Isolation and Molecular Characterization of the *COP1* Gene Homolog from Rice, *Oryza sativa* L. subsp. *Indica* var. Pusa Basmati 1

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Abstract

The *COP1* (*CONSTITUTIVE PHOTOMORPHOGENIC 1*) gene has been identified earlier from dicot species namely *Arabidopsis*, tomato and pea. The protein encoded by this gene acts as a molecular switch that negatively regulates the transition from the skotomorphogenic to the photomorphogenic mode of plant development. We have isolated and characterized the *COP1* homolog from a monocot species, i.e. rice (var. Pusa Basmati 1). All the functional domains (Zn-binding RING finger motif, coiled-coil region, WD-40 repeats, cytoplasmic/nuclear localization sequences and protein-protein interaction domains) that are known in the COP1 proteins from dicots are conserved in COP1 from rice as well. The transcript levels of *COP1* vary in various tissues of the rice plant. These variations were found to be development-dependent and do not solely depend on the light conditions.

Key words: *COP1* (*CONSTITUTIVE PHOTOMORPHOGENIC 1*); photomorphogenesis; rice; spatial regulation

Light plays a vital role in regulating various aspects of growth and development in plants starting from the onset of seed germination, stem growth inhibition, leaf expansion, chloroplast development and induction of flowering. This photomorphogenically active light is perceived by sensory photoreceptors like phytochromes, cryptochromes and phototropin, and the signal is transduced downstream through an intricate network of signaling components. Both, biochemical studies and genetic analysis have contributed to the identification of these components. Genetic analysis of *Arabidopsis* mutants that are defective in photomorphogenic development have identified 11 pleiotropic *COP/DET/FUS* loci,[1,2] which are responsible for the repression of photomorphogenesis in the absence of light. Among these, COP1 was identified as the rate-limiting component in mediating the repression of photomorphogenesis[3,4] and its activity was correlated with its partitioning between the nucleus and the cytosol.[5] In dark, COP1 protein is localized primarily in the nucleus and, with the onset of the light, it is depleted from the nucleus and becomes abundant in the cytosol, although the total cellular level of the protein remains constant.[6] It is now believed that COP1 negatively regulates several transcription factors that are involved in light-regulated gene expression and development.[7] This may involve COP1-mediated and targeted degradation of transcription factors via the 26S proteosome.[8]

Apart from *Arabidopsis*, *COP1* gene homologs have been identified from some other dicot plants namely pea[9,10] and tomato (accession no. ACC98912) as also from the mammalian system.[11] The contribution of light in regulating the pattern of development in different plants is highly variable. This difference is reflected both at the morphological as well as at the molecular levels.[1,2] The variation is expected to be more pronounced between monocots and dicots, which evolutionarily diverged more than 100 million years ago. The interaction of light-dependent and developmental signals in rice has already been shown to influence gene expression in a species-specific manner.[12] How and why plants respond differently to the similar light conditions is an interesting and important aspect of plant development that deserves attention. The *COP1* gene, which is known to act as a negative regulator of photomorphogenesis in

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higher plants,[6] is an ideal subject for this kind of a study. To have a better understanding of the involvement of COP1 in photomorphogenic development of monocots, the *COP1* gene homolog was isolated and characterized from *indica* rice (var. Pusa Basmati 1).

