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Genome-wide *Insilico* analysis of plant two component signaling system in woody model plant *Populus trichocarpa*

Gurpreet Singh* and Ruchi Kumar

P.G. Department of Biotechnology, Lyallpur Khalsa College, GT Road, Jalandhar Punjab INDIA
Pin-144001*Corresponding author Email: s_gp@rediffmail.com, s_gp1012@hotmail.com,

The two-component system (TCS) which works on the principle of histidine-aspartate phosphorelay signaling, is known to play an important role in diverse physiological processes in lower organisms and emerged as an important signaling system in both prokaryotic as well as eukaryotic systems including plants. Employing various bioinformatics tools, we have characterized TCS signaling candidate genes in the genome of *Populus trichocarpa*. In this study we present an overview of TCS gene families in *P.trichocarpa*, including gene structures, conserved motifs, chromosome locations, and phylogeny. This analysis indicates a total of 49 genes out of which at least 12 belongs to histidine kinases (8 histidine kinase (PtHK) and 4 ethylene responsive (PtETR)), 12 pseudo-phosphotransfers (PtHPT) and 9 type-A (PtRRA), 11 type-B (PtRRB), and 5 pseudo-response regulator proteins (PtPRR) present on different chromosomes. Fourteen genes encode 22 putative histidine kinases with a conserved histidine and other typical histidine kinase signature sequences, five phosphotransfer genes encoding seven phosphotransfer proteins, and 32 response regulator genes encoding 44 proteins. Structural and phylogenetic analyses of populus TCS members with their Arabidopsis and rice counterparts revealed similar architecture of their TCSs. Our analysis provides insights into the conservation and divergence of this important signaling machinery in woody plants.

Keywords: Two Component System, Poplar, *Insilico* analysis, signaling molecule.

Two-component and phosphorelay signal transduction systems are the major means by which bacteria recognize and respond to a variety of environmental stimuli (Hoch 2000; Gao *et al.* 2007). The existence of a bacterial-type HK in plants was initially reported by Chang *et al.* (1993). Since then, many plants have been documented to possess genes encoding two-component regulators, and their participation in the

perception and integration of various extracellular and intracellular signals has been reported (Lohrmann and Harter, 2002; Oka *et al.* 2002; Grefen and Harter, 2004; Hass *et al.* 2004; Monicha *et al.* 2010 and references thereon). Two-component systems consists of a histidine protein kinase that senses the input and a response regulator that mediates the output control signal transduction pathways in many prokaryotes and in some

eukaryotes (Fig 1). In bacteria, yeast, slime moulds and plants, the so-called multiple His-to-Asp phosphorelay makes use of a 'hybrid' kinase that contains both a His-kinase (HK) domain and a receiver domain (Rec) in one protein. The TCSs also include a His-containing phosphotransfer (HPT) domain, which functions as a signaling module that connects to the final RRs (Schaller *et al.* 2008). Two-component systems (TCSs) control many of the biological processes such as cell division, cell growth and proliferation, and responses to environmental stimuli and growth regulators in both eukaryotic and prokaryotic cells (Hwang and Sheen, 2001; Pils and Heyl, 2009; Urao *et al.* 2000, 2001). Computational analyses have confirmed that two-component signaling elements are absent from the genome sequences of *Homo sapiens*, *Drosophila melanogaster* and *Caenorhabditis elegans* (Schultz *et al.* 2000).

Trees among the plant species are unique as they display distinctive developmental, physiological and anatomical characteristics such as phase change from juvenile to mature stage-dependent adaptation to the environment and a secondary cambium that produce wood and bark (Wullschlegar *et al.* 2002). Poplars are paleopolyploids (polyploids that have undergone diploidization) and all species have a haploid chromosome number of 19 (Soltis and Soltis, 2000; Brunner *et al.* 2004). Poplar was chosen as a model tree for the molecular biology studies due to its small genome size of ca. 520 Mbp and its availability for routine transformation (Bradshaw *et al.* 2002; Tuskan *et al.* 2006). This study is an attempt to characterize different components of TCS in the genome of *Populus trichocarpa*

Materials and Methods

The initial step to identify genes/proteins encoding TCS elements from the annotations of the *Populus trichocarpa*, the protein sequence data set of HKs, HPTs and RRs of Arabidopsis was used as the query against the poplar proteome data set (<http://www.phytozome.net/poplar>) with the BLASTP program of the NCBI BLAST with default parameters.

