365-313x.2002.01359.
22. Wang, J., Chen, L., Wang, Y., Zhang, J., Liang, Y. and Xu, D., A computational systems biology study for understanding salt tolerance mechanism in rice. *PLoS ONE*, 2013, **8**(6), e64929; doi:10.1371/journal.pone.0064929
23. Kobayashi, S., Satone, H., Tan, E., Kurokochi, H., Asakawa S., Liu, S. and Takano, T., Transcriptional responses of a bicarbonate-tolerant monocot, *Puccinellia tenuiflora*, and a related bicarbonate-sensitive species, *Poa annua*, to $NaHCO_3$ stress. *Int. J. Mol. Sci.*, 2015, **16**(1), 496–509; doi:10.3390/ijms16010496.
24. Sun, X. *et al.*, A heavy metal-associated protein (AcHMA1) from the halophyte, *Atriplex canescens* (Pursh) nutt., confers tolerance

to iron and other abiotic stresses when expressed in *Saccharomyces cerevisiae*. *Int. J. Mol. Sci.*, 2014, **15**(8), 14891–14906; doi: 10.3390/ijms150814891.
25. Huang, D. W., Sherman, B. T. and Lempicki, R. A., Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protoc.*, 2008, **4**, 44–57.
26. Bahieldin, A. *et al.*, RNA-Seq analysis of the wild barley (*H. spontaneum*) leaf transcriptome under salt stress. *C. R. Biologies*, 2015; http://dx.doi.org/10.1016/j.crvi.2015.03.010.
27. Zsigmond, L. *et al.*, Arabidopsis PPR40 connects abiotic stress responses to mitochondrial electron transport. *Plant Physiol.*, 2008, **146**(4), 1721–1737; doi:10.1104/pp.107.111260.
28. Miller, G., Shulaev, V. and Mittler, R., Reactive oxygen signaling and abiotic stress. *Physiol. Plant.*, 2008, **133**(3), 481–489; doi:10.1111/j.1399-3054.2008.01090.x
29. Liu, J. and Zhu, J., A calcium sensor homolog required for plant salt tolerance. *Science*, 1998, **280**(5371), 1943–1945; doi:10.1126/science.280.5371.1943.
30. Walia, H., Wilson, C., Ismail, A. M. and Close, T. J., Comparing genomic expression patterns across plant species reveals highly diverged transcriptional dynamics in response to salt stress. *BMC Genom.*, 2009, **10**, 398.
31. Walia, H. *et al.*, Comparative transcriptional profiling of two contrasting rice genotypes under salinity stress during the vegetative growth stage. *Plant Physiol.*, 2005, **139**(2), 822–835.
32. Wang, J. *et al.*, Transcriptomic profiling of the salt-stress response in the halophyte *Halogeton glomeratus*. *BMC Genom.*, 2015, **16**, 169; doi:10.1186/s12864-015-1373-z
33. Jin-Long, G., Li-Ping, X., Jing-Ping, F., Ya-Chun, S., Hua-Ying, F., You-Xiong, Q. and Jing-Sheng, X., A novel dirigent protein gene with highly stem-specific expression from sugarcane, response to drought, salt and oxidative stresses. *Plant Cell Rep.*, 2011, **31**(10), 1801–1812.
34. Ma, S., Gong, Q. and Bohnert, H. J., Dissecting salt stress pathways. *J. Exp. Bot.*, 2006, **57**(5), 1097–1107.
35. Min, J. H., Chung, J. S., Lee, K. H. and Kim, C. S., The CONSTANS-like 4 transcription factor, AtCOL4, positively regulates abiotic stress tolerance through an abscisic acid-dependent manner in *Arabidopsis*. *J. Integr. Plant Biol.*, 2014, **30**, 123–134.
36. Kosova, K., Vitamvas, P., Urban, M. O., Klima, M., Roy, A. and Prasil, T. M., Biological networks underlying abiotic stress tolerance in temperate crops – a proteomic perspective. *Int. J. Mol. Sci.*, 2015, **16**, 20913–20942; doi:10.3390/ijms160920913.
37. Pais, S. M., Téllez-Iñón, M. T. and Capiati, D. A., Serine/threonine protein phosphatases type 2A and their roles in stress signalling. *Plant Signal Behav.*, 2009, **4**(11), 1013–1015.
38. Ferreira, C. *et al.*, A member of the sugar transporter family, stl1p is the glycerol/h+ symporter in *Saccharomyces cerevisiae*. *Mol. Biol. Cell*, 2005, **16**(4), 2068–2076; doi:10.1091/mbc.E04-10-0884.
39. Sairam, R. K. and Tyagi, A., Physiology and molecular biology of salinity stress tolerance in plants. *Curr. Sci.*, 2004, **86**(3), 407–421.

ACKNOWLEDGEMENT. We thank ICAR-Indian Agricultural Statistics Research Institute, New Delhi and Indian Council of Agricultural Research, New Delhi for support.

Received 19 October 2015; accepted 7 October 2016

doi: 10.18520/cs/v112/i06/1257-1262

An interactive computer vision system for tree ring analysis

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Tree rings (growthing) analysis provides useful information about the age of a tree and the past climatic conditions. Analysis of tree rings manually is a herculean task and requires a domain area expert. The present work proposes a soft technique to analyse tree rings. Effective canny edge detection approach was utilized to process high-quality digital images of tree rings. The developed program successfully performs interactive tree-ring image analysis with MATLAB Image Processing Toolbox. It generates information about the width of earlywood and latewood of the growth ring. The information generated may be further utilized by domain area expert to deduce the age of a tree. The development of such a system will ease the human analysis efforts.

Keywords. Canny edge detection, digital image processing, pixel labelling, tree rings.

DENDROCHRONOLOGY was developed during the first half of the 20th century, by astronomer A. E. Douglass, founder of the Laboratory of Tree-Ring Research at the University of Arizona, USA.

Each year, new cells are formed in a tree. These cells are arranged in concentric circles called annual growth rings, which show the amount of wood produced during one growing season. In summer, dark wood also known as latewood is produced because growth is slow, whereas in spring growth is fast and light wood also known as earlywood is produced. An alternate layer of light and dark wood appears on the cross-section when a tree is cut down. One year of growth is therefore represented by a ring consisting of a light part (earlywood) and a dark part (latewood). The older rings are near the centre of the tree. Width of a growth ring depends upon duration of growing season of the tree. The study of tree growth rings provides a glimpse of the past climatic conditions.

The automation of analysis of tree rings requires image analysis and processing. The task is difficult as the images contain high levels of noise. The appearance of tree rings has a greater contrast when the tree grows in an environment where climate is influenced by seasonal weather change; for example, temperate-zone tree rings compared to tropical-zone tree rings. Therefore, seasonal changes cause the tree to grow at different rates.

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