For isolation of the cDNA clone of *COP1* from *Oryza sativa* L. subsp. *Indica* variety Pusa Basmati 1, a cDNA library of roots from light-grown rice plants, constructed using the ZAP Express™ and Gigapack III® Gold cDNA synthesis kits (Stratagene Cloning Systems, USA), was screened with a heterologous gene-specific cDNA probe from *Arabidopsis*.[4] Comparison of the nucleotide sequence of a clone thus obtained (accession no. AF261992) with that of the known sequence of *COP1* cDNA from *Arabidopsis* indicated that the clone is truncated at the 5′ end.[13] Subsequently, a 1.5-kb region of the cDNA from rice, starting from a *Bam*HI site towards the 3′ end of this cDNA clone, showing high level of homology to the cDNA of *Arabidopsis*, was used as a probe to screen the genomic DNA library of *indica* rice (*Oryza sativa* L. var. Pusa Basmati 1) prepared in Lambda Dash® vector (Stratagene Cloning Systems, USA). After three successive rounds of screening, two clones gave a positive signal. Southern analysis of these two clones indicated that both represent the same gene but differ in the size of the flanking regions. The *Eco*RI-digested fragments of one of the clones were sub-cloned and used for sequencing. It was revealed that the genomic DNA region (5.36 kb) encompassing *COP1* in *indica* rice contains 12 introns and 13 exons (accession no. AF289544), which were established on the basis of a *COP1* partial cDNA sequence of *indica* rice and a cDNA sequence of *japonica* rice (accession no. AB040053) (Fig. 1). The introns range from 74 bp to 614 bp in size and are evenly distributed throughout the gene. Of the 12 introns, the boundaries of at least 10 introns show conserved GT-AG motifs.[14] The size of the exons varies from 100 bp to 456 bp. When compared with *Arabidopsis*, a high degree of conservation is reflected in the number of introns (12) and their relative positions (Fig. 2).[15] The coding region of rice *COP1* has an overall similarity index of 60.1 with that of *Arabidopsis* at the nucleotide sequence level. When the sequence of the coding region of *COP1* from *indica* rice is compared with that of *japonica* (available in database), a high degree of similarity (99.9 units) is observed. There are essentially three substitutions at positions 2045 (T → C), 2076 (T → C) and 5303 (T → A) in the sequence of the *indica* rice. All but one of the substitutions are silent substitutions. Only the substitution at nucleotide 2076 leads to a change in the amino acid (leucine → proline).

Restriction analysis of the complete genomic region containing *COP1* from *indica* rice revealed that it does not have any site for *Sal* I and *Xba* I, has one site for *Bam*HI and two sites each for *Bgl* II and *Eco*RI. When Southern analysis of the rice genomic DNA digested with these enzymes was done employing a 1.5-kb partial cDNA from rice as a probe, only a single band was observed when the DNA was digested with *Sal* I, *Bam*HI and *Xba* I, two prominent bands were seen with *Eco*RI (third being very faint due to limited overlap with the probe), and three bands were seen with *Bgl* II, indicating that the *indica* rice genome harbors a single copy of the *COP1* gene (Fig. 3). In the *Arabidopsis* and pea genomes too, the *COP1* gene is present as a single copy.[4,10]

The assembled coding region of the gene is capable of encoding a polypeptide of 685 amino acids with an estimated molecular weight of 76.4 kDa and a pI of 6.99. When the deduced amino acid sequence of the COP1 protein in *indica* rice is compared with that of *Arabidopsis*, a significant level of homology (similarity index 70.3) is observed throughout the protein (Fig. 4). Among dicot species, similarity indexes of 74.9% and 75.3%, with respect to *Arabidopsis*, has been found in the case of pea and tomato, respectively. *Arabidopsis* COP1 protein is known to consist of three major functional domains, an N-terminal Zn-binding ring finger, a putative coiled-coil domain and the C-terminal region containing WD-40 repeats.[15] The rice COP1 protein shows much higher homology with *Arabidopsis* COP1 in these domains. There is 81% homology in the Zn-binding bipartite cysteine-rich ring finger (amino acids 55–102), which is known to bind two Zn^{++} ions in *Arabidopsis*.[16] The putative coiled-coil (amino acids 136–217) and WD-40 repeat (amino acids 396–629) regions have homology of 72% and 86%, respectively. Besides major functional domains, the COP1 protein is also known to contain a cytoplasmic localization/retention signal (CLS) and a nuclear localization signal (NLS), which play an important role in the light-induced nucleo-cytoplasmic partitioning of the protein.[15] In *Arabidopsis*, the COP1 protein has a bipartite nuclear localization signal and any mutation in the sequence of either of the two core modules results in the loss of activity, indicating that both the modules work in cooperation.[17] In rice, the module shares a 75% homology with that of *Arabidopsis* as there is a substitution of lysine with arginine at position 3 of the first module and position 1 of the second module, but this is not expected to affect the activity of the modules since both amino acid residues are basic.[18] Similarly, the CLS of rice shows homology of 73% with that of *Arabidopsis*. Significant similarity is also observed in the regions responsible for the interaction of the COP1 protein with the COP1 interactive proteins - CIP1 (73%), CIP7 (71.2%) and CIP8 (71.8%).[19–21] Compared to pea and tomato, rice shows a lower degree of homology to all the functional domains known in *Arabidopsis* (Table 1). This could be attributed to the evolutionary divergence of rice, a monocot, from these dicot plants.