These sequences were then aligned by using ClustalX (1.83) multiple alignment tool using Weight Matrix BLOSUM for proteins (Thompson *et al.* 1997). The alignments were then analyzed for differences in their amino acid at specific positions. To confirm the structures of protein domains conserved in each *Populus* TCS member, InterProScan and the InterPro database (<http://www.ebi.ac.uk/interpro/>) were applied, and HMM (Hidden Markov Model) profiles corresponding to domains annotated as CCT, CHASE, HPT, HisKA, MYB, PHY, RR (RRA or RRB or PRR) were used for HMMER searches (Mochida *et al.* 2009). The unrooted phylogenetic trees of these aligned sequence were constructed by the neighbour-joining method using MEGA4 software (Tamura *et al.* 2007).

Results and Discussion

In the last few years, the composition and function of TCS system have been studied extensively in several plant species viz. Arabidopsis, rice and more recently in Lotus, soybean (Hwang *et al.* 2002; Pareek *et al.* 2006, Schaller *et al.* 2007; Mochida *et al.* 2010). It has been reported that the total TCS members varied from 45-75 in different plant species (Table 1). Genome-wide analysis of *P. trichocarpa* indicates that around 49 genes of the TCS members are present on different chromosomes having strong homology with HK, HPT and RR genes of other plant species (Table 2).

Table 1: Comparative analysis of two component system in different plant genomes

Species	HK	HPT	RRA type	RRB type	Pseudo RR	Total	References
<i>Arabidopsis thaliana</i>	8	6	10	13	9	46	Hwang <i>et al.</i> , 2002
<i>Oryza sativa</i>	8	5	13	15	8	49	Pareek <i>et al.</i> , 2006, Schaller <i>et al.</i> , 2007
<i>Glycine max</i>	21	13	18	18	13	83	Mochida <i>et al.</i> , 2010
<i>Populus trichocarpa</i>	12	12	9	11	5	49	Present study

Table 2. Detailed analysis of the two component system in the Populus along with their Amino acid length and chromosome location

GENE NAME	GENE ID	CHR.LOC	AA	DOMAIN
Ethylene receptor	PtETR1	LGII	636	GAF, HisKA, HATPase, REC
	PtETR2	LGX	768	GAF, HisKA, HATPase, REC
	PtETR3	LGVIII	762	GAF, HisKA, HATPase, REC
	PtETR4	LGXIII	763	GAF, HisKA, HATPase, REC
Histidine kinase cytokinin receptor	PtHK1	LGI	1020	CHASE, HisKA, REC
	PtHK2	LGIII	1029	CHASE, HisKA, REC
	PtHK3	LGVIII	1007	CHASE, HisKA, REC
	PtHK4	LGX	1006	CHASE, HisKA, REC
	PtHK5	LGXIV	1234	CHASE, HisKA, REC
	PtHK6	LGXIII	1001	HisKA, HATPase, REC
	PtHK7	LGXVIII	945	PAS, HisKA, HATPase, REC
	PtHK8	LGVI	923	HisKA, REC
Histidine phosphotransfer protein	PtHPT1	LGXVIII	137	HPT
	PtHPT2	LGI	147	HPT
	PtHPT3	LGI	150	HPT
	PtHPT4	LGI	224	HPT
	PtHPT5	LGVI	152	HPT
	PtHPT6	LGVIII	154	HPT
	PtHPT7	LGIX	245	HPT
	PtHPT8	LGX	154	HPT
	PtHPT9	LGX	164	HPT
	PtHPT10	LGXIII	152	HPT
	PtHPT11	LGXIV	139	HPT
	PtHPT12	LGXVI	152	HPT
TypeA response regulator	PtRRA1	LGI	203	REC
	PtRRA2	LGII	243	REC
	PtRRA3	LGIII	193	REC
	PtRRA4	LGVI	233	REC
	PtRRA5	LGVIII	258	REC
	PtRRA6	LGVIII	247	REC
	PtRRA7	LGXIX	151	REC
	PtRRA8	LGXIX	154	REC
	PtRRA9	LGXVI	227	REC
TypeB response regulator	PtRRB1	LGVI	588	REC
	PtRRB2	LGVIII	642	REC, mybDNA binding domain
	PtRRB3	LGVIII	303	REC, mybDNA binding domain
	PtRRB4	LGX	634	REC, mybDNA binding domain
	PtRRB5	LGX	250	REC, mybDNA binding domain

	PtRRB6	LGX	633	REC, mybDNA binding domain
	PtRRB7	LGXII	658	REC, mybDNA binding domain
	PtRRB8	LGXV	716	REC, mybDNA binding domain
	PtRRB9	LGXVIII	588	REC, mybDNA binding domain
	PtRRB10	LGXVIII	517	REC, mybDNA binding domain
	PtRRB11	LGXVIII	661	REC, mybDNA binding domain
Pseudo response regulator	PtPRR1	LGII	458	REC, MYB
	PtPRR2	LGII	694	REC, CCT
	PtPRR3	LGVIII	711	REC, CCT
	PtPRR4	LGXIV	717	REC, CCT
	PtPRR5	LGXV	687	REC, CCT

The Histidine Kinase (HK) protein family

P. trichocarpa has at least 12 distinct Histidine kinases belonging to different gene families viz. the typical cytokinin receptor homologs (PtHKs) and the ethylene receptor (PtETR). Table 2 summarizes the relevant genomic information for these genes and their products. The predicted proteins ranged from 923-1234 amino acids in PtHKs homologs and 636-768 in PtETR homologs. Domain analysis of these PtHKs confirmed that all the PtHKs have a typical hybrid HK-type structure with a conserved HK domain which contains the conserved His phosphorylation site. These PtHKs also contain a complete Rec domain, which contains a highly conserved Asp as the phosphoacceptor, although the number of the TM domains is variable. Besides the typical HK domain, the PtHK1,2,3,4,5 contains the conserved cyclases/HK-associated sensory extracellular (CHASE) domain. PtHK7 contains a PAS domain [Per (period circadian protein), Arnt (ah receptor nuclear translocator protein), Sim (single-minded protein)] domains along with the HK and Rec domains, PAS domains are present in many signaling proteins in archaea, bacteria, and eukaryotes, and act as signal sensor domains (Ho *et al.* 2000). PtETR contains a typical GAF domain (cyclic GMP adenylyl cyclase FhlA) domain. GAF domains bind molecules such as cAMP and cGMP. However CHASE Domain present in the HK members are not

present in the ethylene receptor family of histidine kinase. ETRs and phytochromes (PHYs) are the only plant proteins known to contain GAF and HK-related domains (Chang and Shockey, 1999; Karniol *et al.* 2005). However, the PtHK members share an overall 27% to 78% similarity in amino acid sequence and 30% to 66% identity. These minor structural and functional variations may have arisen through the gene duplication events and modification of gene functions as needed in evolution. In Arabidopsis, a specific member of the HK group plays an important role as an osmosensor. The gene AtHK1 (or AHK1) has high structural similarity with the yeast osmosensing HK SLN1 (synthetic lethal of N-end rule) which is able to functionally complement with the yeast double mutant lacking its two osmosensors, i.e. *sln1* and *sho* (Urao *et al.* 1999) thus indicating the functional conservation of the osmosensing machinery between the two systems. AtHK1 has also been shown to interact physically with AHP2 suggesting that the transduction of the stress signal could occur via a multistep phosphorelay (Urao *et al.* 2000b). Our analysis of the *P.trichocarpa* genome has indicated that the overall percent identity of PtHKs with AtHK1 ranges from 23% to 49% with PtHK6 being the closest relative of AtHK1 (Fig.2a).