To assess the expression pattern of *COP1* at the steady-state transcript level, as influenced spatially, temporally or under conditions of illumination, an

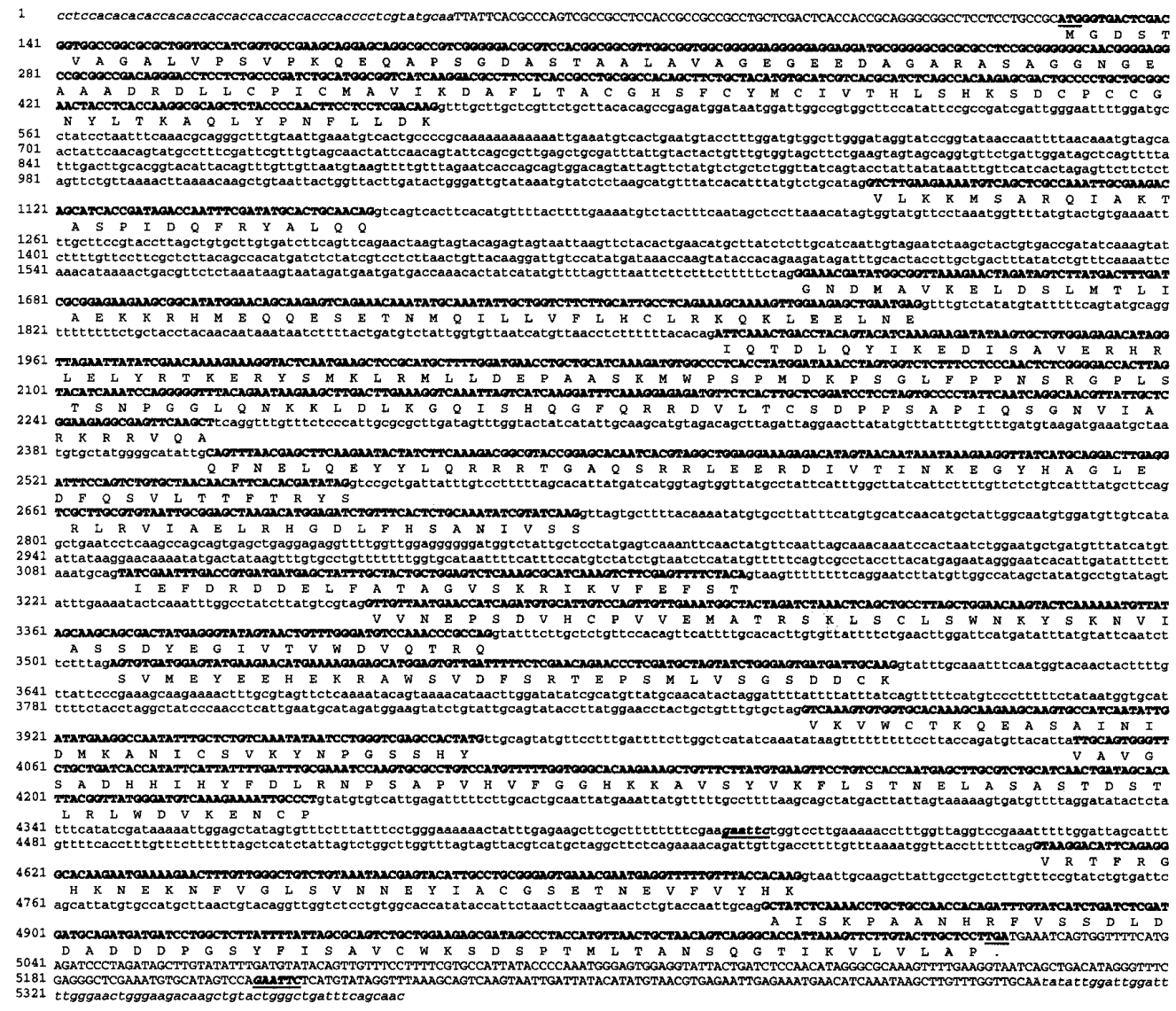

Figure 1. The nucleotide sequence of the genomic DNA from *indica* rice encompassing *COP1*. The sequence of exons is given in capital letters and regions encoding amino acids are given in bold. The 5′ end of the first exon and 3′ end of the 13th exon are defined on the basis of cDNA alignment, and sequences beyond these points are shown in italicized lower case letters. Introns are given in lower case. The deduced amino acid sequence is given in single letter code. Subclones of cDNA and genomic clone were sequenced using either the standard T3 and T7 primers or gene-specific primers. Sequencing was done employing an automated DNA sequencer, ABI Prism 377 (Perkin-Elmer, USA), or through the sequencing facility provided by Microsynth GmbH, Switzerland.

RNase protection assay was performed (Fig. 5). Total RNA from young green leaves (YL), young etiolated leaves (YEL) and young roots (YR) of 1-week-old plants and also from green leaves (ML), root (MR), pre-pollination inflorescence (PP) and post-fertilization inflorescence (PF) from mature plants was used for the analysis. In *Arabidopsis*, various sensory photoreceptors perceive the light signal to regulate the activity of COP1[2,22] by controlling its nucleo-cytoplasmic partitioning[4] but without any significant effect on its transcript levels. Comparison of lanes 3 (YEL) and 6 (YL) reveals that light causes an increase in the steady-state transcript levels of *COP1* in rice. On the other hand, comparison of lane 1 (YR) and 2 (MR) and that of lanes 6 (YL) and 7 (ML) shows that there is a significant decrease in the levels of mRNA in the leaf and root of the adult plant as compared to the young seedling. Besides, a spatial variation in the expression of *COP1* was also observed. Although in the young seedling (1-week-old), the levels of *COP1* mRNA are similar in root and leaf tissue (lanes 1 and 6) but, in mature plants, there is a marked difference, with roots showing a signif-

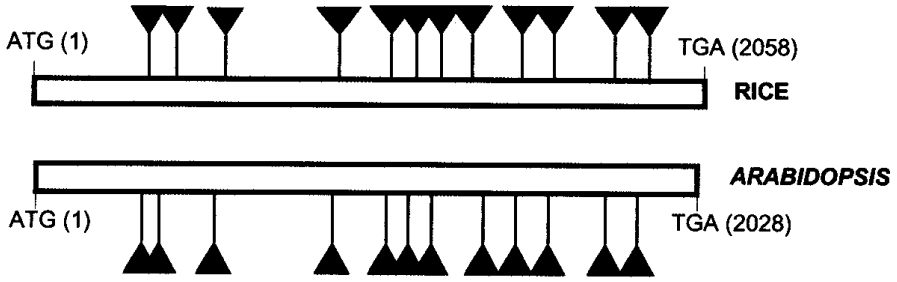

Figure 2. Relative distribution of the introns in the *COP1* gene of rice and *Arabidopsis*. Scale: 20 bp = 0.9 mm.