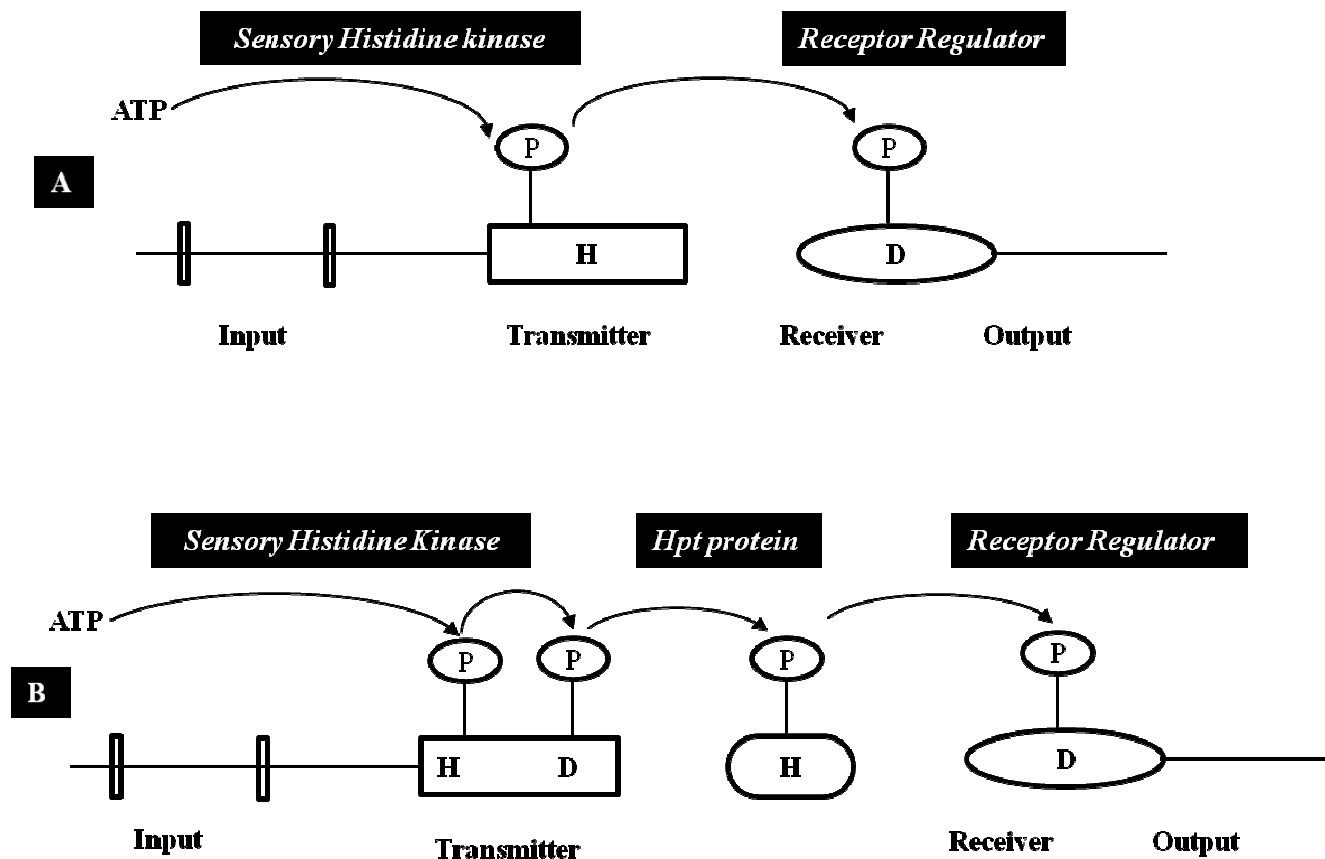


Figure 1. Basic machinery of TCS. A, A simple TCS member. Sensing of an extracellular signal is initiated by the input domain of sensory HK, phosphorylating the conserved His in its transmitter domain. In the next step, the conserved Asp in the RD of the RR is autophosphorylated, resulting in the signal output. B, A hybrid-type TCS in which the conserved His and Asp are found in the same protein, which serves as the sensory HK and is usually membrane bound. The Hpt acts as a mediator for the transfer of the phosphoryl group between the HK and the RR. The vertical bars depicted Transmembrane domains.

The Histidine phosphotransfer (HPT) protein family

In prokaryotes, Histidine phosphotransfer (HPt) are almost exclusively components of hybrid kinases, whereas in eukaryotes, they are found as separate proteins components. HPt domains are ~120 amino acids in length and contain a His residue capable of participating in phosphoryl transfer reactions. The HPT domains do not exhibit kinase or phosphatase activity (Tsuzuki *et al.* 1995) and are ideally suited to serve as specific cross-

communication modules between different proteins (Stock *et al.* 2000). Survey on *P.trichocarpa* genome indicates that it contains 12 genes (PtHPT1-12) with the amino acids length varying from 137-245 and are located on different chromosomes (Table 2). The 12 putative PtHPTs contain a typical phosphotransfer intermediate sequence with the conserved His phosphorylation site. Except for PtHPT2 and PtHPT3 all other members of this family shares a common sequence homology with Arabidopsis and soybean genome HQXKGSSXS(I/V)G

consensus sequence that contains the conserved His residue and share a common four-helix bundle motif without any TM domain indicating their cytoplasmic location. These putative PtHPTs lacking H but showing conserved HPT domain may have

some alternative degenerated phosphorelay role in cell signaling. The unrooted relationship tree for these different PtHPTs is shown in Fig. 2b, indicating the absence of any clustering among them.