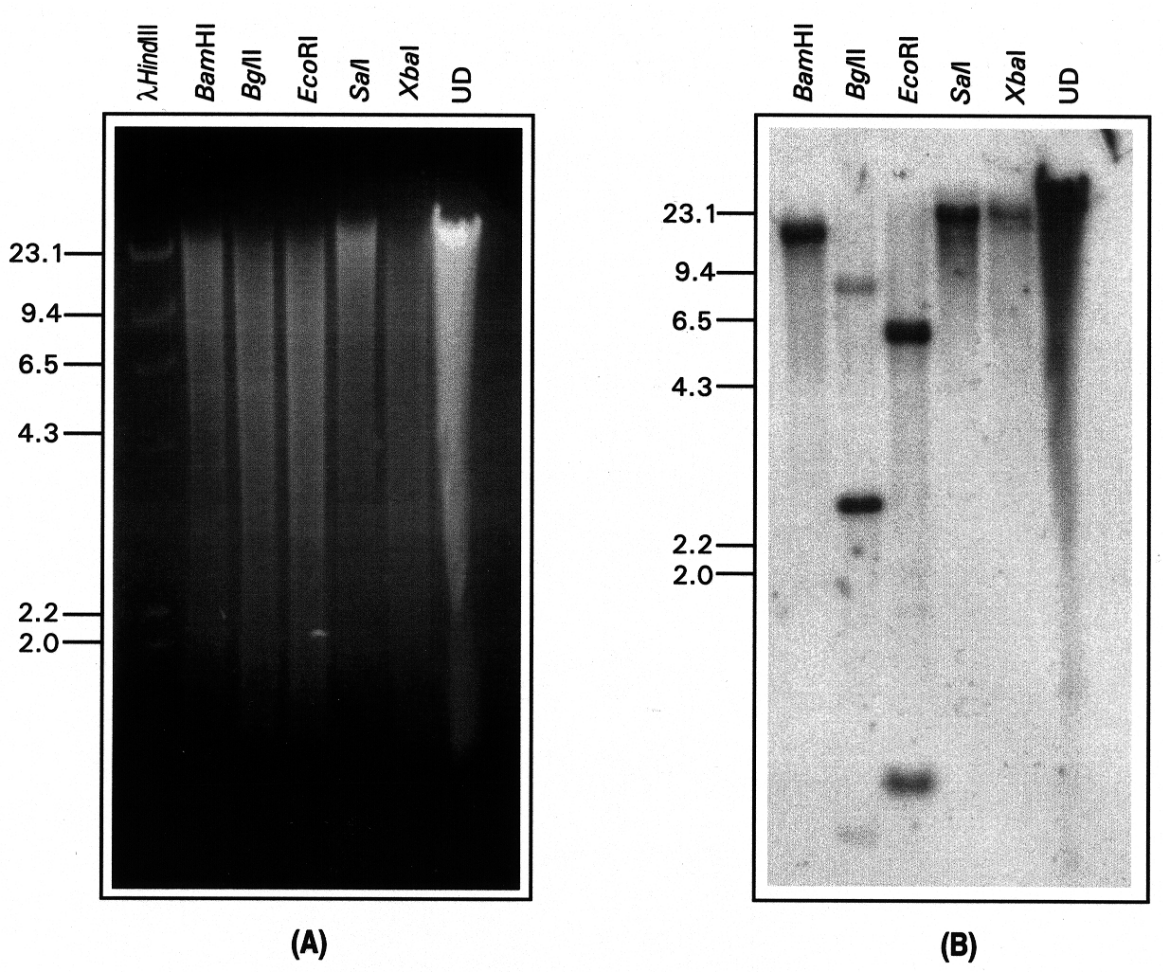

Figure 3. Southern analysis to ascertain the copy number of the *COP1* gene in rice. A. Digested DNA. B. Autoradiogram. Total genomic DNA was isolated following the protocol of Dellaporta et al.[26] Genomic DNA in 5-μg aliquots was digested with *Bam*HI, *Bgl* II, *Eco*RI, *Sal* I, and *Xba* I. Southern blotting and hybridization were done as described in Sambrook et al.[27] The blot was probed with the 1.5-kb *Bam*HI/*Xba* I fragment of the rice cDNA clone.

icant decrease as compared to the mature leaves (lanes 2 and 7). Similarly, if various tissues from a mature plant are compared, a gradation in the level of *COP1* mRNA is observed in the following order: PP > PF > ML > MR (lanes 5, 4, 7 and 2, respectively). This indicates that development-dependent and spatial cues are capable of affecting the mRNA levels of *COP1* in rice. Indeed, the requirement of COP1 in mediating the switch from the