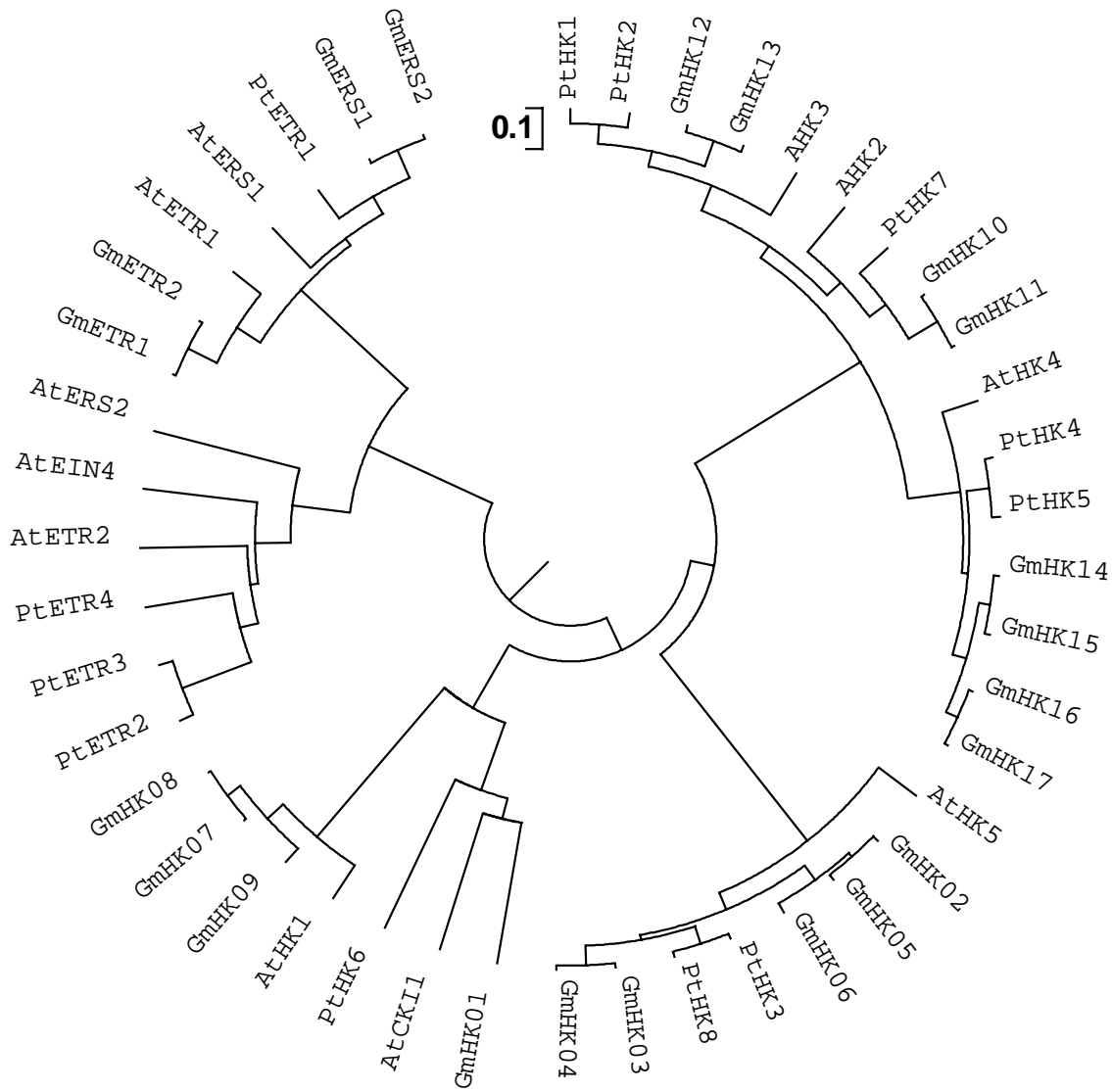


Figure 2a: Phylogenetic analysis of histidine Kinase in different plant species

The Response Regulator (RR/PRR) proteins family

Both the prokaryotic and eukaryotic systems have response regulators at their ends. They represent the terminal component

of the TCS pathway which functions as phosphorylation activated switches that catalyze the phosphoryl transfer from the phospho-His of the HK to a conserved His in its own regulatory domain. Small molecules

such as acetyl phosphate, carbamoyl phosphate, imidazole phosphate, and phosphoramidate serve as phosphodonors to

RRs thus indicating that the RR catalyzes the phosphoryl transfer independently of assistance from an HK (Lukat *et al.* 1992).

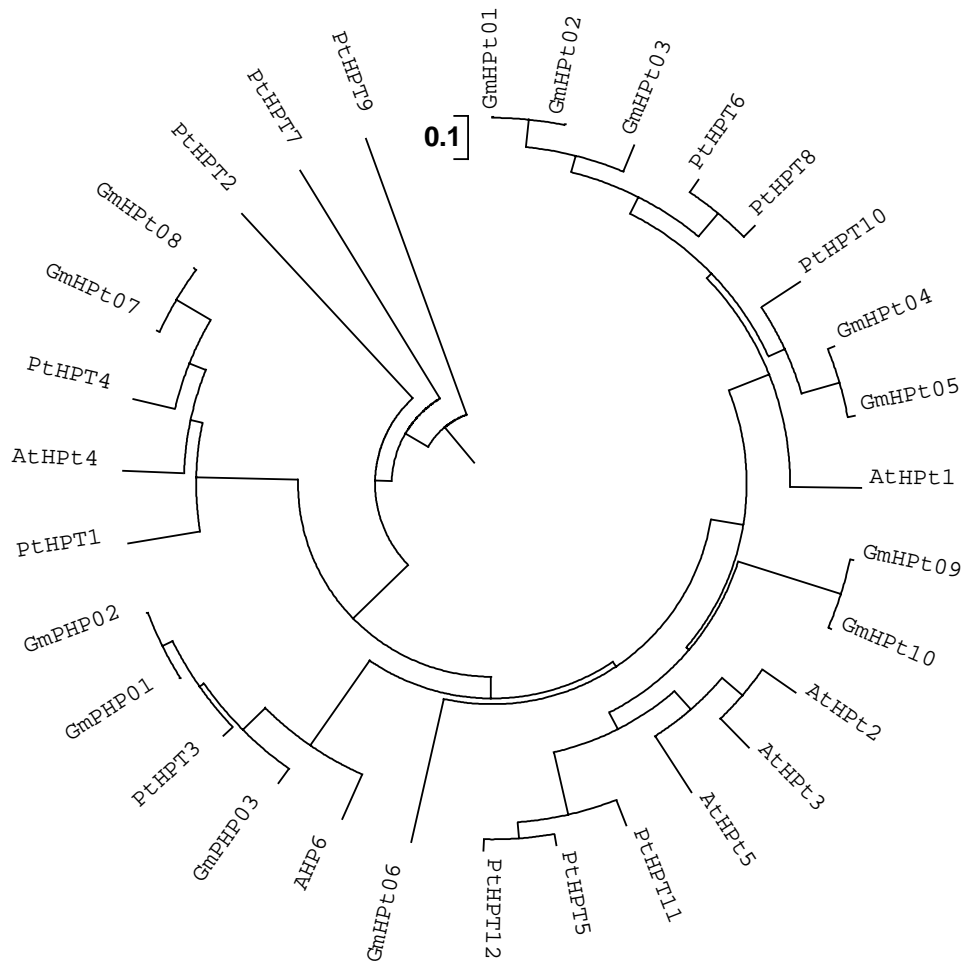


Figure 2b: Phylogenetic analysis of histidine Phosphotransfer (HPT) in different plant species

Genome-wide analysis in different plant species viz. Arabidopsis, rice, and soybean has indicated the existence of 32 - 49 genes encoding putative RRs and related proteins (Table 1). The majority of these RRs have two main domains: a conserved N-terminal regulatory domain and a variable C-terminal effector domain. Depending upon the predicted structural similarities, response regulators are divided into three types viz. PtRR-A proteins, containing only the receiver domain (REC); PtRR-B, which have a receiver

(REC) and a DNA-binding domain (Myb-DNA Binding Domain) and PtPRR which contains a receiver-like (or Pseudo-receiver) domain at the N-terminal end followed by another common motif of about 50 amino acid at the very C-terminal end (CCT domain). *In silico* analysis of the Populus genome indicates the presence of total 9 type A, 11 type B and 5 pseudo response regulators in the *P.trichocarpa* genome (Table 2). Each of the poplar type A-RRs which contain a receiver domain (REC) along with a

divergent C-terminal extension. The phylogenetic tree developed from the RRs collected from Arabidopsis, soybean and Populus indicates closed relationship among the type-A RRs of the three species, which might suggest similar functions for the soybean type-A PtRR. The PtRR-1, 3, 4, and 9 shows a high homology with AtRR-5 and

GmRR9 (Fig. 2c). Several type-A ARR-RRs have shown to be involved in the regulation of light response, circadian and meristem size (To *et al.* 2004; Salome *et al.* 2006; Nieminen *et al.* 2008). The homology of the PtRRAs with ARR5 may provide an indication of the role of these components in poplar growth.