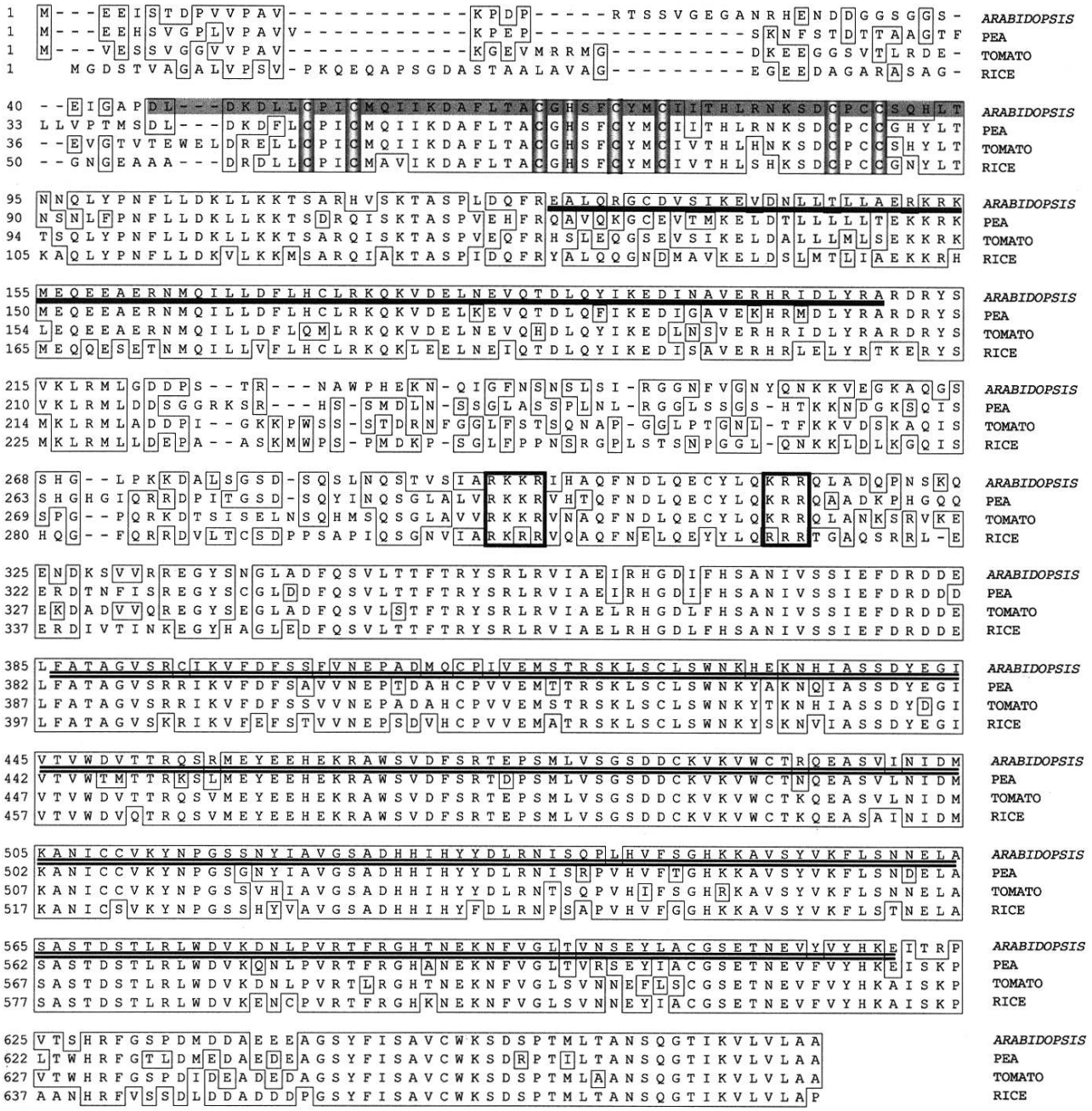

Figure 4. Alignment of the COP1 protein of pea, tomato and *Arabidopsis* with that of rice. The major functional domains are marked. The highlighted residues constitute the Zn-binding ring finger domain (note the cysteine and histidine residues involved in Zn-binding domain), residues underlined with thick line form the coiled-coil region, and the WD-40 repeats are underlined with a double line. The two modules of the bipartite NLS are marked with bold boxes. The amino acid sequence comparison was done by the Jotun Hein method (for multiple sequence alignment) provided by the Lasergene Sequence Analysis Software (DNASTAR, Inc., USA).