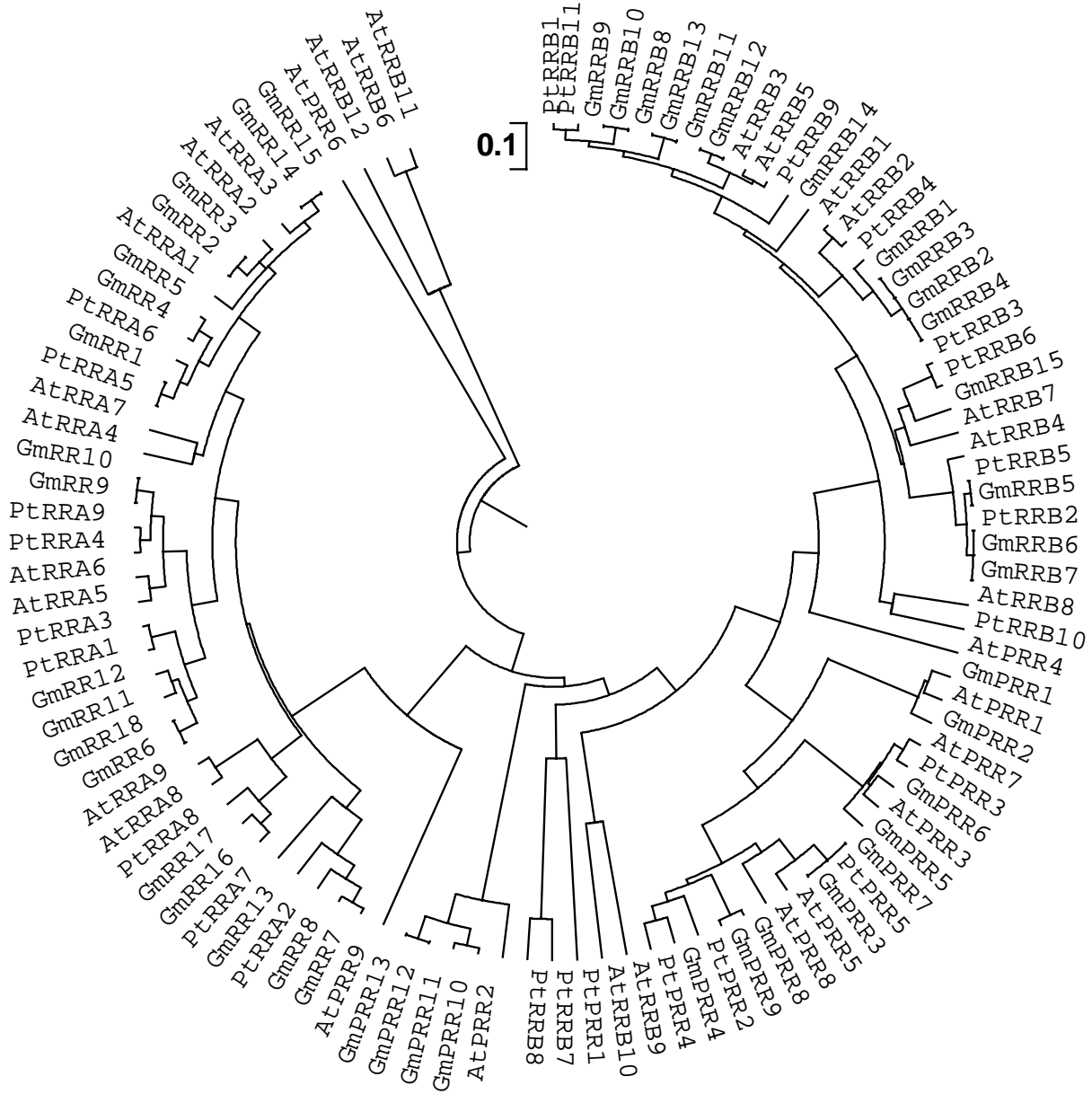


Figure 2c: Phylogenetic analysis of response regulator in different plant species

In *Populus* there are 12 type B response regulators compared to 11 in *Arabidopsis* and 15 in soybean each of these PtRRBs are characterized by the presence of an N-terminal receiver domain and a long C-terminal extension with an Myb-like DNA binding domain (GARP domain) and are widely distributed on the different chromosomes of *P.trichocarpa*. The presence of type-B RRs from unicellular algae, moss and lycophytes to higher land plant species suggests that they might be involved in regulation of photosynthesis, besides Cytokinin signal transduction and ethylene signaling (Doi *et al.* 2004; Mason *et al.* 2005; Pils and Heyl, 2009).

We identified five pseudo-response regulators and designated as PtPRR1, PtPRR2, PtPRR3, PtPRR4 and PtPRR5 whereas it has been reported that in *Arabidopsis*, rice and soybean the number of PRRs varied from 8-9 which are mainly biological clock associated (Mizuno, 2005; Schaller *et al.* 2007). Each has a common structural design containing the pseudo-RD of about 120 amino acids at its N terminus and a short CCT motif of about 50 amino acids at the C-terminal end. The phylogenetic analysis has revealed that PtPRR3 shows a high homology with AtRR7 and PtPRR5 with AtPRR5. It has been shown that AtPRR5 is regulated under the abiotic stress conditions (Nakamichi *et al.* 2009). Expression of the CCT motif PRR genes also varied in a circadian manner and loss-of-function mutants have shown the altered circadian periods thus indicating the roles of these pseudo response regulators to circadian rhythmicity in plants. Nakamichi *et al.* (2005a,b). The unrooted tree generated from the members of the RR family from *Populus*, *Arabidopsis* and soybean has shown that the three distinct groups, each comprised of RRA-type, RRB-type, and PRR members have independent coevolution of these distinct subspecies in these plant genomes (Fig. 2c).

The plant two-component and phosphorelay signal transduction pathways play an important role in diverse physiological processes in both prokaryotic as well as eukaryotic systems including plants for various components such as cytokinin, ethylene and abiotic stress. The present study is an attempt to elucidate the genome of tree *Populus* for the presence of two component system, however the exact proportion and role of these components *vis-à-vis* their role under natural habitat is still a question of further study.

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