skotomorphogenesis to the photomorphogenesis is known to be development dependent.[23]

The conservative nature of the gene indicates that, as in the case of dicots, *COP1* is probably an essential gene even for monocots and may play an important role in plant development.[1–3] It has been found that COP1 and other pleiotropic COP/DET/FUS proteins are highly conserved among diverse eukaryotes ranging from *Arabidopsis* to humans.[24] This fact led to the suggestion that these proteins, along with COP1, are involved in a much broader signal transduction network, a part of which has become specialized in plants for the

Table 1. Comparative analysis showing sequence similarity of various domains of COP1 proteins from rice, pea and tomato *vis-a-vis* protein from *Arabidopsis*. Comparison at the amino acid level is given as percentage similarity to the corresponding domain in *Arabidopsis*.

Arabidopsis COP1 domains (amino acids)	Percent identity with *Arabidopsis*		
	Rice	Pea	Tomato
Zn-binding RING finger motif (45–95)	81.0	95.0	84.3
Coiled-coil region (128–209)	72.0	81.0	78.8
WD-40 repeats (386–619)	86.0	88.0	90.0
Nuclear localisation signal (294–297; 312–314)	75.0	100	100
Cytoplasmic localisation signal (67–177)	73.0	80.0	78.0
Sub-nuclear localisation signal (120–177)	70.6	75.0	71.9
CIP1 interacting domain (105–205)	73.0	79.0	80.0
CIP7 interacting domain (128–215)	71.2	83.0	79.3
CIP8 interacting domain (39–103)	71.8	71.8	76.0
HY5 interacting domain (386–619)	86.0	88.0	90.0

light-mediated responses.[25] Further characterization of *COP1* from rice is in progress and that would help understand various aspects of COP1-mediated signal transduction network, specifically in monocots.

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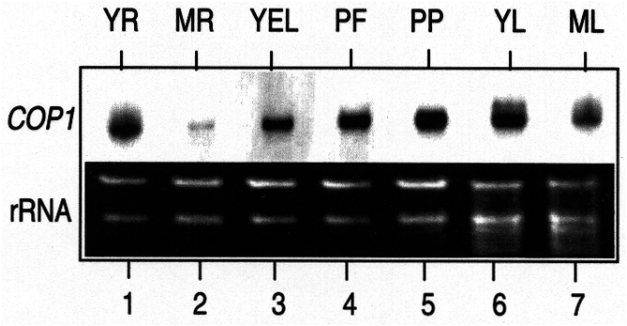

Figure 5. Steady-state transcript levels of *COP1* in rice as detected by RNase protection assay. The lower panel shows ethidium-bromide stained ribosomal RNA from the same samples. Young root (YR), Mature root (MR), Young etiolated leaf (YEL), Post-fertilization inflorescence (PF), Pre-pollination inflorescence (PP), Young leaf (YL) and Mature Leaf (ML). Total RNA was isolated from 1-week-old rice plants grown at $28 \pm 1°C$ in dark or under light provided by fluorescent tubes (Philips TL 40W/54) at a fluence rate of 70 μmol $m^{-2}s^{-1}$. Mature plants were grown in the field. RNA isolation was essentially done according to the protocol prescribed by Logemann et al.[28] An *Eco*RI genomic DNA fragment (4425–5206 bp) was cloned in the vector pSPT18 (Roche Molecular Biochemicals, Germany). The construct was digested with *Hin*dIII (4723 bp) and then transcribed in the presence of [α-^{32}P]CTP with T7 RNA polymerase, which resulted in a 483-bp labeled riboprobe in the antisense orientation. An RNase protection assay was carried out using the kit from Roche Molecular Biochemicals, Germany. Each reaction was done with 80 μg of the total RNA. After RNase treatment, a 363-bp band is expected to be protected.

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